Aminotransferase Activities and Involution of the Thymus in Rats Bearing AH 130 Tumors¹

Fumihide Isohashi,2 Kazue Tsukanaka, Masako Terada, Yoko Nakanishi, Sadanao Tani, and Yukina Sakamoto

Department of Biochemistry, Institute for Cancer Research, Osaka University Medical School, Fukushima-1-chome, Fukushima-ku, Osaka 553, Japan

ABSTRACT

Involvement of the thymus was observed in rats bearing AH 130 (solid-type) tumors. The thymus weight decreased with tumor growth. Daily injection of a pharmacological dose of hydrocortisone into normal rats resulted in decrease of the thymus and marked increase in alanine aminotransferase activity. This treatment also caused slight increase in the activity of tyrosine aminotransferase but not of aspartate aminotransferase in these animals. Involvement of the thymus in tumor-bearing rats, however, was not accompanied by appreciable increases in the activities of these aminotransferases, even at an advanced stage of tumor growth when the plasma corticosterone level was very high and significant increase in the activities of all these enzymes was observed in the liver. Furthermore, additional injections of hydrocortisone into rats with tumors weighing more than 5% of the body weight did not cause any appreciable change in alanine aminotransferase activity in the thymus, although in rats with smaller tumors it slightly increased the enzyme activity in the thymus. Furthermore, in normal rats, increase in alanine aminotransferase activity in the thymus with involution of the glands was observed with a dose of corticosterone close to the physiological range attained in rats with tumors in an advanced stage.

INTRODUCTION

Administration of adrenocorticotrophic hormone or glucocorticoid to animals has been found to cause a decrease in the mass of lymphoid tissue and in the numbers of lymphoid cells (6—8, 10, 13), and addition of glucocorticoid to lymphoid tissue or lymphoid cells in vitro causes lymphocytolysis (3, 21, 35). The mechanism of this action of glucocorticoid on lymphoid tissue is unknown, but regression of lymphoid organs and cells is reported to be accompanied by decrease in the uptake of glucose (25), uptake of amino acids and nucleic acid precursors (23), and then inhibition of protein synthesis (2, 9, 31, 32). Although most of these effects of glucocorticoid on lymphoid tissues are opposite to the effects of glucocorticoid on the liver (20), daily injection of glucocorticoid into rats for 1 week increased the activity of alanine aminotransferase [L-alanine: 2-oxoglutarate aminotransferase (EC 2.6.1.2)] markedly in both liver and thymus, with 95% involution of the latter (30). The activities of various aminotransferases of rat liver cytosol, such as tyrosine aminotransferase [L-tyrosine: 2-oxoglutarate aminotransferase (EC 2.6.1.5)], alanine aminotransferase, and aspartate aminotransferases [L-aspartate: 2-oxoglutarate aminotransferase (EC 2.6.1.1)], which are readily induced by glucocorticoids (20), increased in an advanced stage of growth of AH 130 solid-type (14) and AH 130 ascites-type (27) tumors. Specific receptors for dexamethasone in the cytosol of the liver of tumor-bearing rats were found to increase in an advanced stage of tumor growth (14). In contrast to this, dexamethasone receptors in the cytosol of the thymus of tumor-bearing rats that had been adrenalectomized 3 days previously decreased in an advanced stage of tumor growth (17). Hypertrophy of the adrenal gland occurred during the tumor-bearing process with increase in adrenocortical function (27) and in the level of plasma corticosterone (26, 28). In tumor-bearing rats, adrenalectomy restored the binding capacity of glucocorticoid receptors and the weight of the thymus to nearly the normal values (17). These findings show that glucocorticoid is largely, if not entirely, responsible for involution of the thymus in tumor-bearing rats (17).

This paper, however, reports that in tumor-bearing rats involution of the thymus is not accompanied by increases in aminotransferase activities. A preliminary report of part of this work has been presented (16).

MATERIALS AND METHODS

Animals and Treatment. Male albino Donryu rats, weighing 55 to 65 g, were given laboratory chow and water ad libitum and allowed to adapt to laboratory conditions (temperature 25°, with lighting from 6 a.m. to 6 p.m.). When they reached 100 to 110 g body weight, they were given s.c. injections of 10⁷ to 10⁸ cells of AH 130 ascites tumor in the right hip and were killed 2 weeks later. When specified, hydrocortisone acetate (2.5 mg per 100 g body weight per day) was injected i.m. into rats with or without tumors. Some rats that were adrenalectomized 1 day before the beginning of glucocorticoid injection were given injections of corticosterone (0.4 mg/100 g body weight) every 3 hr and were sacrificed at the times indicated in Charts 5 and 6.

Preparation of Cytoplasmic Extracts. Animals were decapitated, and their thymuses were rapidly removed, weighed, and cooled in ice-cold 50 mM Tris-HCl buffer (pH 7.55) containing 250 mM sucrose, 25 mM KCl, 3 mM MgCl₂, and 1 mM mercaptoethanol. Then it was homogenized in 2 volumes of the same buffer, and the homogenate was centrifuged at 105,000 × g for 1 hr at 2° to obtain the cytoplasmic supernatant fraction (cytosol).

Enzyme Assay. Tyrosine aminotransferase activity in the cytosol was measured by the method of Diamondstone (5). Alanine aminotransferase and aspartate aminotransferase activities were measured with a Wako Transaminase B test kit (Wako Pure Chemical Industry, Ltd., Osaka, Japan), essentially...
as described by Reitman and Frankel (29). Protein was determined by a modification (11) of the method of Lowry et al. (22), with bovine serum albumin as a standard.

Assay of Plasma Corticosterone. Animals were decapitated, and their blood was collected in heparinized glass beakers and centrifuged to obtain the plasma. Corticosterone was extracted and purified from the plasma as described previously (4, 24). Plasma corticosterone was measured fluorometrically (28).

RESULTS

Chart 1 shows the effects of daily i.m. injection of hydrocortisone acetate (2.5 mg per 100 g body weight per day) on the thymus weight and the activities of various aminotransferases in the cytosol of the thymus of normal rats. The weight of the thymus decreased steadily. A higher dose of hydrocortisone caused more rapid involution of the thymus, and even a single injection caused some involution (data not shown). These findings are consistent with results on mouse thymus reported elsewhere (10, 13). Alanine aminotransferase activity in the thymus increased significantly on daily injection of hydrocortisone (Chart 1). The activity of tyrosine aminotransferase also increased slightly, but that of aspartate aminotransferase did not change significantly (p > 0.05) (Chart 1). The activities of these enzymes in the cytosol of rat thymus were much lower than those reported for the cytosol of rat liver (14, 27).

Chart 2 shows the involution of the thymus observed in rats bearing AH 130 solid-type tumors. Similar results have been obtained in rats bearing ascites-type tumors (26). The extent of involution of the thymus in rats with AH 130 solid-type tumors was proportional to the tumor weight. Previous reports from our laboratory showed that the activities of various aminotransferases in the liver cytosol increased in the advanced stage of growth of ascites-type (27) and solid-type (14) tumors. In contrast, in the thymus these aminotransferase activities did not increase at any stage of tumor growth (Chart 3). Moreover, alanine aminotransferase activity decreased slightly in the thymus of tumor-bearing rats, but no correlation was found between its activity and the extent of tumor growth (Chart 3).

Chart 4 shows the endogenous levels of corticosterone in the plasma of rats with and without tumors that had been adapted to standard laboratory conditions as described in ‘Materials and Methods.’ The plasma corticosterone level of control rats shows a typical circadian rhythm which is consistent with previous reports (12). In rats with tumors, these circadian rhythms gradually disappeared, and the plasma corticosterone level increased with tumor growth (Chart 4).

The results in Chart 1 were not obtained under physiological conditions; hydrocortisone, which is not the main glucocorticoid hormone secreted by the adrenal cortex of rats, was injected intermittently, and the pharmacological dose used was far in excess of the endogenous corticosteroid level. Thus, it was of particular interest to determine whether increase in alanine aminotransferase activity could be observed with a
Aminotransferases in Thymus of Tumor-bearing Rats
dose of corticosterone close to the physiological range in rats with advanced stage tumors and in rats with continuous endogenous secretion of glucocorticoid. Therefore, in adrenalectomized rats, we tried to obtain a plasma corticosterone level which was similar to that in rats with advanced-stage tumors by injecting corticosterone (0.4 mg/100 g body weight) every 3 hr (Chart 5). The results in Chart 6 show that the weight of the thymus in these animals decreased steadily and that the alanine aminotransferase activity in the thymus increased significantly. The activity of tyrosine aminotransferase increased slightly (Chart 6). It may be noted that this treatment resulted in more rapid involution of the thymus than that shown in Chart 1 and that the activity of aspartate aminotransferase also increased slightly, but the increase of alanine aminotransferase activity was comparable to that in Chart 1.

We also tested whether alanine aminotransferase activity was enhanced by daily i.m. injection of 2.5 mg of hydrocortisone acetate in rats with tumors as shown in Chart 7. Results indicated that in rats with small tumors the enzyme activities increased slightly but that in those with bigger tumors the activities were not increased by injection of hydrocortisone.

DISCUSSION
Injection of glucocorticoid causes marked atrophy of the

---

Chart 4. Plasma corticosterone concentrations over a 24-hr period in normal and tumor-bearing rats. Plasma corticosterone was measured fluorometrically. •, animals bearing tumors weighing about 10 to 15% of the total body weight (means of duplicate determinations on single animals); ○, normal rats (means of values for 4 to 6 animals); bars, S.D.

Chart 5. Plasma corticosterone concentration after i.m. administration of corticosterone. Corticosterone (0.4 mg/100 g) was administered i.m. every 3 hr to adrenalectomized rats (arrows). Animals were decapitated, and their blood was collected. Plasma corticosterone was determined fluorometrically. ○, means of duplicate determinations on single animals.

Chart 6. Changes of thymus weight and activities of various aminotransferases in the cytosol of the thymus of rats given injections of corticosterone (0.4 mg/100 g) every 3 hr as described in Chart 5. Enzymatic activities are expressed as described in Chart 1. Points, means of values for 4 to 7 animals; bars, S.D. ○, thymus weight; •, tyrosine aminotransferase; ▲, aspartate aminotransferase; ▼, alanine aminotransferase.

Chart 7. Activities of alanine aminotransferase in the thymus of tumor-bearing rats treated daily with hydrocortisone acetate (2.5 mg/100 g body weight) for 4 days. Enzymatic activities are expressed as μmol of product formed per min per mg of protein. ●, tumor-bearing rats treated with hydrocortisone acetate (means of duplicate determinations on single samples); □, normal rats treated with hydrocortisone acetate (means of values for 7 animals); bars, S.D.; hatched column, range of S.D. ○, normal rats treated with 0.9% NaCl solution (means of values for 7 animals); bars, S.D.; stippled column, range of S.D.
thymus in mice and rats (10, 13, 20, 30), and the decrease in thymus weight and in the number of thymocytes is proportional to the dose of hydrocortisone injected (13). During atrophy of the thymus on hydrocortisone injection, alanine aminotransferase activity increased greatly (30), tyrosine aminotransferase activity increased slightly, and aspartate aminotransferase activity did not change significantly (Chart 1). It should be pointed out here that the activities of tyrosine aminotransferase and aspartate aminotransferase in the cytosol of rat thymus were much lower than those found in the cytosol of rat liver (14, 27). It is obscure in this paper whether the activities of tyrosine aminotransferase determined in the cytosol of thymus are the same as those of tyrosine aminotransferase or pseudosozyme of tyrosine aminotransferase, which appears to be aspartate aminotransferase (34), although the activities of aspartate aminotransferase and tyrosine aminotransferase in the cytosol of thymus did not change in parallel (Charts 1 and 6).

Involution of the thymus also occurred during growth of AH 130 solid-type tumors without addition of exogenous glucocorticoid, and the extent of decrease in thymus weight was proportional to the tumor weight (Chart 2). However, involution of the thymus in AH 130 tumor-bearing rats was not accompanied by increase in alanine aminotransferase, aspartate aminotransferase, or tyrosine aminotransferase activity at any stage of tumor growth (Chart 3), although the activities of these enzymes increased significantly in the liver of animals with tumors in an advanced stage of growth (14, 27). Administration of a pharmacological dose of glucocorticoid induced these aminotransferase activities in the livers of normal rats, and more especially of tumor-bearing rats (27). Glucocorticoid treatment also increased alanine aminotransferase activity in the thymus of normal rats (Chart 1) (30), but not in the thymus of rats with advanced tumors (Chart 7), although it induced a slight increase in activity in the thymus of rats with tumors in an early stage of growth before involution of the thymus had become pronounced (Chart 7).

Adrenalectomy restored the weight of the thymus to nearly the normal value in rats bearing tumors (17). Duval et al. (10) reported that, 8 days after treatment of adrenalectomized mice with glucocorticoid, the thymus had returned to almost the normal weight, and similar findings have been observed in rats (15). Our results showed that the endogenous glucocorticoid level is very high in rats with tumors in an advanced stage of growth (Chart 4). Similar findings have been made on rats with ascites-type tumors (27) and lymphomas (28). The circadian rhythm of the plasma corticosterone level observed in normal rats was scarcely detectable in tumor-bearing rats (Chart 4). Thus, it is possible that atrophy of the thymus in tumor-bearing rats may be caused by a high level of endogenous glucocorticoid (26, 33). However, the events associated with atrophy of the thymus in normal rats treated with glucocorticoid and in tumor-bearing rats are different (Charts 1, 3, and 6).

There is much evidence that the binding of glucocorticoid to specific cytosol receptors is an essential initial step in specific enzyme induction mediated by glucocorticoid, it involves tissue responsiveness to the hormone (33), and the change in the levels of receptors may be an important mechanism for regulating the effects of hormones on target tissues (33). However, the increases in activity of alanine and tyrosine aminotransferases in the thymus of glucocorticoid-treated rats were not correlated with the binding capacities of specific receptors for glucocorticoid in the cytosol of these glands, since the binding capacities decrease during thymus involution in rats (15) and mice (10). Glucocorticoid receptors in the thymus of tumor-bearing rats also decrease during atrophy of the thymus (16, 17). The apparent discrepancy between the induction of the enzymes by glucocorticoid treatment and decrease in cytosol receptors in this gland, however, may be explained in other ways, namely, that on glucocorticoid treatment since the receptors translocate into the nucleus which is necessary for enzyme induction, or that since the cytosol receptors are filled with glucocorticoid, reduced [3H]glucocorticoid binding can be demonstrated. These explanations, however, are unlikely for the following reasons. The level of cytosol receptors in the thymus is still very low on Day 3 after adrenalectomy in tumor-bearing rats and in glucocorticoid-treated rats when corticosterone was undetectable in the plasma or thymus (15), and the receptors in the liver cytosol show minimal binding capacity in control (1, 32), tumor-bearing (14), and glucocorticoid-treated (15) rats. Moreover, dichloromethane extracts (10) of the plasma and thymus of glucocorticoid-treated and tumor-bearing rats which had been adrenalectomized 3 days previously did not significantly influence dexamethasone binding in the cytosol of the thymus from adrenalectomized control rats (15). Thus, it is hard to explain the increases in aminotransferase activities in the thymus of glucocorticoid-treated rats directly on the basis of the action of glucocorticoid through specific cytosol receptors. Conceivably, secondary factors produced by glucocorticoid, or a different mechanism of action of glucocorticoid, are involved in the change of these enzyme activities in the thymus of glucocorticoid-treated rats. It was reported (18, 19) that change in the cell population of the thymus in tumor-bearing animals is difficult to interpret, since it cannot be accurately related to the normal cell mass. The loss of cells from the thymus of tumor-bearing mice was strikingly similar to the simple involution observed after short-term glucocorticoid treatment of normal mice. However, anti-β susceptibility of thymus cells decreased during cortisone involution, whereas it showed little or no change during tumor bearing (18, 19). Moreover, the thymus cells increased in H-2 representation in tumor-bearing mice, but the proportion of low-density cells decreased (18, 19). Therefore, it is clear that the changes observed in tumor-bearing mice and those noted after cortisone-induced acute involution are not identical (18, 19). The inverse correlation between the activity of alanine aminotransferase and involution of the thymus in glucocorticoid-treated rats, and the absence of this correlation in the thymus of tumor-bearing rats could be similarly explained by differences in the populations of thymus cells in glucocorticoid-treated and tumor-bearing rats. Further studies are necessary to elucidate these differences from various aspects, such as the immunological state, effect of substances released from the tumor cells, the nutritional state, and the actions of hormones other than glucocorticoid in tumor-bearing rats.

ACKNOWLEDGMENTS

The authors would like to thank Drs. Y. Nakata, T. Higashi, K. Higashi, and F. Wada, Osaka University Medical School, for discussion and encouragement; Dr. H. Fukushima, M. Hirai, and Y. Hashimoto (undergraduate students of Osaka University Medical School) for their assistance; and M. Kitagawa for secretarial assistance.
REFERENCES


Aminotransferase Activities and Involution of the Thymus in Rats Bearing AH 130 Tumors

Fumihide Isohashi, Kazue Tsukanaka, Masako Terada, et al.


Updated version

Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/40/3/877

E-mail alerts

Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.