Studies on the Pathogenesis of Plasma Cell Tumors:
Effects of Sex Hormones on the Development of Plasma Cell Tumors

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SUMMARY

The effects of sex hormones on the development of plasma cell tumors in BALB/c mice were studied. Mice of both sexes were injected i.p. with mineral oil. Plasma cell tumors in female mice exhibited a lower incidence and a longer period of latency than those of male mice. Testosterone administration to female mice strikingly accelerated development of mineral oil-induced plasma cell tumors and the growth of transplanted tumors. Progesterone administration suppressed tumorogenesis and increased the number of i.p. mast cells in these mice. Gonadectomy of males stimulated and gonadectomy of females retarded the development of plasma cell tumors. These results demonstrate extensive influence of plasma cell tumorogenesis by sex hormones.

INTRODUCTION

BALB/c mice are highly susceptible to mineral-oil-induced plasma cell tumors (8, 22, 23, 30). The incidence of tumor development is much higher in males than in females and very sensitive to endocrine factors (26-29). We have previously reported that glycoprotein pituitary hormones (follicle-stimulating hormone, luteinizing hormone, and thyrotropic-stimulating hormone) suppressed oil-induced plasma cell tumor formation and increased the number of i.p. mast cells. Suppression of tumorogenesis was attributed to the phagocytic ability of the mast cells (28). The inhibitory mechanism of these hormones and the action of endogenous hormonal steroids were considered separately. In view of the evidence implicating sex hormones in the pathogenesis of plasma cell tumors, a study was undertaken to explore the effects of the endogenous and exogenous sex hormones on oil-induced plasma cell tumor development in BALB/c mice. The effects of steroid hormones on the growth of established plasma cell tumors were also studied.

MATERIALS AND METHODS

Development of Plasma Cell Tumors. Both sexes of BALB/c mice (Microbiological Associates, Inc., Washington, D.C.) received 3 i.p. injections of 0.5 ml mineral oil (Prime Oil 355, white heavy, from Humble Oil and Refining Co., New York, N. Y.) at 2, 4, and 6 months of age. Mice were housed in plastic cages and were given tap water and food pellets (Wayne Lab Blox, Allied Mills, Chicago, Ill.) ad libitum.

Hormone Treatment. All mice received i.p. mineral oil injections at 2, 4, and 6 months of age as a tumorigenic stimulus and were divided into four major groups:

Group 1. Control I. Received only i.p. injections of mineral oil.

Group 2. Control II. Received steroid suspending vehicle (containing 9 mg sodium chloride, 5 mg sodium carboxymethyl cellulose 7LP, 0.004 ml polysorbate 80, 0.009 ml benzyl alcohol in 1 ml water) s.c., 0.1 ml per day, 5 times a week.

Group 3. Administration of Steroid Hormones. Received the following hormones suspended in 0.1 ml vehicle: (a) testosterone (NSC 9700E), 0.01 mg daily to female mice; (b) estradiol-17β (NSC 9856E), 0.01 mg daily to male mice; (c) progesterone (NSC 9704E), 0.1 mg daily to male mice. The suspending vehicle and hormones were administered s.c. five times a week. Injections commenced on the same day as the first i.p. injection of mineral oil and continued throughout the entire experimental period.

Group 4. Gonadectomy. Orchidectomy and ovariectomy were performed under ether anesthesia two weeks prior to the first injection of mineral oil on 1.5-month-old mice.

Determination of Incidence of Plasma Cell Tumors. Peritoneal fluid was obtained every month from each mouse. Smears were stained with Wright-Giemsa and examined microscopically. Tail blood was collected for electrophoretic analysis of serum proteins on polyacetate cellulose strips. The diagnosis of plasma cell tumors was based on the appearance of characteristic malignant plasma cells in the ascitic smears and the electrophoretic pattern of the serum protein. The diagnosis was confirmed by postmortem histologic examination.

Effects of Sex Hormones on Established Plasma Cell Tumors. The plasma cell tumor used for this experiment was developed in a male BALB/c mouse following mineral oil treatment. This tumor (T-PC 2) secretes IgA type immunoglobulin and is maintained by successive transplantation. Transplantation was accomplished by suspension of tumor cells in Eagle's medium by means of a loose-fitting glass homogenizer. The suspension was filtered with glass fiber and 10⁷ cells in 0.2 ml of medium were transplanted s.c. in the abdominal wall of 2-month-old female BALB/c mice. The mice were divided into 4 groups and injected with steroid hormones s.c. each day from transplantation until death. Hormones, 0.1 mg daily, were administered s.c. in 0.1 ml suspending medium. Control mice received injections of the medium alone. The size of the tumor was measured in 3 dimensions by a caliper and the weight calculated from the average diameter.

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RESULTS

Description of Plasma Cell Tumor. All mice with primary plasma cell tumors developed bloody ascites. The volume of ascites gradually increased up to approximately 20 ml. The ascitic fluid contained typical malignant plasma cells. Tumor plasma cells are large (15–50 μ) and irregular in size and shape. The eccentric nucleus is almost always either lobulated or duplicated and relatively large. Abundant mitotic figures are seen. Cytoplasm is dark blue. A juxtanuclear clear area, varying in size and shape, is seen in the cytoplasm. Microscopically, the peritoneal surface of the mesentery is studied with many granulomatous and tumor nodules of varying size. Paraffin section of a tumor mass shows sheets of neoplastic plasma cells. Each cell resembles morphologically the tumor cells found in the ascites. The so-called “cartwheel” nucleus characterized by the tendency for the chromatin to attach to the rim of the nuclear membrane-like spokes of a wheel is observed in fixed tissue preparations. Serum or ascitic fluid from plasma cell-tumor-bearing mice showed an abnormal peak of immunoglobulins, IgG or IgA. Many of the tumor-bearing mice secrete Bence Jones protein in urine.

The tumor nodule can be transplantable to BALB/c mice and a new transplantable tumor line secretes the same type of abnormal immunoglobulin as the primary tumor. These observations are entirely confirmatory to those of Potter and MacCardle (23).

Hormonal Influence on Plasma Cell Tumorigenesis

Sex Difference. The incidence of plasma cell tumors was higher in control males treated with mineral oil only (28/30, 93%) than females (21/30, 70%) (P < 0.05). The tumor had a longer period of latency in female mice than in male mice (Chart 1). Malignant plasma cells began to appear in the peritoneal fluid of male mice 6 months after the initial injection of oil. The peak incidence is about 10 months in male and 12 months in female mice after the initial mineral oil injection.

Effects of Suspending Vehicle. The incidence of induced plasma cell tumor treated with suspending vehicle was the same as that in control oil-treated mice (Table 1). By 1 year of age, 57% (20/35) control and 50% (5/10) suspending vehicle-treated male mice had developed the tumor (P > 0.05), while 26% (9/35) control and 40% (4/10) suspending vehicle-treated female mice had developed tumors (P > 0.05).

Effects of Gonadectomy. By 1 year of age, 57% (20/35) control male mice treated with only mineral oil and 29% (7/24) orchiectomized mice had developed tumor (P < 0.05), while 26% (9/35) control female and 61% (20/33) ovariectomized mice had developed plasma cell tumors (P < 0.01). Gonadectomy prolonged the latency period in males and shortened it in females (Charts 2, 3).

Effect of Testosterone Administration. Testosterone administration to female mice accelerated tumor development strikingly (Chart 2). By 11 months of age, 88% (22/25) testosterone-treated mice and only 14% (5/35) of control female mice had developed plasma cell tumors (P < 0.01). Malignant plasma cells appeared and ascitic volume increased only 4 months after the initial mineral oil injection in testosterone-treated mice. Within a month after the detection of malignant plasma cells in the ascites, all the mice died from extensive tumor proliferation. In control mice treated with mineral oil only, the volume of ascites increased more slowly and death usually occurred within 2 to 3 months after the detection of tumor cells in ascites.

Effect of Estradiol Administration. Estradiol treatment resulted in no significant change in tumor incidence in male mice (Table 1).

Effect of Progesterone Administration. Progesterone administration to male mice suppressed plasma cell tumor development almost completely (Table 1, Chart 3). Ten months after the initial oil injection, the number of i.p. mast cells increased.

![Chart 1. Sex difference in oil-induced plasma cell tumor (PCT) incidence in BALB/c mice. Mice received 0.5 ml mineral oil injection i.p. at 2, 4, and 6 months of age. Thirty-five mice of each group were tested. All male mice died before 17 months of age and all female mice died before 18 months of age.](chart1.png)
TABLE 1

Effect of Sex Hormones on the Incidence of Plasma Cell Tumors Induced by Mineral Oil in BALB/c Mice at 12 Months of Age

<table>
<thead>
<tr>
<th>Group</th>
<th>Sex</th>
<th>No. of mice at start</th>
<th>No. of mice bearing plasma cell tumors</th>
<th>Incidence of plasma cell tumors (%)</th>
<th>No. alive at 12 mo. of age</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control (mineral oil i.p. only)</td>
<td>M</td>
<td>35</td>
<td>20</td>
<td>57</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>35</td>
<td>9</td>
<td>26</td>
<td>27</td>
</tr>
<tr>
<td>2. Suspending vehicle, 0.1 ml/day</td>
<td>M</td>
<td>10</td>
<td>5</td>
<td>50 ($P &gt; 0.05^a$)</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>10</td>
<td>4</td>
<td>40 ($P &gt; 0.05$)</td>
<td>7</td>
</tr>
<tr>
<td>3. Gonadectomy</td>
<td>M</td>
<td>24</td>
<td>7</td>
<td>29 ($P &lt; 0.05$)</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>33</td>
<td>20</td>
<td>61 ($P &lt; 0.01$)</td>
<td>22</td>
</tr>
<tr>
<td>4. Testosterone, 0.01 mg/day</td>
<td>F</td>
<td>25</td>
<td>22</td>
<td>88 ($P &lt; 0.01$)</td>
<td>5</td>
</tr>
<tr>
<td>5. Estradiol, 0.01 mg/day</td>
<td>M</td>
<td>13</td>
<td>3</td>
<td>23 ($P &gt; 0.05$)</td>
<td>7</td>
</tr>
<tr>
<td>6. Progesterone, 0.01 mg/day</td>
<td>M</td>
<td>10</td>
<td>0</td>
<td>0 ($P &lt; 0.01$)</td>
<td>9</td>
</tr>
</tbody>
</table>

* All mice were given 0.5 ml i.p. mineral oil injections, 3 times at 2, 4, and 6 months of age. Gonadectomy was performed 2 weeks prior to the initial oil injections. Steroids and suspending vehicle were given s.c. 5 days a week from the day of initial oil injection and continued throughout the experiment.

$^a$ $P$ values were determined by $x^2$ test with Yate's correction between control and each group of the same sex.

CHART 2. Effects of gonadectomy and testosterone administration on oil-induced plasma cell tumor (PCT) incidence in female mice. All mice received 0.5 ml mineral oil i.p. injection at 2, 4, and 6 months of age.

These mast cells appeared similar to those induced by glycoprotein pituitary hormone administration (28). This increase of i.p. mast cells was only observed in mice treated with progesterone. No mast cells were observed in groups treated with the other hormonal steroids. A comparison of ascites smears of a suspending vehicle-treated mouse and a progesterone-treated mouse is shown in Figs. 1 and 2. The number of mast cells in the ascites of progesterone-treated mice were about $10^2$ to $10^3$ cells per cu mm. This was not quite as extensive as what we had encountered in mice treated with glycoprotein pituitary hor-
Pathogenesis of Plasma Cell Tumors

Chart 3. Effect of gonadectomy and progesterone administration on plasma cell tumor (PCT) incidence in male BALB/c mice. All mice received 0.5 ml mineral oil i.p. injection at 2, 4, and 6 months of age.

Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Day of 1-gm tumor</th>
<th>Average survival period (days) after transplantation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control suspending vehicle, 0.1 ml/day</td>
<td>20.5 ± 1.4</td>
<td>29.8 ± 1.9</td>
</tr>
<tr>
<td>2. Testosterone, 0.1 mg/day</td>
<td>15.0 ± 1.2 (P &lt; 0.05)</td>
<td>27.4 ± 2.2 (P &gt; 0.05)</td>
</tr>
<tr>
<td>3. Estradiol, 0.1 mg/day</td>
<td>16.5 ± 0.9 (P &lt; 0.05)</td>
<td>22.9 ± 2.3 (P &lt; 0.05)</td>
</tr>
<tr>
<td>4. Progesterone, 0.1 mg/day</td>
<td>19.3 ± 1.3 (P &gt; 0.05)</td>
<td>32.1 ± 1.7 (P &gt; 0.05)</td>
</tr>
</tbody>
</table>

* Ten mice of each group were tested; 106 tumor cells in 0.2 ml Eagle’s medium were transplanted s.c.

† The rate of growth was expressed as that day when the tumor was 1 gm in size, calculated from the average diameter.

+ Mean ± S.E.

# P values were determined by Student’s t tests with comparison to control animals.

Sex hormones (10^4 to more than 10^6 cells per cu mm). The average life span of the mice treated with progesterone was longer than control mice treated with mineral oil only.

Effects of Sex Hormone Administration on the Growth of Established Plasma Cell Tumor. Daily administration of testosterone accelerated the growth of plasma cell tumor significantly (Table 2). Estradiol also exhibited a slight stimulatory effect which shortened the life span. However, this shortened life span may be attributed to the toxic effect of high dosages of estradiol as well as malignant tumor growth. Progesterone was found to have no effect on the growth of the established tumor and on the life span. A similar stimulatory effect of testosterone on plasma cell tumor growth was also observed in 3 other established tumor lines tested (T-PC3, T-PC6, and T-PC10).

DISCUSSION

These results are consistent with our prior reports on powerful endocrine mechanisms in the pathogenesis of plasma cell tumors (27–29). Sex hormones are implicated in the development and growth of mammary, pituitary, genital, and other neoplasms (1, 14, 21, 33). The present studies may be analyzed with respect to the role of sex steroids in the pathogenesis of leukemia (3, 6, 9, 10, 13, 31). Administration of androgen retards (5–7, 12, 13, 20) and estrogen stimulates (4, 5, 15–17) the development of spontaneous and radiation-induced leukemia in mice. Although ovariectomy of female mice was reported to reduce the incidence of some spontaneous leukemia (19), castration of male mice has little influence (11, 13, 18, 32). Progesterone administration has no consistent results (13). The biochemical basis for the apparent difference in sex hormone effects in plasma cell and leukemia tumorigenesis is not clear at present.

Previously, we reported that follicle-stimulating hormone, luteinizing hormone, and thyrotropic-stimulating hormone administration almost completely suppressed plasma cell tumor development and increased the number of mast cells in peritoneal fluid (28). Luteinizing hormone might have been ex-
pected to stimulate tumor development by virtue of increased testosterone secretion. The inhibitory effect of gonadotropic pituitary hormones on the tumorigenesis should be considered separately from the effects of hormonal steroids, and, in fact, may have a nonhormonal mechanism. Selye reported that chronic administration of carboxymethyl cellulose s.c. resulted in the development of numerous cells in the adrenal medulla which look like mast cells and stained metachromatically (24). It is possible that true mast cells like "these pseudo mastocytes" may develop from phagocytosis of metachromatically staining materials (25). However, mast cells could not be observed by feeding carboxymethyl cellulose to macrophages in tissue culture (2). In our experiments, chronic administration of suspending vehicle containing carboxymethyl cellulose did not result in the suppression of plasma cell tumors. The suppression of tumor development by progesterone administration is striking. The exact relationship of this suppression to mast cell proliferation is under further study.

Estradiol exhibited no significant effect on tumorigenesis. However, even a dose of estradiol of 0.01 mg per day was found to be too toxic for mice. Further studies are necessary to evaluate the effect of estrogen before arriving at a definite conclusion to this problem.

REFERENCES

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**FIG. 1.** Smear of peritoneal fluid of a mouse treated with i.p. mineral oil injection and progesterone, 0.1 mg in 0.1 ml suspending vehicle per day s.c. Smear taken 10 months after the initial mineral oil injection. Numerous cells containing metachromatic granules in cytoplasm (mast cells) are observed. No malignant plasma cells were observed. Wright-Giemsa stain, X 1200.

**FIG. 2.** Smear of peritoneal fluid of a mouse treated with i.p. mineral oil and 0.1 ml/day s.c. of a suspending vehicle containing carboxymethyl cellulose. Smear taken 10 months after the initial mineral oil injection. Malignant plasma cells are seen similar to those from mice treated with mineral oil only. Only very occasional mast cells could be seen. Wright-Giemsa stain, X 1200.
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