

CHANGES IN H-ALLOANTIGEN-RECOGNITION FUNCTION OF LYMPHOCYTES AFTER HYDROCORTISONE ADMINISTRATION IN MICE

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The ability of spleen, thymus, and bone-marrow cells of intact (control) and hydrocortisone-treated (experiment) CBA mice to induce a lymph node graft versus host reaction (GVHR) in (CBA \times C57BL) F_1 hybrids was compared. After injection of hydrocortisone into the donors in a dose of 2.5 mg per mouse their spleen cells induced a more active GVHR, as shown by an increase in the lymph node indices and in the percentage of immunoblasts in the regional (popliteal) lymph node compared with the control. After transfer of thymus cells of hydrocortisone-treated donors the number of immunoblasts was higher than, but the weight of the lymph node was almost the same as in the control. Conversely, after injection of bone marrow cells from hydrocortisone-treated donors, the lymph node enlarged whereas the percentage of immunoblasts did not increase above the control. Consequently, when the increase in the hydrocortisone level is exogenous in nature, the cell populations of the spleen and thymus contain a higher proportion of T lymphocytes, which respond by proliferation to contact with H alloantigens.

KEY WORDS: *T lymphocytes; hydrocortisone; H alloantigens; graft versus host reaction.*

The H-alloantigen-recognition function of lymphocytes from spleen, thymus, and bone marrow of mice treated with hydrocortisone was assessed in this investigation by the use of a lymph node model of the graft versus host reaction (GVHR) [3].

EXPERIMENTAL METHOD

CBA mice aged 3-4 months were used as donors and (CBA \times C57BL) F_1 hybrids of the same age as recipients; both groups of mice were obtained from the Stolbovaya nursery, Academy of Medical Sciences of the USSR. Hydrocortisone (from Richter) was injected intraperitoneally into the donors in a dose of 2.5 mg per mouse 48 h before cells were taken from them [2]. Spleen and thymus cells were injected in a dose of $2 \cdot 10^6$ and bone marrow cells in a dose of $5 \cdot 10^6$ in 0.05 ml medium No. 199 subcutaneously into the recipient's left foot. The intensity of the GVHR was assessed by the lymph-node index (LI), i.e., the ratio between the weights of the left and right popliteal lymph nodes, and accumulation of immunoblasts in the regional lymph node. The numerical results were subjected to statistical analysis by Student's *t* test.

EXPERIMENTAL RESULTS

As Table 1 shows, after transplantation of $2 \cdot 10^6$ spleen cells from normal CBA donors LI of the (CBA \times C57BL) F_1 recipients was considerably increased in 3 days, but later it remained substantially unchanged throughout the rest of the period of observation. If the same dose of spleen cells from donors treated with hydrocortisone was injected, LI started to increase after the third day, reached a maximum by the fifth to seventh day, and was a little lower on the ninth day. LI on the 7th day was significantly higher than in the control ($P < 0.05$). After transplantation of $2 \cdot 10^6$ thymus cells from normal donors maximal values of LI were determined on the seventh to ninth day. Transplantation of the same dose of thymocytes from mice treated with hydrocortisone led to a significant increase in size of the regional lymph node after 3 days, but later LI did not change significantly. Bone marrow cells from donors

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TABLE 1. Lymph Node Indices for (CBA × C57BL)_F₁ Recipients After Transplantation of Spleen, Thymus, and Bone Marrow Cells from Intact (control) and Hydrocortisone-Treated (experiment) CBA Donors (M ± m)

Time after transplanta- tion, days	Composition of transplanted cells					
	2×10 ⁶ spleen cells *		2×10 ⁶ thymus cells *		5×10 ⁶ bone marrow cells	
	control	experiment	control	experiment	control	experiment
3	1,8±0,19	2,1±0,15	1,1±0,05	1,4±0,10	—	—
5	1,9±0,14	2,9±0,39	1,2±0,10	1,4±0,12	—	—
7	1,7±0,13	2,8±0,28	1,4±0,13	1,3±0,37	1,2±0,08	1,7±0,10†
9	2,0±0,27	2,3±0,17	1,3±0,16	1,7±0,35	—	—

*Recipients in control received injection of mixture of cells from 3-4 spleens or 6-8 thymus glands, experimental recipients received cells from 5-6 spleens and 30-40 thymus glands of donors.

†P < 0.05.

TABLE 2. Percentage of Immunoblasts in Regional Lymph Node of (CBA × C57BL)_F₁ Recipients After Transplantation of 2·10⁶ Spleen Cells, 2·10⁶ Thymus Cells, and 5·10⁶ Bone Marrow Cells from Intact (control) and Hydrocortisone-Treated (experiment) CBA Donors Subcutaneously into the Foot (M ± m)

Time after transplan- tation, days	Source of cells					
	spleen		thymus		bone marrow	
	control	experiment	control	experiment	control	experiment
3	1,8±0,50	2,4±0,62	0,3±0,11	0,7±0,04	—	—
5	1,9±0,43	2,7±0,51	—	—	—	—
7	1,6±0,22	3,1±0,61	1,3±0,32	2,2±0,59	0,4±0,10	0,3±0,22
9	1,8±0,83	2,8±0,54	—	—	—	—

Legend. Immunoblasts counted in 10,000 cells in squash preparations of lymph node stained with azure II-eosin. Number of immunoblasts in popliteal lymph node of intact mouse 0.15 ± 0.05%.

treated with hydrocortisone caused more marked enlargement of the lymph nodes than in the control (P < 0.05).

There are indications that following injection of hydrocortisone [5] or in systemic stress [1] characteristic changes take place in the T-lymphocyte population. They are connected, on the one hand, with death of the cortisone-sensitive pool in the thymus and peripheral lymphoid organs in situ, and on the other hand, with increased migration of cortisone-resistant T lymphocytes from the thymus in the spleen, lymph nodes, and bone marrow [7]. Cortisone-resistant T lymphocytes are considered to behave more actively in tissue incompatibility reactions in vitro [4] or in vivo [6]. In the present experiments a single injection of hydrocortisone strengthened the ability of the spleen cells to induce a local form of GVHR, as was manifested not only by the relative increase in LI, but also by the higher percentage of immunoblasts in the regional lymph node (Table 2). Meanwhile, after transplantation of the thymus cells of intact and hydrocortisone-treated donors the relative sizes of the regional lymph nodes were about the same, but the number of blast cells in the second situation was a little higher (Table 2). Conversely, after transplantation of bone marrow cells of the experimental mice LI values were significantly higher than in the control, whereas the percentage of immunoblasts was the same in the experimental and control series. Under the influence of hydrocortisone the relative proportion of cells responding by proliferation to stimulation by H alloantigens in the cell populations of the spleen and thymus was thus increased by hydrocortisone. Under these circumstances the population of cortisone-resistant cells of the spleen induced the local GVHR more actively than the cortisone-resistant thymocytes.

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