Growth Hormone-secreting Variants of a Mammotropic Tumor

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SUMMARY

Three investigators described independently somatotropic variants (mutants) of the originally typical mammotropic MtT.W5, which was isolated from a rat that had been X-rayed (800 R) over the head and neck. Studies of one of these variants (W5/St.H) led us to conclude that the three variants are basically identical; all possess high somatotropic hormone (growth hormone, StH) and very low mammotropic hormone (prolactin, MtH) activity. However, persistence of secretion of some MtH is indicated by our tests with the highly sensitive radioimmunoassay and immunohistochemical staining.

The mammary gland stimulation of the variant studied by us is characterized by moderate alveolar hyperplasia with some milk secretion, periglandular and perivascular fibrosis, some ability to support the growth of a hormone-responsive mammary tumor, and serum levels of MtH elevated 8 to 23 times normal. The variant is stable and fully autonomous, growing equally well in rats of both sexes.

StH secretion was about as high as that of the original tumor as judged by similar criteria. StH serum levels were increased to about 220 times normal in rats of both sexes.

Immunohistochemical staining of the tumors was distinct for StH but not for MtH. Staining of the pituitaries of tumor hosts for MtH was reduced as compared with normal pituitaries. These findings suggest that the quantity of this hormone secreted per cell in the tumors was very small.

This study supports our earlier findings of the intimate relation of MtH and StH production and is consistent with the hypothesis that the same neoplastic acidophils can produce both hormones.

INTRODUCTION

Primary pituitary tumors arising in acidophils are predominantly mammotropic. When transplanted in isologous hosts, they gain in body and organ weight and exhibit marked somatotropic and some adrenotropic potency (6). Upon successive transplantation, the ability to secrete these hormones greatly decreases but is rarely lost completely.

It is widely held that in rodents MtH, StH, and AtH are secreted by different cells. If so, these pituitary tumors should be composed exclusively or predominantly of mammotropes, somatotropes, and adrenotropes. On the contrary, in our experience all of the many pituitary tumors arising in acidophilic cells secrete MtH and StH and some also AtH in varying ratios.

It was reported independently from 3 laboratories that MtT.W5 that originated in a female W/Fu rat which had been irradiated over the head with 800 R (22) and was transplanted in series in normal W/Fu female rats lost the ability to produce MtH but not StH. MacLeod et al. (11) discovered this by the failure of the rat to support the growth of a MtH-dependent mammary tumor and by the character of the hormone profile in polyacrylamide gel electrophoresis of the pituitary extracts. Tashjian et al. (19, 20) isolated a similar variant by cloning cell cultures of this tumor, and Hollander and Hollander (7) did so from an ovarian metastasis.

Because of the theoretical and practical importance of having monomorphous, transplantable, monohormonal neoplasms, we have undertaken a characterization of the Hollander variant with conventional and more recently introduced sensitive techniques.

MATERIALS AND METHODS

Origin and Variants of MtT.W5. MtT.W5 was isolated in 1961 from a rat 563 days after the rat had received 800 R over the head and neck (22). When the tumor became fast growing and metastasizing, Hollander and Hollander (7) isolated from an ovarian metastasis a variant named MtT.W5/OM. Earlier, the similar variants isolated from this tumor in the 2 other laboratories were named StW5 (11) and GH3 (20), respectively. Following the conventional terminology of geneticists, we propose to name the 3 somatotropic variants of MtT.W5 as follows: W5/St.M, W5/St.T, and W5/St.H. Characters following the shilling indicate the character of the variant (St = somatotrope) followed by the initial letter of the senior investigator.

In our laboratory, W5/St.H was transplanted s.c. in 6 consecutive passages of males and in 8 consecutive passages of females, in a total of 47 normal and 4 ovariectomized females and 49 normal and 4 castrated males. The grafted tumors were palpable at about 30 days and grew very fast in both females and males, reaching approximately 3 cm across (weighing about 21 g) in about 50 days. They grew equally well in castrated female and male rats.

At autopsy, all endocrine glands and their target organs were carefully examined, sectioned, and stained by hematoxylin and eosin routinely. IHS was performed in some
tumors and pituitaries of the tumor-bearing hosts. All mammary pads were examined at autopsy; but for precise comparative histological studies, only the right inguinal pad, including the lymph node, was sectioned.

RIA. Serum STH and MtH values were measured by means of the double-antibody technique (17). The biological potencies of rat MtH (NIAMD-RP-I-1) and of STH (NIAMD-RGH-I-1) were 30 and 1.5 units/mg, respectively. The corresponding antithormones were designated NIAMD-Anti-RP-S-I and Anti-RGH-S-I. The basic technical details used have been described (8). Because of its simplicity, the charcoal method (15) was used to duplicate the conventional double-antibody assays for STH in 5 tests.

In each assay, sera with known high and low values were tested with the unknowns. All serum samples were tested in duplicate, some in 2 or 3 dilutions; the values stated were averages. Some sera were tested repeatedly. The standard curve was made with hormone concentrations from 0 to 25 ng/0.1 ml. Five tests were made for MtH and 5 were made for STH. The most sensitive areas of the curve on which the calculations were based were between 0.5 to 10 ng/0.1 ml.

IHS. This was done by peroxidase-labeled antibodies following the technique of Nakane and Pierce (14) with tumors, pituitaries of the tumor hosts, and normal pituitaries as controls. The tissues were fixed in Bouin solution and embedded in paraffin as recommended by Baker et al. (1). Rabbit antithormones to rat STH, rat MtH (18), and human STH/VA were prepared by us. The goat anti-rabbit y-globulin was conjugated with horseradish peroxidase, and the substrate used was 3,3'-diaminobenzidine.

Test for MtH for Stimulation of MtH-responsive MT. MtT.W5/St.H was tested for stimulation of MtH-responsive MT. A hormone-responsive mammary tumor (MtT.W9A) was derived from a combined treatment of subcarcinogenic doses of 3-methylcholanthrene and MtH (9). It is transplantable in normal female rats but grows much faster in MtT-bearing (MtH-stimulated) hosts.

Four experiments were performed. The MtT mince was injected s.c. in the back of the neck, and MT was injected in the right groin. Each experiment contained 2 additional groups of rats. In one, MT was coincubated with the high-MtH-secreting MtT.W10, and in the other, normal females received no pituitary tumor graft. MT and MtT were grafted at the same time but MtT grew faster than MT. In Experiments 1 and 2, the rats were sacrificed in extremis. In Experiments 3 and 4, the rats were sacrificed in matched “trios” when the MtT was very large.

RESULTS

Transplantation and Anatomical Evidence of MtH and StH Secretion. Transplantation of W5/St.H was successful in all 104 rats, and none of the tumors regressed. The rate of growth was equal in males and females and was not affected by castration.

In the original tumor (MtT.W5), the tumor cells were fairly uniform in size and shape (Fig. 1). The only distinct morphological change in W5/St.H was a moderate anaplasia. This is indicated by much greater variability in the size and shape of the tumor cells, especially their nuclei and an increased number of mitoses (Fig. 3). In some areas the tumor cells were detached, superficially resembling reticulum cell sarcomas.

The marked increase in body weights and a proportional increase in organ weights indicated StH secretion in W5/St.H (Table 1). The increases in weights are based on those of rats not grafted with MtT's. These figures indicate a marked somatotropic activity of the variant W5/St.H. The changes noted were in direct relation to tumor size and tumor age (Table 2). When the normal control females weighed about 328 g, the W5/St.H-bearing female rats weighed 455 g and the males weighed 518 g. The thymus weight in this experiment rose from a range of 180 to 200 mg to 540 mg in rats with tumors weighing 4 to 6 g and measuring 1.5 to 2.0 cm in

Table 1

<p>| Tumor, organ, and carcass weights of rats in Experiment 1 in Table 3 |
|---------------------------------|--------|--------|</p>
<table>
<thead>
<tr>
<th>No or rat</th>
<th>W5/St.H in host</th>
<th>W10 in host</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Carcass (g)</td>
<td>202 ± 7.3</td>
<td>327 ± 10</td>
</tr>
<tr>
<td>MT (g)</td>
<td>0.21 ± 0.06</td>
<td>0.69 ± 0.34</td>
</tr>
<tr>
<td>MtH (g)</td>
<td>15.0 ± 2.0</td>
<td>36.0 ± 5.2</td>
</tr>
<tr>
<td>Thymus (mg)</td>
<td>188 ± 10</td>
<td>381 ± 41</td>
</tr>
<tr>
<td>Adrenal (mg)</td>
<td>36 ± 6.7</td>
<td>54 ± 7.0</td>
</tr>
<tr>
<td>Ovary (mg)</td>
<td>55 ± 7.6</td>
<td>112 ± 11.2</td>
</tr>
<tr>
<td>Uterus (mg)</td>
<td>204 ± 4.6</td>
<td>329 ± 98</td>
</tr>
<tr>
<td>Spleen (g)</td>
<td>0.44 ± 0.08</td>
<td>1.06 ± 0.18</td>
</tr>
<tr>
<td>Kidney (g)</td>
<td>0.75 ± 0.03</td>
<td>1.08 ± 0.06</td>
</tr>
<tr>
<td>Liver (g)</td>
<td>7.0 ± 0.3</td>
<td>17.2 ± 2.1</td>
</tr>
</tbody>
</table>

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both StH and MtH was progressively increased with the tumor weights increased disproportionately. However, microscopic examination failed to indicate the type of stimulation by AtH as seen in AtH-secreting MtT's. In Table 1, the weight changes in W5/St.H rats are contrasted with those of the usual type of MtH-, 5tH-, and AtH-secreting, MtT.W10-bearing rats.

Mammary Gland Hyperplasia (Figs. 5 to 7). This was often missed at autopsy in rats with small tumors because a fibromuscular fascia obscured the underlying mammary gland. It became, however, conspicuous at autopsy only when the tumor-bearing period was long and the tumors were large. Milk secretion, which is characteristic of the usual MtT strain (Fig. 2), was slight or absent. Diffuse periductal and perivascular fibrosis is characteristic of these tumors (Figs. 6 and 7), in contrast to usual MtT's. In Table 1, the weight changes in W5/St.H rats are contrasted with those of the usual type of MtH-, StH-, and MtH-secreting, MtT.W10-bearing rats.

Table 2

<table>
<thead>
<tr>
<th>Rat</th>
<th>Tumor</th>
<th>Biological features</th>
<th>StH</th>
<th>MtH</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>Sex</td>
<td>Time (days)</td>
<td>Size (cm)</td>
<td>Body weight</td>
</tr>
<tr>
<td>14</td>
<td>F</td>
<td>0</td>
<td>1.0</td>
<td>200</td>
</tr>
<tr>
<td>1</td>
<td>F</td>
<td>0</td>
<td>2.0—3.2</td>
<td>320 ± 24.2</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>66 (44—78)</td>
<td>4.0—6.0</td>
<td>455 ± 2.6</td>
</tr>
<tr>
<td>16</td>
<td>M</td>
<td>0</td>
<td>3.2</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>116 (79—190)</td>
<td>-5.0</td>
<td>518 ± 40.0</td>
</tr>
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<td></td>
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</table>

*a Mean and range.

*b Mean ± S.E.

average diameter. With further growth of the tumor, the thymus weight decreased to about 40 mg. This may be due to “stress”-induced AtH secretion. At the same time, adrenal weights increased disproportionately. However, microscopic examination failed to indicate the type of stimulation by AtH as seen in AtH-secreting MtT's. Hollander and Hollander (7) observed the same behavior of their variant, derived from an ovarian metastasis.

Test for Ability of W5/St.H to Support the Growth of a Hormone-responsive Mt. Failure to stimulate the growth of the hormone-dependent Mt9 led MacLeod et al. (11) to the discovery of his StH. Hollander and Hollander (7) observed the same behavior of their variant, derived from an ovarian metastasis.

The ability of Mt.W5/St.H to stimulate growth of the hormone-responsive Mt9A was tested by us in 4 experiments, using for comparison the MtH-secreting Mt.W10 and normal females (with no MtT cогrafts). Two of these experiments are detailed in Table 3. All grafted Mt's grew progressively. The growth promotion of the hormone-responsive MT by the high-MtH-secreting Mt.W10 is indicated by shortened latency period of MT in Experiments 1 and 2 and by marked increase of the size of MT in Experiment 1. In Experiments 3 and 4.

Table 3

Hormones in sera

<table>
<thead>
<tr>
<th>Hormones in sera</th>
<th>StH</th>
<th>MtH</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats</td>
<td>14</td>
<td>43</td>
</tr>
<tr>
<td>Level (µg/ml)</td>
<td>0.04 ± 0.02</td>
<td>0.057 ± 0.01</td>
</tr>
<tr>
<td>No. of rats</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Level (µg/ml)</td>
<td>0.03 ± 0.01</td>
<td>0.014 ± 0.001</td>
</tr>
</tbody>
</table>

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Viral antigen was identified. No disease occurring in the W/Fu were in any way influential in the findings here described. These experiments suggest that both the high-MtH-secreting MtT.W10 and the low-MtH-secreting W5/St.H can promote the growth of the hormone-responsive MT. However, MtT.W10 does it more efficiently than the predominantly StH-secreting W5/St.H.

INCIDENTAL OBSERVATIONS WITH COMMENTS

Staining Human Pituitaries with Anti-Human Growth Hormone. Postmortem Bouin-fixed human somatotropic cells in pituitaries can be well visualized, not only by anti-human growth hormone (Fig. 19) but also by anti-rat and anti-bovine growth hormone by means of IHS. Since the human growth hormone has inherent prolactational activity, we also tried to stain the human pituitary with anti-rat MtH. In simultaneous tests, rat pituitary stained intensely while the human pituitary failed to do so.

Virus in Mammosomatotropic Tumors. Chopra and Taylor (4) found C-type viral particles in both the original W5 strain and its W5/St.H variant. There is no evidence at present that these viral particles play any role in tumorigenesis in the rat or in any way influential in the findings here described. From MtT.W10 culture fluids, collected in our laboratory, gs3 viral antigen was identified. No disease occurring in the W/Fu strain could be connected with this virus.

Extensive experiments aimed at acceptance by W/Fu rats of administered Bittner's mammary tumor virus have been unsuccessful (G. Ueda and J. Furth, unpublished data).

DISCUSSION

The Problem. Since the discovery that all estrogen-induced pituitary tumors, originally named mammotropic, also possessed somatotropic properties (6), large numbers of spontaneous, variously induced and transplanted pituitary tumors were studied in our laboratory, but none was found that did not secrete some of both hormones.

The constant association of MtH with StH puzzled us because of the currently accepted view that these hormones are secreted by 2 different types of acidophils, "orangophils," which secrete StH, and "carminophils," which secrete MtH [Lacour (10)]. Lacour's findings have been confirmed by electron microscopists, who noted ultrastructural differences between somatotropic and mammotropic cells (16). However, immunofluorescent labeling of rat pituitaries with anti-MtH by Emmart et al. (5) showed bright fluorescence of all normal acidophils. This tends to support the view that one cell may have overlapping MtH and StH activity. In contrast, by the use of IHS some differences were noted between mammotropic and somatotropic cells (1, 14). Conclusive experiments to settle this problem are wanting.

The problem was further complicated by the findings of high levels of AtH in some MtT's. Studies of Mt.T.F4 with very high AtH activity (2, 12) led us to conclude that this tumor derived from acidophils is composed of cells which can secrete 3 hormones: MtH, StH, and AtH (J. Furth and P. Moy, unpublished data). With the W5/St.H variant, IHS failed to disclose the secretion of AtH. The adrenal are only slightly enlarged, and the thymus becomes involuted only when the tumors are large. This is a common event in rats with large transplanted tumors of diverse types. This atrophy is attributed to adrenotrope-mediated, stress-induced thymus involution by the glucocorticoids of the host (21).

Our observations suggest the hypothesis that neoplastic "acidophils" can secrete both MtH and StH and that some can also secrete AtH. Neoplastic cells are transformed normal cells; in the course of transplantation some cells can undergo further modification.

On the basis of extensive experience, the natural history of MtT's appears to be as follows. With primary tumors the dominant anatomic feature is hyperplasia of the mammary gland. However, RIA's (8) reveal elevated levels of both MtH and StH in the sera of rats with primary tumors, although in varying ratios. In the course of transplantation, StH activity usually becomes manifest as early as the 1st transplant generation. In the course of further transplantations, the trend is increase in the rate of reproduction, and increase in anaplasia of tumor cells, with diminution of secretion of both MtH and StH, predominantly the former. Some tumors lose ability to secrete hormones altogether; others become stable at a fixed secretory rate which may include AtH. The latter seems to be associated with MtH. Why, when, and how AtH is acquired are unknown.

Somatotropic Pituitary Tumor Variants. All 3 somatotropic tumors reported (7, 11, 20) were derived from the same MtT.W5. The original tumor is being preserved in liquid nitrogen in the Hormone Related Tumor Bank of the National Cancer Institute. It was resuscitated upon our request by Dr.
A. E. Bogden, who found that it still possessed high MtH activity.

The 3 reports on StH are not overlapping and supplement each other remarkably. The 3 variants have reported 2 basic features in common: secretion of large quantities of StH and diminution or possibly complete loss of ability to secrete MtH. Two basic issues remain to be clarified. Did W5/St.H lose completely the ability to secrete MtH or was it composed of 2 cell types (mammotropic and somatotropic) of which one (the mammotrope) was lost in the course of transplantations? The techniques used by others were not sensitive enough to prove complete loss of MtH secretion. Reasons for the possible failure of others to detect mammary gland stimulation morphologically have already been stated. Disc electrophoresis in acrylamide gel detects only about 10 to 20 μg protein per gel of MtH and StH, whereas RIA detects 1 to 10 ng (μg).

It is well known that circulating hormones depress the production of the corresponding hormone in the pituitary. This has been well documented for StH by MacLeod et al. (11). In our study with IHS of the pituitaries of rats bearing larger tumors, the depression was only moderate. This remains to be explained.

The Biological Potentialities of StH. We found no report pertaining to the question of whether StH alone can stimulate the mammary gland but suppose that it may do so on prolonged administration. If it does, in what way does it differ morphologically from stimulation by MtH?

Bates et al. (3) found that bovine StH administered to rats for 10 days failed to increase the weight of the mammary gland; while ovine MtH, similarly administered, increased it 4 times normal without visible milk production. The combination of MtH, StH, and AtH increased the weight of the gland 10-fold, and the alveoli were filled with milk.

Tests for MtH by promotion of the growth of hormone-responsive MT is supported by the findings of Nagasawa and Yanai (13), who found that bovine MtH but not bovine StH strongly stimulated hormone-responsive MT.

Mt.T.W5/St.H secretes homologous, nonantigenic StH in much higher quantities and over longer periods of time than hitherto administered StH. Therefore, it is possible that StH alone accounted for some mammary gland stimulation in our studies of W5/St.H. However, the elevated RIA levels for MtH and the secretion of milk in the mammary alveoli suggest some MtH activity. The marked periductal and perivascular fibrosis in the mammary gland of Mt.T.W5/St.H may be due to StH. The effects of StH on the mammary gland with and without glucocorticoids remain to be studied.

ACKNOWLEDGMENTS

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18. Takizawa, S., Moy, P., and Furth, J. Relation of Mammotropes to...

Figs. 1 to 7 are stained with H & E. All sections are from female rats.
Fig. 1. The original MtT.W5. [Reproduction of Fig. 4 of Yokoro et al. (22).] × 1000.
Fig. 2. Mammary gland of a rat bearing the original MtT.W5. Note the marked hyperplasia with moderate secretion. [Reproduction of Fig. 9 of Yokoro et al. (22).] × 50.
Fig. 3. Variant MtT.W5/St.H. Note the increased anaplasia with mitotic figures and a giant cell. × 1000.
Fig. 4. The mammary gland of a young adult normal female in proestrus. × 50.
Fig. 5. Mammary gland of a rat bearing MtT.W5/St.H, showing minimal hyperplasia, rated ±. × 50.
Fig. 6. Mammary gland of a rat bearing the same type of tumor, showing moderate hyperplasia, rated +, showing marked periglandular fibrosis and numerous mast cells. × 50.
Fig. 7. Mammary gland of a rat bearing the same type of tumor, showing marked hyperplasia, rated ++ with slight fibrosis and moderate milk secretion. × 50.
Figs. 8 to 11. IHS of tumors and pituitaries of MtT.W5/St.H-bearing female rats. × 390.
Fig. 8. Tumor stained for STH.
Fig. 9. Pituitary stained for STH.
Fig. 10. Tumor stained for MTH.
Fig. 11. Pituitary stained for MTH.
Figs. 12 and 13. IHS of pituitaries of a normal female rat. × 390.
Fig. 12. Pituitary stained for STH.
Fig. 13. Pituitary stained for MTH.

Figs. 14 to 17. IHS of tumors and pituitaries of MtT.W5/St.H-bearing male rats. × 390.
Fig. 14. Tumor stained for STH.
Fig. 15. Pituitary stained for STH.
Fig. 16. Tumor stained for MTH.
Fig. 17. Pituitary stained for MTH.
Fig. 18. IHS for ATH of a MtT.W5/St.H tumor. × 390.
Fig. 19. Human female pituitary stained for STH. × 390.
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