RESPONSES OF LYMPHOCYTES OF HYDROCORTISONE-STIMULATED AND ADRENALECTOMIZED MICE TO H ALLOANTIGENS

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The ability of spleen, bone marrow, and thymus cells of intact and adrenalectomized CBA mice, and of CBA mice receiving single or repeated doses of hydrocortisone, to induce a lymph node "graft versus host" reaction (GVHR) in (CBA × C57BL)F_1 hybrids was determined. The ability of the spleen and bone marrow cells to induce GVHR was increased two days after administration of 2.5 mg hydrocortisone, whereas the ability of the thymus cells was unchanged. Seven days and, in particular, 15 days after injection of hydrocortisone, the spleen cells were less active. Activity of the thymocytes in GVHR was increased two days after repeated daily injections of the hormone in a dose of 0.25 mg for 18 days, whereas activity of the spleen and bone marrow cells was unchanged.

KEY WORDS: hydrocortisone; adrenalectomy; graft versus host reaction; T lymphocytes.

It has recently been shown that the function of recognizing H alloantigen is connected with thymus-derived lymphocytes. It is usually studied in models of graft versus host reactions (GVHR) which are initiated by T lymphocytes [9, 11]. The T cell population is distinguished by high sensitivity to glucocorticoids [4, 6]. Under the influence of these hormones it undergoes characteristic reorganization. In particular, injection of pharmacological doses of hydrocortisone leads to lysis of immature cortisone-sensitive cells in the cortex of the thymus and of other lymphoid organs [5] and to a redistribution of cortisone-resistant T cells in the bone marrow [1]. Meanwhile the dependence of activation of T cells in allogeneic recognition reactions on adrenocortical function has been inadequately studied. In the present investigation a lymph-node form of GVHR was used to assess the H-alloantigen-recognizing function in intact and adrenalectomized mice and in mice stimulated once or repeatedly with hydrocortisone.

EXPERIMENTAL METHOD

The donors were CBA mice and the recipients were (CBA × C57BL)F_1 hybrids, females weighing 18-22 g. Details of the method of producing lymph-node GVHR in this combination of lines of mice were described previously [3]. In series I the donors were given hydrocortisone once only in a dose of 2.5 mg per mouse, in series II hydrocortisone was given daily for 18 days in a dose of 0.25 mg intraperitoneally (total dose 4.5 mg per mouse). In series III the donors underwent bilateral adrenalectomy under ether anesthesia. Intact donors served as the control. In series I, a cell suspension was obtained from the spleen, thymus, and bone marrow 2, 7, and 15 days, and in series II, two days after the final injection of the hormone, and in series III 8 days after bilateral adrenalectomy. The cells were injected in a dose of $5 \cdot 10^6$ or $5 \cdot 10^7$ in 0.05 ml medium No. 199 subcutaneously into the left foot of F_1 hybrids. The reaction was assessed by a lymph node index (LI), namely the ratio of the weight of the regional (experimental) popliteal lymph node (left) to the weight of the contralateral (control) lymph node. Statistical analysis was carried out by Student's t-test.

EXPERIMENTAL RESULTS AND DISCUSSION

After a single injection of the hormone the weight of the spleen and thymus was significantly reduced on the 2nd day and regained its initial value on the 15th day (Fig. 1).

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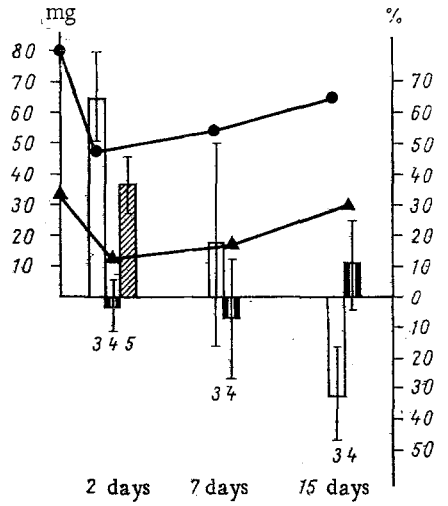

Fig. 1. Weight of spleen (1) and thymus (2) of donors at different times after the single injection of hydrocortisone and the percentage change in LI of recipients after transplantation of $5 \cdot 10^6$ spleen (3) and thymus (4) cells and $5 \cdot 10^7$ bone marrow cells (5) of donors treated once with hydrocortisone.

LI after translantation of $5 \cdot 10^6$ spleen cells obtained on the 2nd day after injection of hydrocortisone, was significantly higher than in the control. On the 7th day after injection of hydrocortisone, LI was indistinguishable from its values after transplantation of spleen cells from intact mice, but on the 15th day they were lower than in the control. Thymus cells, in a dose of $5 \cdot 10^6$, preserved the same ability to reduce a local GVHR as normal thymus cells on the 2nd, 7th and 15th days after injection of the hormone. Bone marrow cells obtained 2 days after injection of hydrocortisone were more active in inducing a local GVHR than normal bone marrow cells. After repeated injection of hydrocortisone the ability of thymus cells to induce the GVHR was appreciably increased (Table 1). When spleen and bone marrow cells obtained after repeated injection of the hormone were transplanted, the same values of LI were recorded as after transplantation of cells from the same sources from intact mice. Adrenalectomy on the donors led to a significant decrease in LI during transplantation of $5 \cdot 10^7$ bone marrow cells compared with the control but had little effect of the ability of thymocytes and spleen cells to induce a local GVHR (Table 2).

The results thus indicate that thymus and spleen cells active in the GVHR were cortisone-resistant, in agreement with observations by other workers [4-6]. It can be assumed that bone marrow and, in particular, spleen cells were more active in inducing the GVHR on account of migration of cortisone-resistant T lymphocytes into these organs. Meanwhile, ability to induce lymph-node GVHR was not increased in thymocytes from donors receiving a single dose of hydrocortisone. The fact that in a systemic GVHR cortisone-resistant thymocytes induced a greater degree of splenomegaly in immunologically immature F_1 hybrids than intact thymocytes [6], whereas in the present experiment the local GVHR was not intensified, can evidently be explained as follows. In the systemic GVHR in the $P \rightarrow F_1$ combination splenomegaly develops not so much on account of proliferation of the donor's immunocompetent cells in the spleen as because of the accumulation of precursors of hematopoiesis and cells of the histiomonocyte serine in it [2]. In the lymph-node GVHR, the lymph node enlarges mainly because of primary proliferation of the donor's cells and secondary involvement of the recipient's lymphocytes, dependent on it, in proliferation [7]. It is interesting to note that the weight of the spleen on the 15th day after a single injection of hydrocortisone returned to its initial level, but the ability of the spleen cells to induce GVHR was low. Probably at this stage the weight of the spleen is restored mainly on account of proliferation of cells that are inactive in the GVHR. After repeated injection of hydrocortisone the thymocytes behaved much more actively than after single hormonal stimulation. Conversely, the ability of the spleen cells to induce GVHR in this situation was appreciably weakened. The possibility cannot be

TABLE 1. Weight of Lymph Nodes (in mg) and LI after Injection of Spleen, Bone Marrow, and Thymus Cells from Intact (1) CBA Donors and CBA Donors Repeatedly Treated with Hydrocortisone (2) into (CBA × C57BL)F_1 Recipients

Type of transplantation		No. of recipients	Regional lymph node (experiment)	Contralateral lymph node (control)	LI
Spleen cells	1	9	3,3±0,4	1,6±0,25	1,9±0,3
	2	10	4,8±0,36	2,3±0,16	2,0±0,19
Bone marrow cells	1	8	2,0±0,19	1,6±0,16	1,4±0,4
	2	11	2,5±0,25	1,7±0,18	1,5±0,23
Thymus cells	1	8	2,4±0,23*	1,8±0,2	1,4±0,17†
	2	12	4,0±0,37*	1,7±0,2	2,8±0,42†

Legend. 1. *) $P<0.05$; †) $P<0.005$. 2. In the control a mixture of spleen cells from three or four intact animals, thymocytes from five or six animals, and bone marrow cells from five or six animals was injected; after repeated treatment with hydrocortisone, a mixture of spleen cells from 8 to 10 animals, of thymus cells from 35-40 animals, and of bone marrow cells from 15-20 animals was injected into the recipients.

TABLE 2. Weight of Lymph Nodes (in mg) in (CBA × C57BL)F_1 Recipients and LI after Transplantation of $5 \cdot 10^7$ Spleen, Thymus, and Bone Marrow Cells from Intact (1) and Adrenalectomized (2) CBA Mice (M±m)

Type of transplantation		No. of recipients	Regional lymph node (experiment)	Contralateral lymph node (control)	LI
Spleen cells	1	18	3,5±0,22	1,9±0,14	2,0±0,18
	2	18	3,0±0,19	1,8±0,07	1,7±0,1
Bone marrow cells	1	18	2,5±0,22	1,7±0,1	1,4±0,1
	2	18	2,7±0,3	1,8±0,09	1,6±0,17
Thymus cells	1	18	2,4±0,19	1,7±0,11	1,5±0,12*
	2	18	2,0±0,3	1,8±0,23	1,1±0,07*

Legend. 1. *) $P<0.05$. 2. A mixture of spleen, thymus, or bone marrow cells from 6 to 8 intact or adrenalectomized donor mice was injected.

ruled out that this was connected with accumulation of cells of the macrophage series which, under certain conditions can inhibit GVHR, in the spleen of mice repeatedly stimulated by hydrocortisone [10]. There are indications that adrenalectomy causes the transfer of cells active in reactions of cellular immunity from the bone marrow into the regulating pool [8]. To some extent this can explain the weakening of activity of bone marrow cells obtained in the present experiment from adrenalectomized donors in the local GVHR.

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CHANGES IN ACTIVITY OF SOME MECHANISMS OF SPECIFIC AND NONSPECIFIC IMMUNITY IN VITAMIN B_1 DEFICIENCY

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The effect of thiamine deficiency on the immune response and activity of certain mechanisms of natural immunity was studied in adult rats. Thiamine deficiency was simulated experimentally by a single injection of hydroxythiamine, a vitamin B_1 antagonist. Administration of hydroxythiamine caused a marked decrease in complement activity, phagocytic activity of the peripheral blood leukocytes, bactericidal activity of the serum, and antibody production in response to immunization with sheep's red blood cells. Conversely, lysozyme activity increased. In vitamin B_1 deficiency the intensity of incorporation of [^{14}C]leucine into liver protein synthesis was reduced.

KEY WORDS: thiamine deficiency; hydroxythiamine; factors of natural immunity; immune response.

Data in the literature on the effect of vitamin B_1 deficiency on the activity of mechanisms of specific and nonspecific immunity are few in number and contradictory in nature [1]. One reason for this is the difficulty encountered by research workers when attempting to simulate alimentary avitaminosis B_1. A considerable period of time elapses between exclusion of thiamine from the diet and the appearance of any marked metabolic disturbances, and during that time various compensatory reactions disturbing the purity of the biochemical changes are able to develop. These defects are overcome by the nowadays widespread method of administration of thiamine antimetabolites in order to obtain B_1 avitaminosis in a short time [2, 3].

The object of this investigation was to study the effect of vitamin B_1 insufficiency caused by administration of hydroxythiamine (HT), a powerful thiamine antimetabolite, which has no vitamin activity, on certain mechanisms of specific and nonspecific immunity.

EXPERIMENTAL METHOD

Experiments were carried out on 60 noninbred male rats weighing 250-300 g. Acute thiamine insufficiency was produced by a single subcutaneous injection of HT in a dose of 400 mg/kg. HT synthesized in the Department of Regulation of Metabolism, Academy of Sciences of the Belorussian SSR, was used. The compound was kindly presented by A. N. Martinchik. To study the state of nonspecific immunity, blood was taken from the animals before and 48 h after injection of HT. According to the literature data, the biochemical changes reflecting B_1 avitaminosis reach their maximum at this time, but compensatory changes are still insignificant [2]. The serum bactericidal activity was studied by a nephelometric method, using *Staphylococcus aureus* (strain Zhaev) as the test organism; complement activity was determined relative to 50% hemolysis. The serum lysozyme content was determined by Dorofeichuk's method. The phagocytic activity of peripheral blood neutrophils relative to *Staph. aureus*, strain Zhaev, was determined by the usual method, by counting the total number of phagocytic cells and calculating the phagocytic index. To study the effect of thiamine insufficiency on antibody production, 48 h after injection of HT the animals were immunized intraperitoneally with 2 ml of a 10% suspension of sheep's red blood cells. The antibody titer was determined in the usual way by the passive hemagglutination test. Activity of the protein synthesizing function was judged from the intensity of incorporation of [^{14}C]leucine into liver protein metabolism. The radioactive amino acid was injected intraperitoneally two days after injec-

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