Studies on the Pathogenesis of Plasma Cell Tumors
II. The Role of Mast Cells and Pituitary Glycoprotein Hormones in the Inhibition of Plasma Cell Tumorigenesis

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Summary

The effect of administration of glycoprotein pituitary hormones, thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), and luteinizing hormone (LH), on the development of plasma cell tumors in BALB/c mice was studied. Mice of both sexes were given i.p. injections of mineral oil. By 17 months of age, 93% male and 70% female control mice treated with mineral oil alone had developed plasma cell tumors. Daily s.c. injections of bovine albumin with mineral oil treatment did not modify the tumor incidence. Mineral-oil-treated mice receiving daily s.c. injections of 0.1 mg of either TSH, FSH, or LH showed extensive proliferation of mast cells in the peritoneal cavity at the age of 14 months. Phagocytosis of malignant plasma cells by mast cells was observed in some mice. Mast cell proliferation then subsided, and no plasma cell tumors appeared thereafter. By 17 months of age, 20% of the males and 0% of the females had developed plasma cell tumor.

These results indicate the presence of a mast cell stimulating factor in the glycoprotein pituitary hormones and the inhibitory role of these substances or of the mast cells on the plasma cell tumor development.

Introduction

Several tumorigenic stimuli were: the BALB/c strain of mice highly susceptible to plasma cell tumor development (16, 17, 19, 25). Potter and Boyce (17) reported that i.p. injections of mineral oil led to a 68% incidence of plasma cell tumors in female BALB/c mice. We have confirmed this high susceptibility of BALB/c mice to plasma cell tumors and have demonstrated that male BALB/c mice displayed more than a 90% incidence of plasma cell tumors when submitted to the same treatment (23). This tumorigenesis is very sensitive to endocrine factors. Administration of growth hormone and testosterone stimulate tumor formation, while cortisol and progesterone retard its development (22, 24).

We have found that i.p. injection of mineral oil combined with daily s.c. administration of the glycoprotein pituitary hormones, thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), and luteinizing hormone (LH), greatly increased proliferation of mast cells in the peritoneal fluid of BALB/c mice and almost completely suppressed the development of plasma cell tumors. We attributed this suppression of plasma cell tumor development to the function of mast cells.

Materials and Methods

Both sexes of BALB/c mice (Microbiological Associates, Inc., Washington, D. C.) received 3 i.p. injections of 0.5 ml of mineral oil (Prime Oil 355, white heavy U.S.P. from Humble Oil and Refining Co., New York, N. Y.) at 2, 4, and 6 months of age. They were housed in plastic cages which contained a maximum of 10 mice per cage and were given tap water and food pellets (Wayne Lab Blox, Allied Mills, Chicago, Ill.) ad libitum. The mice were divided into 6 groups to be injected as follows:

GROUP 1. Control: This group received only i.p. injections of mineral oil.
GROUP 2. Albumin: Bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.) was given, 0.5 mg in 0.1 ml of saline solution per day.
GROUP 3. ACTH: Corticotropin U.S.P. (Parke Davis and Co., Detroit, Mich., 35-98-1, 1 U.S.P. unit per mg protein) was given, 0.1 mg in 0.1 ml of saline solution per day.
GROUP 4. TSH: (NIH-TSH-B2, Bovine) 0.1 mg (0.352 unit, U.S.P.) was given in 0.1 ml of saline solution per day.
GROUP 5. FSH: (NIH-FSH-P1, Porcine) 0.1 mg (0.076 unit) was given in 0.1 ml of saline solution per day.
GROUP 6. LH: (NIH-LH-S8, Ovine) 0.1 mg (0.073 unit) was given in 0.1 ml of saline solution per day. (See Table 3 for unit description.)

All solutions were administered s.c., 5 times a week. Injection of the solutions commenced on the same day as the 1st i.p. injection of mineral oil and continued throughout the entire experimental period, except where otherwise noted.

Ascitic fluid from all mice was examined microscopically every 2 months. The smears were stained by Wright-Giemsa's method. Tail blood was collected for electrophoretic analysis of serum protein. Electrophoresis was carried out on polyacrylamide cellulose strips (0.05 x barbital buffer, pH 8.6, 1 ma, 200 volts per 2.5-cm wide strip, 90 min). The strips were stained with 0.2% Ponceau 2R in 5% acetic acid.

Antemortem diagnosis of plasma cell tumors was accomplished by the demonstration of characteristic malignant plasma cells (18) in ascites smears and an increase of IgG (7SG) or IgA (7G) globulin in the serum protein. The diagnosis was confirmed by...
postmortem histologic examination or transplantation study of nodules. Mast cell proliferation was determined by the huge number of mast cells (more than 10⁴ mast cells/cu mm) in ascitic fluid and the increased occurrence of mast cells in paraffin sections of granulomatous tissue from the peritoneal cavity, stained with toluidine blue or Wright-Giemsa stain.

Three mice of each group and 7 normal mice of both sexes were sacrificed at 17 months of age to study the effects of long-term administration of hormones. The endocrine organs were examined histologically.

**Results**

**Plasma Cell Tumor Development by the Administration of Mineral Oil**

Fifteen months after the initial injection of mineral oil, at 17 months of age, 93% of the male and 70% of the female control mice (Group 1) had developed plasma cell tumors. Bovine albumin (Group 2) injected s.c. as a control for pituitary hormone administration did not modify plasma cell tumor development. Table 1 shows no significant difference of tumor incidence between control and albumin groups. Although ACTH (Group 3) suppressed the onset of the tumor, it did not entirely prevent plasma cell tumor development. Only a few mast cells could be detected in the control and albumin-treated groups.

**Effects of Glycoprotein Pituitary Hormones on Plasma Cell Tumor Development**

The effects of glycoprotein pituitary hormones on the mice treated with mineral oil were completely different from effects observed in those groups previously described. By 17 months of age, 82% of the control mice (both sexes) treated with mineral oil alone and only 10% of the mice treated with glycoprotein pituitary hormones (both sexes) had died from plasma cell tumor. The incidence of plasma cell tumor is summarized in Table 1. About 8 months after the initial mineral oil treatment, more mast cells were evident in the ascitic fluid of these groups treated with glycoprotein pituitary hormones compared with the control groups. The number of these mast cells increased gradually. Almost all of the mice 14 months old, which were treated with these hormones, showed extensive proliferation of mast cells in their peritoneal fluid (Fig. 1 and Table 1). These cells were extremely variable in size (15-80 μ), were full of metachromatic granules, and had a nucleus often hidden under the granules. Metachromasia was evident by either Wright-Giemsa or toluidine blue stain. The mast cells were fluorescent either with or without formal fixation in the fluorescent microscope. Electrophoresis of serum from mice with mast cell proliferation did not show the characteristic increase of IgG or IgA globulin as did that of mice bearing plasma cell tumors. There is no significant difference of tumor incidence and mast cell proliferation among the 3 glycoprotein pituitary hormone-treated groups. The number of mast cells in ascitic fluid of mice treated with glycoprotein pituitary hormones reached a maximum at about 14 months of age and then gradually began to decrease, in spite of continued hormone administration. However, no plasma cell tumor appeared thereafter. Most of those mice showing mast cell proliferation had no evidence of plasma cell tumor. The peak period of the abnormal plasma cell appearance or the mast cell proliferation in the peritoneal fluid is almost the same, i.e., about 1 year after the initial mineral oil treatment. In order to study the relationship between abnormal plasma cells and mast cells, ascitic smears of TSH-treated male mice were carefully examined twice a week after 10 months of treatment. Two mice at this age displayed a large number of malignant plasma cells in ascitic fluid and a small number of mast cells. By 14 months of age the number of mast cells in the peritoneal fluid had greatly increased, and a large

**TABLE 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>Material (injected s.c.)a</th>
<th>Sex</th>
<th>No. of mice at start</th>
<th>Cumulative No. of mice dead from PCT</th>
<th>Death from other causesb</th>
<th>No. alive at 17 mos. of age</th>
<th>Cumulative No. of mice with MCP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (mineral oil only)</td>
<td>M</td>
<td>30</td>
<td>28</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>30</td>
<td>21</td>
<td>4</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Albumin</td>
<td>M</td>
<td>10</td>
<td>9 P &gt; 0.05</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>10</td>
<td>9 P &gt; 0.05</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>ACTH</td>
<td>M</td>
<td>20</td>
<td>5 P &lt; 0.01</td>
<td>7</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>10</td>
<td>3 P &gt; 0.05</td>
<td>4</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>TSH</td>
<td>M</td>
<td>10</td>
<td>2 P &lt; 0.01</td>
<td>4</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>10</td>
<td>0 P &lt; 0.01</td>
<td>7</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>FSH</td>
<td>M</td>
<td>10</td>
<td>2 P &lt; 0.01</td>
<td>4</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>10</td>
<td>0 P &lt; 0.01</td>
<td>3</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>LH</td>
<td>M</td>
<td>10</td>
<td>3 P &lt; 0.01</td>
<td>2</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>10</td>
<td>0 P &lt; 0.01</td>
<td>3</td>
<td>7</td>
<td>9</td>
</tr>
</tbody>
</table>

a Refer to text for doses used. ACTH, adrenocorticotropic hormone; TSH, thyroid-stimulating hormone; FSH, follicle-stimulating hormone; and LH, luteinizing hormone.

b Death was attributed primarily to respiratory infection.

P values were determined by x² test with Yate's correction between control and each group of the same sex.
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number of these cells was observed phagocytizing abnormal plasma cells (Fig. 2). One mast cell can phagocytize several plasma cells. The phagocytizing action of mast cells on malignant plasma cells was also observed by phase microcinematography. The phagocytosis of abnormal plasma cells was not observed in control and albumin- or ACTH-treated groups.

At 12 months of age, malignant plasma cells were evident in the ascitic fluid of a mouse receiving FSH. At 14 months of age, these malignant plasma cells were completely replaced by mast cells. This mouse died with mast cell proliferation in ascitic fluid. However, autopsy revealed no gross tumor mass. Only an oil granulomatous tissue that commonly accompanies the injection of mineral oil was evident. Although paraffin sections of this granuloma revealed no evidence of plasma cell tumors, they did show an increased number of mast cells in the granulomatous and peri-granulomatous connective tissue. Granuloma containing a large number of mast cells was transplanted s.c. and i.p. to normal BALB/c mice. These mice received daily s.c. injections of 0.1 mg of FSH. Two months after the transplantation, microscopic examination of a transplanted granulomatous nodule revealed no evidence of either plasma cell tumor or mast cell tumor. No gross tumor growth occurred. Further observation of these mice is still in progress.

Three male mice, being treated with LH, were sacrificed at the ages of 10 and 11 months because of extensive accumulation of ascitic fluid containing malignant plasma cells. Paraffin sections revealed the presence of plasma cell tumors and a large number of mast cells in the granuloma adjacent to these tumors.

**Effect of Cortisol Administration on Mast Cell Proliferation**

After 12 months of LH administration, 9 female mice which had developed mast cell proliferation were divided into 3 groups. The 1st group received continued administration of LH (0.1 mg/d, s.c.) throughout the study. In the 2nd group administration of LH was discontinued. The 3rd group received 0.5 mg of cortisol, s.c., each day for a 2-week duration without LH administration. Cortisol was reduced to 0.1 mg/day at the 3rd week, and this dosage was maintained throughout the study without LH administration. A few days after the administration of cortisol a clumping and condensation of the granules, accompanied by a decrease in mast cell number and size, occurred. A progressive degranulation was observed. After 2 weeks of cortisol administration all mast cells had been degranulated. Although a small number of cells with vacuoles, morphologically similar to macrophages, were present, no typical mast cells were seen at this time. Cortisol administration resulted not only in the rapid disappearance of mast cells but also in a decrease in ascitic volume. Mast cell proliferation was unchanged in the ascitic fluid of the group receiving continued LH. This proliferation, although still apparent at 15 months of age, gradually subsided. At the age of 17 months almost all mast cells disappeared from the ascitic fluid. In the group in which administration of LH was discontinued, degranulation of the mast cells occurred sooner than in the 1st group receiving continued LH administration. However, degranulation following cortisol administration was much faster than either. At 17 months of age, a mouse from the group being given cortisol and 1 from the group which had stopped LH died from respiratory infection. In both cases there was no gross or microscopic evidence of plasma cell tumors. The remaining 7 mice had already lived an average life-span for BALB/c mice and are still without any evidence of plasma cell tumors.

**Effects of Prolonged Administration of Pituitary Hormones on Endocrine Organs**

Mice treated with hormones for 15 months were sacrificed at 17 months of age for a complete examination of the endocrine glands. Female mice, 17 months old, treated with mineral oil only and no hormone administration were also studied, but since male mice treated with mineral oil alone died before 17 months of age, the examination of this group was carried out on another experimental group, 10 months of age. Control mice, 17 months old, without any treatment were also examined for comparison of the effects of hormones on the various endocrine glands.

TSH-treated mice showed considerable hair loss. Exophthalmos was generally found in this group, and 1 of the female mice showed lagophthalmos. Due to technical difficulties the weight of the thyroid gland could not be measured accurately. Microscopic examination of the thyroid gland revealed that the follicle cells showed a flattened atrophic epithelium change. However, even prolonged administration of TSH did not cause complete atrophy. Some parts of the thyroid retained almost normal follicle cells. The adrenals maintained a normal appearance. Testes, prostate, ovaries, uterus, and pituitary gland showed considerable atrophy. FSH- and LH-treated animals showed no hair loss and no exophthalmos as did TSH-treated animals. Atrophy of sex organs was pronounced in these groups. Although thyroid gland showed atrophy, it was not as extensive as that in the animals treated with TSH. The adrenals were normal. The weights of the endocrine organs are summarized in Table 2. ACTH-treated animals had almost normal-sized adrenals and much less granulomatous tissue in the peritoneal cavity than did any other group. The thymus showed almost complete atrophy. The thyroid gland and the sex organs showed neither atrophy nor stimulation. There was no significant change in the endocrine organs between control animals and mineral-oil-treated animals without hormone administration.

The glycoprotein pituitary hormones used had very small amounts of the other pituitary hormones. The purity of these hormones is summarized in Table 3.

**Discussion**

Cortisol administration prevents plasma cell tumorigenesis in the mineral-oil-treated mouse (23). Since the amount of ACTH activity assayed in the FSH, LH, and TSH is quite small (Table 3), it doubtful that the prevention of mineral-oil-induced plasma cell tumors by these hormones is due to the contamination of ACTH. Cortisol inhibition resulted from, or at least was accompanied by, striking anti-inflammatory effects. The lipogranuloma on the peritoneal surfaces were markedly diminished, and the peritoneal fluid cell count was very low compared with i.p. oil-injected control mice receiving no steroid.

Mice receiving the glycoprotein hormones had abundant granulomatous reaction, and the peritoneal fluid was extremely cellular. However, the most striking difference between the action of ACTH and glycoprotein pituitary hormones lies in the
TABLE 2

WEIGHT OF BODY AND ORGANS AT 17 MONTHS OF AGE

<table>
<thead>
<tr>
<th>Sex</th>
<th>Material</th>
<th>No. of mice</th>
<th>Body wt. (gm)</th>
<th>Adrenals (mg)</th>
<th>Testes (mg)</th>
<th>Ovaries (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Control</td>
<td>7</td>
<td>28.5 ± 0.6 (S.E.)</td>
<td>6.1 ± 0.3 (S.E.)</td>
<td>159 ± 7 (S.E.)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mineral oil*</td>
<td>3</td>
<td>36.2 ± 2.2</td>
<td>5.2 ± 0.3 (P &gt; 0.05)*</td>
<td>178 ± 6 (P &gt; 0.05)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TSH</td>
<td>3</td>
<td>25.1 ± 2.8</td>
<td>4.5 ± 0.3 (P &lt; 0.05)</td>
<td>80 ± 11 (P &lt; 0.01)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FSH</td>
<td>3</td>
<td>26.0 ± 1.5</td>
<td>4.6 ± 0.6 (P &gt; 0.05)</td>
<td>87 ± 10 (P &lt; 0.01)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LH</td>
<td>3</td>
<td>25.9 ± 2.3</td>
<td>6.4 ± 1.0 (P &gt; 0.05)</td>
<td>99 ± 17 (P &gt; 0.01)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ACTH (F)</td>
<td>3</td>
<td>31.2 ± 1.5</td>
<td>6.7 ± 0.7 (P &gt; 0.05)</td>
<td>149 ± 17 (P &gt; 0.05)</td>
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<td></td>
<td></td>
<td></td>
<td>P &gt; 0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>Control</td>
<td>7</td>
<td>27.1 ± 0.8 (S.E.)</td>
<td>8.2 ± 0.6 (S.E.)</td>
<td>159 ± 7 (S.E.)</td>
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</tr>
<tr>
<td></td>
<td>Mineral oil*</td>
<td>3</td>
<td>25.6 ± 1.8</td>
<td>6.5 ± 1.4 (P &gt; 0.05)</td>
<td>178 ± 6 (P &gt; 0.05)</td>
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<tr>
<td></td>
<td>TSH</td>
<td>3</td>
<td>23.5 ± 0.5</td>
<td>5.9 ± 0.2 (P &gt; 0.05)</td>
<td>80 ± 11 (P &gt; 0.01)</td>
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<td>P &gt; 0.05</td>
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<td></td>
<td></td>
<td></td>
<td>P &gt; 0.05</td>
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</tbody>
</table>

* The age of mineral-oil-treated mice was 10 months. No mice lived past 17 months of age. TSH, thyroid-stimulating hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; and ACTH, adrenocorticotropic hormone.

TABLE 3

CONTAMINATING ACTIVITIES OF PITUITARY HORMONES

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Source</th>
<th>Mean Relative Potency (U.S.P. units/mg protein)</th>
<th>Contaminating Activities (units/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>TSHb</td>
</tr>
<tr>
<td>TSH</td>
<td>NIH-TSH-B2</td>
<td>3.52</td>
<td>0.012</td>
</tr>
<tr>
<td>FSH</td>
<td>NIH-FSH-P1</td>
<td>0.76</td>
<td>0.036</td>
</tr>
<tr>
<td>LH</td>
<td>NIH-LH-S8</td>
<td>0.73</td>
<td>0.0090</td>
</tr>
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</table>

* From the report of the Endocrinology Study Section, Pituitary Hormone Distribution Program, NIH.

Stimulation of appropriate endocrine glands by glycoprotein pituitary hormones with subsequent secretion of hormones which prevent plasma cell tumor formation must be considered. The effect of thyroid hormones has not been evaluated yet, but LH administration to male mice should have increased testosterone production (8, 11). A small dose of this steroid markedly enhances tumor production and reduces the latent period (24). If, on the other hand, the action of the LH in the female mice were to stimulate progesterone secretion (8, 12), the increased steroid production might explain the protection from tumor development. No consistent explanation of the present observations can be made in terms of the trophic functions of the hormones.

The role of the mast cell proliferation is under active study. Mast cells are not thought to be phagocytic, although the phagocytosis of red blood cells by mast cells has been reported (5, 26). At least in 2 mice in this experiment, plasma cell tumors were apparently induced, yet the intervention of mast cell prolifera-
tion resulted in disappearance of tumor. It is not known if the phagocytosis demonstrated is specific for tumor cells, but it may be relevant that Swartzendruber (21) reported the phagocytosis of plasma cells by macrophages following the administration of antigen. Studies to elucidate the chemical nature of the mast cell granules and the basis for plasma tumor cell ingestion are in progress.

Acknowledgments

The authors are grateful to Dr. Thelma B. Dunn of the NIH and to Dr. Arthur F. Goldberg and Dr. Howard D. Dorfman of the Hospital for Joint Diseases for their generous aid in the morphologic studies and fruitful discussions. We are also indebted to Wesley B. Mason (medical student, University of Pennsylvania) and Una Brown for their skilled technical assistance.

References

FIG. 1. The smear of peritoneal fluid in an LH-treated female mouse at the age of 15 months. A large number of mast cells are visible. Wright-Giemsa stain, X 600.

FIG. 2. The smear of peritoneal fluid in 1 of the TSH-treated male mice at the age of 12 months. This mast cell has phagocytized 2 malignant plasma cells. RBC in upper right field allows an estimate of cell size. Plasma cells were stained dark blue in cytoplasm and perinuclear clear areas still remained. Wright-Giemsa stain, X 1000.
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