Heterotransplantation of Human Prostatic Adenoma Cells, MA160, into Nonimmunosuppressed Hamsters

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SUMMARY

MA160 cells, derived in tissue culture from a benign prostatic adenoma, can produce large tumors in nonimmunosuppressed hamsters. These tumors contain bone and hematopoietic precursors. Chromosome analysis of the tumors showed them to be human in genetic makeup, and serological tests failed to implicate any of the common DNA or RNA hamster tumor viruses. The data indicated that this is a "human" tumor growing in a heterologous host.

Progressive tumor growth in hamsters is related to the presence in their sera of globulin, presumably antibody, which binds strongly with the membrane of living tumor cells as seen in the indirect immunofluorescence test. No membrane fluorescence was seen with sera from hamsters in which tumors had regressed.

INTRODUCTION

The development of cell line MA160 from a benign prostatic adenoma has been previously reported (1). For further definition of the biological characteristics of these cells, heterotransplantation studies were performed with unconditioned golden Syrian hamsters (Mesocricetus auratus). Certain aspects of this heterotransplantation system are unusual, and the relevance of this work can be seen in several areas of human tumor research. The unusual aspects of this system are (a) the capacity of MA160 cells to form large tumors in nonimmunosuppressed animals; (b) the presence of osfication and active hematopoietic elements in the tumors derived from MA160 cells; and (c) the functional relationship of MA160 cells to human, benign, prostatic adenomatous tissue.

MATERIALS AND METHODS

Cell Cultivation and Heterotransplantation Technique

In this study, MA160 cells at passage level 275 were used. The cell line has been cultivated as monolayers on EMEM2 supplemented with 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 10% fetal bovine serum, and antibiotics (100 units of penicillin and 100 µg of streptomycin per ml). For heterotransplantation, cells were cultivated in a suspension culture system, with 5% fetal bovine serum and the additions as outlined above. At the time of its inoculation into the animals, the cell line was mycoplasma negative, and the cell viability, as determined by trypan blue exclusion, was greater than 98%. Prior to inoculation, cells were washed 3 times in EMEM without serum, each wash representing a volume equal to that of the original suspension culture. After they were washed, the cells were concentrated in EMEM to 40 X 10⁷/ml. This suspension (0.25 ml) was inoculated s.c. in the dorsal caudal area of 2-day-old unconditioned golden Syrian hamsters (LVL/LAK randombred strain; Lakeview Hamster Colony, Lakeview, N. J.). Animals were observed daily for tumor formation and/or illness. Those animals that were cannibalized or that died within 28 days of causes unrelated to tumor (usually running) were excluded from evaluation.

Immunological Tests

Because of the peculiarities of this human-hamster heterotransplantation system, various immunological aspects were studied. Two immunological tests were used to examine the hamster response to the challenge of living human cells. (a) The indirect membrane immunofluorescence test with living target cells was used, as we thought that the host reaction to foreign cells would probably be directed at antigenic receptors on the cell membrane. (b) Sera were also assayed for cytotoxicity by means of the complement-dependent cytotoxicity test.

Indirect Immunofluorescence Test. Sera from both animals with tumor and normal, uninoculated hamsters of the same age and strain were tested. The tissue culture cells that were used had been derived from the 1st 2 tumors resulting from MA160 heterotransplantation (see below). Sera were also tested for membrane fluorescence against other cell lines. HeLa and H. Ep. No. 2 cells (human heteroploid, continuous cell lines) and WI38 (human diploid cells) were used. The cells were checked for autofluorescence with the anti-hamster globulin conjugate without serum.

The test was performed as follows. Approximately 10 million cells, freshly cultivated from tissue culture, were used for each serum. The cells were washed 3 times in EMEM to free them of residual calf serum. A pellet of freshly cultivated and washed cells was then mixed with 0.1 ml of serum for 15 to 30 min in a 12-ml conical centrifuge tube at 37°. This mixture was agitated by hand at 5-min intervals while it was incubating. After being washed 3 times in PBS, the cells were then mixed with 0.1 ml of a 1:40 dilution of...
goat anti-hamster globulin conjugate (Baltimore Biological Laboratories, Baltimore, Md.) for 20 min at 37°C with periodic manual agitation. Following another 3 washes in PBS, the cells were resuspended in a 50% glycerine in PBS solution and were examined with the fluorescent microscope for membrane staining. The intensity of membrane staining was graded on a scale of 1 to 4. The percentage of fluorescent cells was also documented.

**Complement-dependent Cytotoxicity Test.** The test was performed essentially as described by Görer and O’Gorman (2). Freshly reconstituted guinea pig sera (Flow Laboratories, Rockville, Md.) served as a source of complement. Cytotoxicity was determined by the inability of nonviable cells to exclude trypan blue. In addition to sera from hamsters with tumors, normal hamster sera and PBS were assayed for cytotoxicity. Again, the cells used were tissue culture cells derived from the 1st tumors induced by MA160 cells. All sera and PBS were tested both with and without complement.

**RESULTS**

**Growth Characteristics of MA160 Cells in Nonimmunosuppressed Hosts.** Small hard nodules, about 3 to 4 mm in diameter, were noted at the site of implantation in 100% of the animals by Days 2 to 5 postinoculation. Most nodules regressed by Days 14 to 18. However, in 2 of 27 inoculated animals, progressively growing masses developed at the site of implantation. These animals were sacrificed in moribund condition on Days 38 and 45 postinoculation, and at that time they were noted to have rock-hard masses, 4 to 5 cm in diameter. There was no evidence of metastasis.

Both tumors were cultured *in vitro* by the methods previously described (1). Luxuriant growth was seen early in all tissue cultures, with proliferation of epithelial cells morphologically identical to that of the parent cell line.

The s.c. implantation of the tissue culture cells derived from the tumors resulted in a higher number of “persistors.” We define persisters as those animals showing persistent and progressive growth at 28 days postimplantation, at least 1 to 2 weeks beyond the time when regression was usually seen. Of 111 animals inoculated with cells derived from tumors induced by MA160, 29 (27%) had actively growing neoplasms at the end of 28 days. Only 2 of 27 (7%) animals that received the parent MA160 cells had persistent tumor.

Of the 29 persisters, 14 continued to manifest progressive tumor growth after 28 days. Six of these 14 were sacrificed at intervals from Days 28 through 66 while still showing progressive growth. The remaining 8 of the 14 animals died from tumor-related causes, usually inanition and infection secondary to the large tumor mass. The tumors in some of the persisters reached large proportions. Several tumors at 99 days postimplantation were 7 to 8 cm in largest diameter. In the other 15 persisters, 6 tumors regressed completely at intervals from Days 35 to 76. Eight tumors regressed but left as residue severe scoliosis (that this was tumor associated was evident from the finding of scoliosis in 2 animals that died of tumor-related causes). One tumor reached a point of static growth followed by complete ossification.

Tumor in one of the “nonpersisters” regressed by Day 28, only to reappear at Day 42, continue growth, and eventually reach a point of static growth followed by ossification.

Histologically (Fig. 1), these heterotransplanted tumors were composed of sheets of anaplastic epithelial cells, arranged in clusters separated by fibrous septa. Nuclear pleomorphism, increased nuclear:cytoplasmic ratios, and numerous mitoses with abnormal spindles were characteristic. Areas of ossification, noted in all of the tumors, contained active hematopoietic elements in some instances. Numerous plasma cells were noted surrounding the tumor and in draining lymph nodes. Only a minimal histiocytic response surrounded the tumor. Foci of reactive lymphoid tissue in the hepatic portal areas and spleen were also seen.

Preliminary electron microscopic studies showed tumor cells with very active dilated ergastoplasm, an abundant endoplasmic reticulum, and polyribosomes. Much amorphous material was noted within the dilated ergastoplasm. No virus particles were seen.

**Immunological Studies of the Heterotransplantation System.** Sera from 6 animals sacrificed with actively growing neoplasms from Days 25 to 80 postinoculation showed brilliant membrane-fluorescent staining with 100% of cells. The staining was characterized by diffuse particulate staining of the entire membrane (Fig. 2). The serum of 1 animal with persistent tumor was still positive (4+) at 132 days postinoculation. Sera of animals with the largest tumors manifested the strongest membrane-fluorescent staining.

Weak fluorescence (1+) was seen in the sera of the nonpersisters when tested at Days 21 to 28 postinoculation. However, this reactivity disappeared by 8 weeks postinoculation. These nonpersisters were from the same litters as were the 6 animals with persistent tumors, the sera of which showed brilliant fluorescence. The presence of membrane staining in sera was consistently associated with the presence of tumor. Sera from normal uninoculated weanling and adult hamsters were unreactive, and no fluorescence was seen with the cells and conjugate without serum.

Sera of all hamsters that had been inoculated with the MA160 cell line showed a weak fluorescence (+/-) with the HeLa, H.Ep. No. 2, and WI38 cell lines. This reactivity was absent in the sera of all hamsters after 1 month postinoculation and was considered to be nonspecific.

The sera from tumors as well as control hamsters did not manifest complement-dependent or -independent cytotoxicity against the tumor-derived tissue culture cell lines.

An attempt was made to demonstrate immunological enhancement by the active immunization of 2-day-old hamsters with either tumor extracts or living tumor cells. No enhancement was seen when these animals were subsequently challenged s.c. at 23 days of age with 10⁷ viable tumor cells. In another experiment, a group of 2-day-old animals was inoculated with 10⁷ cells and, on Day 5, was given pooled serum i.p. from 2 to 3 hamsters with large, actively growing neoplasms. The treated animals manifested a slightly increased incidence of tumor persistence, compared with an identical group of animals that was given tumor challenge at Day 2 and an injection of 0.85% NaCl solution on Day 5.

In this experiment (Table 1), 36 animals received tumor cells and serum. Eleven animals (31%) had persistent tumor at Day 28. Of these 11, 3 regressed from the 6th to the 12th
Table 1

<table>
<thead>
<tr>
<th>No. of animals inoculated</th>
<th>No. of tumors (persistors) at 4 wk postinoculation</th>
<th>No. of persistors regressing after 4 wk</th>
<th>Arithmetic mean of largest tumor diameter (cm) at 4 wk, 16 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>36 (experimental)b</td>
<td>11 (31%)</td>
<td>3</td>
<td>2.8, 4.9</td>
</tr>
<tr>
<td>34 (control)c</td>
<td>8 (24%)</td>
<td>3</td>
<td>2.5, 4.3</td>
</tr>
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</table>

a These data include only those animals with tumors that persisted 16 weeks.
b These animals received serum and $10^7$ cells.
c These animals received 0.85% NaCl solution and $10^7$ cells.

week postinoculation. Of 34 control animals that received cells and 0.85% NaCl solution, 8 (24%) had persistent tumor at 28 days, and 3 tumors in this group also regressed by the 12th week. There was no significant difference in tumor size between serum-treated and control animals.

Human Origin of Neoplasm. To obtain evidence as to whether we were dealing with a "human" tumor growing in a hamster or whether, in fact, these neoplasms were hamster in nature, we performed both chromosome studies and serological tests.

Chromosome studies of the cells, derived in tissue culture from the tumors induced by MA160 heterotransplantation, showed the modal number to be 60 to 61. The parent MA160 culture manifested a modal number of 60. The chromosome complement of both parent- and tumor-derived cell lines was aneuploid. The heterochromatin-staining patterns (kindly provided by Dr. T. C. Hsu, M. D. Anderson Hospital and Tumor Institute, Houston, Texas) of both parent- and tumor-derived cell lines were reported to be human.

Sera from hamsters with tumors did not react by immunofluorescence or CF with the T or neoantigen of SV40, with adenovirus 12 or 18, or with polyoma virus. No CF reaction was noted between sera from animals with tumors and extracts derived from tumors induced with the Moloney sarcoma virus. Concentrated extracts of the MA160-induced hamster tumors did not react by CF with sera from animals bearing any of the above DNA or RNA virus-induced tumors.

The above data suggest that we are dealing with a human tumor growing in a heterologous host.

DISCUSSION

Ofner (3) has demonstrated that MA160 cells contain the 4 C19O2 steroid-metabolizing enzymes present in extracts of human benign prostatic adenomatous tissue and that they thus show a functional similarity to prostatic tissue from which the cell line was derived. Furthermore, the ossification noted in the tumors induced by MA160 cells may be an expression of function and suggests a correlation with the osteoblastic aspects of metastatic prostatic adenocarcinoma in man. Ossification has been reported in cortisone-treated mice that received implants of human heteroploid cells (4), but there has been no report of ossification without cortisone.

Our data indicate that this is a human tumor growing in a heterologous host. We have shown that, in this heterologous tumor transplantation system, progressive tumor growth is associated with a humoral globulin (presumably antibody) response, i.e., globulin that has an affinity for the surface of tumor cells as seen in our indirect membrane immuno-fluorescence test and which is not cytotoxic as measured in our system.

Efforts are being made to study both the nature of the fluorescing surface protein and the questionable role of immunological enhancement in this heterologous tumor transplantation system. Experiments are also being planned to examine the host (hamster) cellular immune response to a progressively growing, foreign (human) tumor.

ACKNOWLEDGMENTS

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REFERENCES

Fig. 1. Preparation from a 35-day hamster tumor that resulted from MA160 heterotransplantation. Bone in center of photomicrograph separates tumor cells on left from hematopoietic cells on right. H & E, X 200.

Fig. 2. Brilliant membrane fluorescence elicited with serum from hamsters with actively growing neoplasms, cells derived in tissue culture from these tumors, and anti-hamster globulin conjugate. X 450.
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