The Biology of Testicular Cancer

II. Endocrinology of Transplanted Tumors*

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It is the object of this paper to present an endocrine analysis of five permanently transplantable human testicular tumors maintained in the cheek pouches of cortisone-treated hamsters. Two of the tumors produce chorionic gonadotrophin and represent the first reported human neoplasms to be functional in heterologous hosts.

The growth characteristics of three of these tumors have been reported (7). The fourth, PITT 100, has only recently been isolated, and its behavior will be described here.

MATERIALS AND METHODS

The methods of transplantation, including the cortisone1 treatment of the hosts, were modifications of those of Lutz et al. (5), Toolan (8), and Patterson (6), and have been described (7). Four to 6-week-old female hamsters weighing 40–60 gm. were used as hosts. When their tumors had attained transferable size, they were killed by decapitation, and their sera were stored at —20° C. until assayed for gonadotrophins in male frogs (4). The gross and microscopic morphology and the weights of the hosts' ovaries and uteri were also used as indicators of endocrine alteration during the tenure of the tumor grafts. The cortisone-treated hamsters were at least 6–9 weeks of age when the data were collected. Their ovaries and uteri were removed in a block by dissection at the cervico-uterine junction, were freed from fat, and were separated from each other. The ovaries were weighed on a Roller-Smith balance to the nearest milligram, as were the uteri after any intraluminal fluid had been expressed. Histologic assessment was performed on uterine, ovarian, and neoplastic tissue that had been fixed in Bouin's fluid and stained with hematoxylin and eosin.

Control series were always treated in the same manner as the experimental, but they did not have tumor grafts.

The frog tests were performed according to the method of Hodgson (4). Paired male frogs (Rana pipiens), weighing from 25–40 gm. each, were injected in the dorsal lymph sac with 1 cc. of hamster serum/10 gm of frog weight. A test was considered positive if spermatozoa appeared in the cloacal fluid of one of the frogs within 4 hours of the time of injection of the serum. The sensitivity of the method, determined by the injection into male frogs of known amounts of human chorionic gonadotrophin in a serum vehicle, indicated that 10 I.U. of chorionic gonadotrophin/cc of serum or 1 I.U./gm of frog was necessary for a positive test. Sera from the hamsters in each tumor group were pooled until a sufficient amount was available to perform a test (two frogs). The test was subsequently repeated on three additional batches of pooled serum from each group, with the exception of PITT 100 and PITT 94, for which sufficient sera were available for only one additional test.

The tumors employed were:

a) PITT 61: embryonal carcinoma, 35th generation (36 months) in the cheek pouches of cortisone-treated hamsters.

b) PITT 89: choriocarcinoma, 25th generation (16 months) in the cheek pouches of cortisone-treated hamsters.

c) PITT 94: embryonal-cell carcinoma, sixteenth generation (13 months) in the cheek pouches of cortisone-treated hamsters.

d) DE AC 3: embryonal-cell carcinoma, acquired from Dr. B. Patterson, Boston, and grown in this laboratory for 2 years.

e) PITT 100: Portions of this tumor, made up of teratomatous and embryonal carcinomatous components (1), primary in the scrotal testis of a 32-year-old male, were transplanted to cortisone-treated hamsters.

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1 The cortisone acetate used in these experiments was supplied in generous amounts by Dr. F. K. Heath, Research Division, Merck, Sharp & Dohme & Co., Rahway, New Jersey.

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treated hamsters. None attained transferable size, although four showed gross and microscopic evidence of proliferation. The patient died 3 months after orchiectomy. An autopsy was performed 5½ hours post mortem. Tissues, for transplantation, were selected from hemorrhagic, friable, pulmonary and liver metastases, and from them a relatively slowly growing but otherwise typical permanently transplantable choriocarcinoma was developed. Tumor takes occurred in only 25 per cent of animals grafted, and approximately 25 days were required for them to attain a weight of approximately ½ gm. Grossly, the tumors were soft and large tumors. The enlarged ovaries were altered by many lutein cysts, and luteinization was found to have occurred even in immature follicles. These findings were considered consistent with those resulting from stimulation by chorionic gonadotrophin. Gonadotrophin content of pooled sera when assayed by injection into male frogs indicated at least 20 I.U./cc of chorionic gonadotrophin in the case of PITT 89 and at least 30 I.U./cc for PITT 100. Sera from cortisone-treated control animals were always associated with negative frog tests. PITT 89 and PITT 100 have shown no measurable decline in functional activity during

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Duration of Exper.</th>
<th>Tumor wt.</th>
<th>Ovary wt.</th>
<th>Uterine wt.</th>
<th>Frog</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor hosts</td>
<td>(days)</td>
<td>(gm.)</td>
<td>(mg.)</td>
<td>(mg.)</td>
<td>test</td>
</tr>
<tr>
<td>PITT 89</td>
<td>84</td>
<td>19.0 ± 4.5</td>
<td>1.05 ± 0.17</td>
<td>76 ± 17.8</td>
<td>468 ± 114.2</td>
</tr>
<tr>
<td>PITT 100</td>
<td>15</td>
<td>23 ± 4.9</td>
<td>0.4 ± 0.16</td>
<td>122 ± 20.3</td>
<td>545 ± 90.4</td>
</tr>
<tr>
<td>DEAC 3</td>
<td>35</td>
<td>27.4 ± 10.2</td>
<td>1.15 ± 0.5</td>
<td>22.9 ± 4.4</td>
<td>74.9 ± 40.4</td>
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<tr>
<td>PITT 61</td>
<td>229</td>
<td>22.9 ± 7.3</td>
<td>1.1 ± 0.5</td>
<td>22.4 ± 6.1</td>
<td>186 ± 84.7</td>
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<tr>
<td>PITT 94</td>
<td>17</td>
<td>25 ± 11.2</td>
<td>0.8 ± 0.4</td>
<td>20.0 ± 6.3</td>
<td>106 ± 66.5</td>
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<tr>
<td>Controls</td>
<td>23</td>
<td>10.4</td>
<td>Nil</td>
<td>95.6 ± 6.4</td>
<td>95 ± 45.4</td>
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* Each result represents the mean of the number of observations ± standard deviation.
† A negative result indicates that there were less than 10 I.U. of chorionic gonadotrophin/cc of hamster serum tested.

TABLE 2

GONADAL RESPONSE OF HAMSTERS BEARING STRAINS OF PITT 61*

<table>
<thead>
<tr>
<th>No.</th>
<th>Duration exper. (days)</th>
<th>Tumor wt. (gm.)</th>
<th>Uterine wt. (mg.)</th>
<th>Frog</th>
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</thead>
<tbody>
<tr>
<td>PITT 61</td>
<td>122</td>
<td>22.9 ± 7.3</td>
<td>1.12 ± 0.5</td>
<td>101 ± 47.7</td>
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<td>PITT 61 A</td>
<td>17</td>
<td>16.9 ± 5.6</td>
<td>1.15 ± 0.4</td>
<td>306 ± 75.4</td>
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* Each result represents the mean of the number of observations ± standard deviation.
† Tests not performed for want of sufficient serum.

hemorrhagic and were found upon microscopic examination to be composed of villi containing both cyto- and syncytiotrophoblast (Figs. 1 and 2). Hemorrhage and areas of necrosis were commonly seen. Metastases in the heterologous hosts have not been noted; indeed, the tumors have always been well encapsulated.

RESULTS

The results of the experiments are summarized in Table 1. PITT 89 and PITT 100, the human choriocarcinomas in heterologous hosts, were invariably associated with polycystic ovaries and hyperplastic uteri that weighed approximately 4-5 times more than their respective control values. There was no correlation between tumor size and the host's gonadal response, since, when a minute choriocarcinoma was present, the host's gonadal response was approximately that associated with the 25 and ten generations, respectively, that they have been maintained in this laboratory.

Animals bearing the embryonal carcinomas (PITT 61, PITT 94, and DEAC 3) had mean gonadal and uterine weights that approximated control values, although uteri associated with PITT 61 were slightly heavier and had a much wider range (30-439 mg.) than those of the controls (40-199 mg.). It was observed that PITT 61 differed from the other two embryonal carcinomas in that occasionally its transplants grew at an increased rate and were associated with large uteri in the hosts.

To compare these aberrant PITT 61 transplants with the majority, the PITT 61 series was divided into two groups according to uterine weight. A large uterus was defined as one, the weight of which exceeded the mean weight of the control uteri plus 3 times the standard deviation of the
weights of the uteri of the control series. A uterine weight greater than this figure (232 mg.) would be expected only once in a population of 378 (2). In this experiment comprising 139 observations, seventeen uteri weighed more than 232 mg., which observation indicated that uncontrolled influences were altering the uteri of the experimental series. This group of tumor-bearing animals with large uteri was named for convenience PITT 61 A. Reference to Table 2 indicates that the uteri of PITT 61 A were approximately 3 times heavier than were those of PITT 61. The mean weights of the tumors produced in the PITT 61 A and PITT 61 series were the same, but the tumors associated with the small uteri took an average of 6 days, or 35 per cent more time, to attain that size (P = <0.01).

DISCUSSION

Although PITT 89 and PITT 100 represent the first reported human tumors to have produced hormone after heterotransplantation, there are many instances of induced tumors of mice that have retained such function after transplantation within inbred lines (3). Some tumors have been shown to lose their ability to secrete hormone with continued transplantation, while others, including PITT 89 and PITT 100, have retained that ability. Indeed, PITT 89 and PITT 100 have maintained their function even though their growth rate has increased. This might suggest that endocrine function may be independent of the other measurable attributes by which progression of malignant change is measured.

Whether or not hormone production can be attributed to one of the embryonal carcinomas, PITT 61 A, because of the associated uterine hyperplasia in the host, remains to be determined.

SUMMARY

1. The characteristics of a second heterotransplantable human testicular choriocarcinoma, PITT 100, are described.
2. Two permanently heterotransplantable choriocarcinomas, PITT 89 and PITT 100, are permanently functional, producing chorionic gonadotrophin in their new hosts.
3. One variant of an embryonal carcinoma demonstrated an increased growth rate and was associated with uterine hyperplasia in the host.

REFERENCES

The Biology of Testicular Cancer: II. Endocrinology of Transplanted Tumors

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