Effect of Antiserum to Human Chorionic Gonadotropin on Growth and Function of Choriocarcinoma in Vivo and in Vitro

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SUMMARY

The effects of antiserum to human chorionic gonadotropin (HCG) on growth and endocrine function of human choriocarcinoma serially transplanted in the cheek pouch of the hamster and maintained in tissue culture were examined. Anti-HCG serum given in amounts sufficient to neutralize the biological effect of the HCG secreted by the tumor significantly reduced tumor size. Since tumor growth was not enhanced by HCG administration or by ovariectomy, the antitumor effect could not be attributed to neutralization of the hormonal effects of the HCG. Anti-HCG serum had no effect on the growth of choriocarcinoma cells in vitro, suggesting that a host factor was required for the in vivo effect of the antiserum on tumor growth.

INTRODUCTION

Many tumors of endocrine and other tissues secrete hormonally active polypeptides that may produce one of a variety of endocrine syndromes (16). Frequently, the presence of an endocrine syndrome constitutes not only the 1st indication of the presence of a cancer, but also a major therapeutic challenge. The ideal therapeutic modality for such endocrine-active tumors would be directed at eradication of the neoplastic tissue with attendant amelioration of the endocrine syndrome. This has been difficult to achieve with most tumors.

Among the various approaches to cancer treatment, both active and passive forms of immunotherapy have been extensively investigated. Although demonstration of passive immunization with antisera to tumor antigens has been possible with murine leukemias (7), in general, results of studies of this approach to tumor therapy have not been promising. In fact, an undesirable result, immunological enhancement of tumor growth, has often been observed (11).

Since hormones secreted by tumors are products of the cellular metabolism rather than cellular structural constituents, antisera generated to hormones might not be expected to produce the immunological enhancement phenomenon. Thus, antihormone sera should neutralize the hormone secreted by the tumor and attenuate the endocrine manifestations without enhancing tumor growth. Accordingly, the effects of antiserum generated to HCG on the growth and function of human choriocarcinoma serially transplanted as a xenograft in the cheek pouch of the hamster (10) and adapted to prolonged survival in tissue culture (13) were examined.

MATERIALS AND METHODS

Female Golden Syrian hamsters (Lakeview Hamster Colony, Newfield, N. J.), 3 to 6 weeks of age, were used for serial transplantation of a human choriocarcinoma in the cheek pouch, as described by Hertz (10). The growth, histological and immunological characteristics, and secretion of HCG of this serially transplanted choriocarcinoma (Reid strain) were similar to those described for the Woods strain of the Erwin-Turner choriocarcinoma (15).

The radioimmunoassay procedures, as well as in vivo and in vitro experiments, were performed with rabbit antiserum made to HCG purified from pregnancy urine. The method of Greenwood et al. (9) was used to iodinate highly purified HCG. The specific activity for the HCG was about 90 μCi/μg, and 85 to 95% of the total counts were immunoprecipitable with excess anti-HCG serum. Plasma HCG levels were determined by a radioimmunoassay method in which sheep anti-rabbit γ-globulin was used to separate bound from unbound radioligand (18).

The approximate half-life of rabbit anti-HCG antibodies in the hamster was determined in 6 non-tumor-bearing animals. After i.p. injection of 0.1 ml of the anti-HCG serum, the hamsters were divided into 2 groups that were anesthetized with pentobarbital on alternate days, and blood was obtained from the orbital sinus. The binding capacities for HCG in hamster plasma and anti-HCG serum were determined by Scatchard analysis (5, 20). The plot of bound-to-free ratio versus bound HCG with the use of the anti-HCG serum is shown in Chart 1. The binding capacity for HCG was approximately 1000 IU HCG/ml antiserum, as determined from the intercept on the horizontal axis. Antibody half-life in hamster plasma was estimated from least-squares linear regression analysis assuming a single exponential fit (Chart 2). The calculated half-life was 2.75 days.

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The abbreviations used are: HCG, human chorionic gonadotropin; IU, international unit of the Second International Standard HCG.
Antiserum and Choriocarcinoma

Chart 1. Plot of bound-to-free ratio versus bound HCG for the anti-HCG serum.

Chart 2. Half-life of HCG-binding activity (rabbit anti-HCG antibody) in hamster plasma. Anti-HCG serum was given by i.p. injection at Time 0. Confidence interval (C.I.) for the half-life is shown.

From the HCG-binding capacity of the administered anti-HCG serum and the half-life of anti-HCG antibodies in the hamster, an antiserum treatment schedule was devised that was calculated to provide a minimum of a 2- or 3-fold excess of antiserum. Thus, the anti-HCG serum was given i.p. on Days 0 (transplant), 2, 4, 6, and 8, in doses of 0.1, 0.2, 0.2, 0.4, and 0.4 ml, respectively. This schedule of increasing doses was not only to conserve antiserum, but also to conform to the HCG production pattern for the growing tumor (15). Presence of excess antibody in hamster serum on Day 10 was demonstrated by immunoprecipitation of added HCG-125I. On Day 10, the hamsters were anesthetized, blood was obtained from the vena cava, and tumor, ovary, and uterus were weighed. Tissue samples were placed in Bouin’s fixative prior to staining with hematoxylin and eosin. Student’s t test (unequal variance assumed) was performed according to the method of Snedecor and Cochran (21).

The choriocarcinoma cells (Reid strain) were adapted to culture after transfer in the hamster cheek pouch and were maintained in serial subculture as previously described for the JEG lines of choriocarcinoma cells (13). The cell culture medium was F-10 (Grand Island Biochemical Company, Grand Island, N. Y.) supplemented with 13.5% horse serum; 3.2% fetal calf serum; streptomycin, 50 μg/ml; penicillin, 50 units/ml; and either anti-HCG or nonimmune (control) rabbit serum. At the start, replicate culture flasks contained 2 million cells per flask. Anti-HCG or nonimmune serum was added to the medium on the 2nd day after plating. Cell counts were performed with a hemocytometer on Days 0, 4, and 8 of antiserum treatment. Trypan blue stain was used to differentiate viable from nonviable cells. Cells that excluded the dye were considered viable.

RESULTS

The effects of anti-HCG serum on the marked ovarian and uterine stimulation in tumor-bearing hamsters are shown in Table 1. Anti-HCG serum had no significant effect on ovarian or uterine weight in animals without tumors. In contrast, treatment of hamsters bearing tumors with anti-HCG serum resulted in complete inhibition of uterine enlargement and partial inhibition of ovarian enlargement. In separate experiments, the uteri of tumor-bearing ovarie-tomized hamsters were atrophic. Ovariectomy had no significant effect on tumor growth or plasma HCG levels. Tumor-bearing hamsters given 0.9% NaCl solution or nonimmune rabbit serum exhibited plasma HCG levels, tumor growth, and ovarian and uterine stimulation similar to those of tumor-bearing hamsters given no treatment.

The effect of the anti-HCG serum on tumor growth is shown in Table 2. Although there was no significant effect on the frequency with which tumor growth was obtained,

<table>
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<th>Table 1</th>
<th>Effect of anti-HCG serum on ovarian and uterine weight in choriocarcinoma-bearing hamsters</th>
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<td>Treatment</td>
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<tr>
<td>Nontumor and nonimmune serum</td>
<td>5</td>
</tr>
<tr>
<td>Nontumor and anti-HCG serum</td>
<td>5</td>
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<tr>
<td>p value</td>
<td>N.S.*</td>
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<tr>
<td>Tumor and nonimmune serum</td>
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<td>Tumor and anti-HCG serum</td>
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<tr>
<td>p value</td>
<td>&lt;0.01</td>
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* Mean ± S.E.
* N.S., not significant.
tumor size was significantly reduced in the group treated with anti-HCG serum. The gross appearance of the tumors in these 2 groups was the same except for size. Histological examination revealed no difference in tumor cell morphology or in the amount of vascularity, hemorrhage, necrosis, or cellular infiltrate.

Since tumor growth occurred in the presence of HCG and was retarded by anti-HCG serum, the effect of exogenous HCG on tumor growth was examined. After administration, for 10 days, of 200 IU HCG/day, hamsters without tumors had plasma HCG levels and uterine and ovarian weights essentially the same as those of the tumor-bearing hamsters. As shown in Table 3, exogenous HCG did not alter either the frequency of growth or the size of the transplanted tumors.

The effect of anti-HCG serum on growth of choriocarcinoma cells in vitro is shown in Chart 3. The anti-HCG serum had no significant effect on the number of viable cells per flask after 4 or 8 days of treatment. Similarly, the number of nonviable cells per flask was not changed by anti-HCG. Presence of the antibody in the anti-HCG-treated culture medium on Days 4 and 8 was demonstrated by precipitation of HCG-125I.

**DISCUSSION**

In this study, antiserum to the secretory product of a tumor caused inhibition rather than enhancement of tumor growth. In previous studies, treatment of animals bearing other types of tumors with humoral antibody to transplanta-

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<th>Treatment</th>
<th>No. of hamsters with tumor/no. treated</th>
<th>Tumor size (mg)</th>
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<tr>
<td>Nonimmune serum</td>
<td>11/14</td>
<td>430 ± 131*</td>
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<tr>
<td>Anti-HCG serum</td>
<td>10/13</td>
<td>127 ± 39</td>
</tr>
<tr>
<td>*p value</td>
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<td>&lt;0.05</td>
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* Mean ± S.E.
* N.S., not significant.

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<th>Treatment</th>
<th>No. of hamsters with tumor/no. treated</th>
<th>Tumor size (mg)</th>
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<tr>
<td>HCG (200 IU/day)</td>
<td>19/29</td>
<td>168 ± 46*</td>
</tr>
<tr>
<td>Diluent</td>
<td>18/26</td>
<td>208 ± 72</td>
</tr>
<tr>
<td>*p value</td>
<td></td>
<td>N.S.*</td>
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* Mean ± S.E.
* N.S., not significant.

Chart 3. Effect of anti-HCG serum on growth of choriocarcinoma cells in tissue culture. Cell culture medium containing anti-HCG, 10 μl/ml, or nonimmune serum was changed daily after Day 2. Vertical bars, S.E. of 4 replicate cultures.

The etiology of the inhibition of tumor growth by the antihormone serum is unclear. Hertz (10) found no evidence for formation of antibodies to HCG in either tumor-immunized or HCG-treated hamsters although isologous sera from tumor-immunized hamsters could produce passive immunization to the tumor. Thus, antibody to HCG does not play a role in the usual hamster immune response to this tumor. There has been considerable evidence of specific binding of heterologous anti-HCG antibodies to trophoblastic neoplasms (17, 19), as well as to normal trophoblastic tissue (17). Normal trophoblast is a homograft with a prolonged survival that seems in part to be due to an extracellular barrier to immunological attack (12). Its susceptibility to host immune mechanisms can be enhanced by pretreatment with the desialylating agent, neuraminidase (3). It is conceivable that the processes involved with the secretion of HCG represent a vulnerable site in this sialomucoprotein barrier. Antisera to HCG could act at this site directly by interfering with membrane function, for example via complement fixation (8), or indirectly by revealing other antigenic sites to immunological attack.
Another explanation for the effect of the anti-HCG serum on tumor growth may relate to the neutralization of circulating HCG. Tumors of a variety of tissues have been shown to retain responsiveness to endocrine influence. An effect of HCG on human placental glycogenolysis has recently been investigated (4). If certain metabolic functions of trophoblastic tissue were HCG modulated, then the tumor could be vulnerable to treatment with anti-HCG serum. Because HCG administration did not result in enhanced tumor growth, loss of an HCG-tropic stimulus on the tumor seems an unlikely explanation for the effect of antisera on tumor size. The failure of anti-HCG serum to inhibit cell growth in vitro supports this contention. Since ovariectomy had no effect on tumor growth or HCG levels, it seems unlikely that the antisera effect on tumor growth was mediated through neutralization of ovarian stimulation. These observations suggest that the antitumor effect of the anti-HCG serum was not hormonally mediated.

Previous evidence strongly indicates that species-specific antigens play an important part in the immune response of hamsters to the cheek-pouch choriocarcinoma (10). The participation of some species-specific factors in the present study seems likely. However, human choriocarcinoma is unique among human cancers since, in its usual clinical setting, it is a homograft. For this reason, xenograft studies of human choriocarcinoma may rest on firmer immunological ground than xenograft studies of other human tumors.

The results show that the peripheral hormonal effects of choriocarcinoma in the hamster can be greatly reduced by treatment with antisera to the secreted hormone. Several factors may have contributed to this effect of the anti-HCG serum. First, neutralization of circulating gonadotropin by formation of antigen-antibody complex is a well-known phenomenon. Second, since a correlation between tumor size and plasma HCG levels has previously been demonstrated (15), it seems likely that the reduced size of the tumors in the anti-HCG serum-treated hamsters resulted in less total HCG secretion in this group. Third, the possibility that the anti-HCG serum had some direct effect on the tumor itself, resulting in decreased HCG secretion, cannot be excluded.

ACKNOWLEDGMENTS

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REFERENCES

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