EFFECT OF T-ACTIVIN AND HYDROCORTISONE ON TRANSPLANTATION IMMUNITY

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UDC 612.6.02.017.1.014.46:615.357.453

KEY WORDS: B mice; T-activin; hydrocortisone; transplantation immunity.

Previous investigations showed that the functions and morphology of the thymus depend on the level of endogenous glucocorticoids (corticosterone) in the inbred lines of mice with opposite reactivity to weak transplantation H-Y antigens [3, 6, 7]. The results of these investigations served to distinguish the thymus–adrenals as a system regulating cellular immunity, in which endogenous glucocorticoids are a connecting link between the adrenals and the thymus; other adrenal hormones (catecholamines), moreover, may be included in a regulatory stage operating through activation of the pituitary and increased ACTH production [5].

The object of this investigation was to study the separate or combined administration of T-activin and hydrocortisone on restoration of transplantation immunity in thymectomized and lethally irradiated mice, protected with syngeneic bone marrow, and treated *in vitro* with T-activin and hydrocortisone.

EXPERIMENTAL METHOD

To obtain B recipients adult CBA mice in whose bone marrow there are virtually no cells with a Θ-marker, were used, for such animals could be obtained with a maximal deficiency of T cells. The method of obtaining B mice and assessing T-cell immunodeficiency was described previously [4]. Bone marrow of syngeneic donors was treated *in vitro* with T-activin and hydrocortisone. T-activin (the active factor of calf thymus, or ATF-6) is a mixture of polypeptides with molecular weight of between 1500 and 6000 daltons and possesses marked stimulating activity in a series of tests [1]. To a suspension of bone marrow cells in medium 199 T-activin in a concentration of 50 μg/ml and hydrocortisone in a concentration of 0.3 mg/ml were added. This dose of hydrocortisone causes increased proliferation of mouse thymocytes in culture [9]. A suspension of bone marrow cells with T-activin and hydrocortisone was incubated at 37°C for 30 min and injected intravenously into thymectomized and irradiated (900 R) mice, each of which received, in a volume of 0.2 ml, 10×10^6 cells with 10 μg T-activin and 0.06 mg hydrocortisone. The doses of hydrocortisone and of T-activin used did not affect the visibility of the bone marrow cells, determined by staining with trypan blue. Bone marrow with cortisol-resistant T lymphocytes was obtained from CBA mice which had been given three injections of hydrocortisone, each in a dose of 2.5 mg [4].

EXPERIMENTAL RESULTS

Bone marrow cells, after treatment with T-activin in a combination with hydrocortisone or alone, induced the development of transplantation immunity in thymectomized recipients in 85 and 80% of cases, respectively (Table 1; groups 1 and 2); reactivity of the recipients to H-2 antigens of skin grafts of C57BL/6 mice in groups 1 and 2, moreover, was identical. Signs of rejection of skin allografts in the recipients of these groups appeared on the 12th-15th day, and complete rejection occurred by the 20th-23rd day after grafting. Bone marrow cells, after exposure *in vitro* to hydrocortisone or simply to incubation at 37°C did not induce reactions of transplantation immunity (groups 3 and 4). When bone marrow from donors treated with hydrocortisone was injected into thymectomized recipients, restoration of transplantation immunity was observed in all the recipients (group 5). The degree of recovery of transplantation immunity in the recipients of group 5, assessed as the mean time of survival of C57BL/6 skin grafts,

Department of Noninfectious Immunology and Department of Molecular Immunology, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR R. V. Petrov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 8, pp. 84-86, August, 1982. Original article submitted February 17, 1982.

0007-4888/82/9408-1111$07.50

TABLE 1. Correction of T-Immunodeficiency in B Mice by Syngeneic Bone Marrow Cells Treated with T-Activin and Hydrocortisone

Group of animals	Number of thymectomized recipients	Dose of irradiation, R	Injection of bone marrow cells (10×10^6)		No. of recipients with rejected C57BL/6 skin grafts 25 days after †	Restoration of immunity, %
			after treatment in vitro	from donors with hydrocortisone in vivo		
1	20	900	T-activin + hydrocortisone	—	17	85
2	15	900	T-activin	—	12	80
3	15	900	Hydrocortisone	—	0	0
4	15	900	—	—	0	0
5	20	900	—	+	20	100
6*	15	900	—	—	15	100

*Mock operation performed without removal of thymus.
†Maximal time of survival of skin allografts in recipients of group 5 was 25 days.

was less (19.1 ± 2.4 days) than in the animals of group 6 (13.6 ± 0.6 days; $P < 0.01$), undergoing a mock operation without removal of the thymus, in which T-cell reactivity was restored during regeneration of the thymus after irradiation.

The experimental results demonstrate the effectiveness of restoration of T-cell reactivity by the use of both bone marrow from donors receiving hydrocortisone and of bone marrow from intact animals treated *in vitro* with T-activin and hydrocortisone. The results agree with the experiments of Fauci [11], in which a subpopulation of cortisol-resistant T2 lymphocytes migrated into the bone marrow after injection of pharmacologic doses of glucocorticoids into mice. He showed that lymphoid cells are more effective in restoring immunologic reactivity in athymic mice than thymosin (fraction 5) [8]. It may be that large doses of thymosin must be injected into thymectomized mice in order to restore their T-cell immunity more completely, in order to compensate for the absence of the thymus, where precursors of cytotoxic T lymphocytes are differentiated [10]. It follows from the results of the present experiments that hydrocortisone, in the dose used, had no antagonistic action on maturation of T lymphocytes from bone marrow precursors, induced by T-activin. The possibility cannot be ruled out that the presence of glucocorticoids is essential, together with thymus factors, for differentiation of cortisol-resistant T2 lymphocytes *in vivo*.

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