Transplantation of Human Malignant Epithelial Cells from Tissue Culture to Rat Brains*

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SUMMARY

The rat brain has been shown to be a useful site for the transplantation of human, malignant cells previously grown in vitro. Although several lines of established cultures of epidermoid carcinoma cells (KB and three lines of HeLa) produced tumors after inoculation into the brains, a difference in the growth capacity of the various lines of cells was observed.

The occurrence of mucin production in the tumors was an unexpected finding, since this feature was not described in the biopsy specimens of the human neoplasms from which the KB and HeLa cells were originally obtained. Also, mucin was not demonstrated in the malignant cells in vitro in this laboratory.

Poliomyelitis virus multiplied in the tumor-bearing brains, but the cytopathogenic effect of the virus was not comparable to the pronounced destructive changes produced by the virus in the neoplastic cells in vitro.

In this laboratory, transplantations of tissues, organs, and cells from tissue culture are being carried out in rats, hamsters, chicks, and chick embryos. The main objective of these experiments has been to study the effects of the internal environment of a host on the multiplication of viruses in the transplants.

The present article is concerned with one aspect of the investigations: namely, the use of the rat brain as a site for the transplantation of human cancer cells previously grown in tissue culture. The cells used in this study were obtained from established cultures of two human epidermoid carcinomas. One of these was a cervical carcinoma from which Gey and associates (2) developed a serial culture passage in vitro since February, 1951 (7). The other was a carcinoma of the floor of the mouth from which a line of cells, designated KB, was established in tissue culture by Eagle (1) following initial planting of material from the original tumor in December, 1954.

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MATERIALS AND METHODS

Weanling Wistar rats weighing 40–60 gm. were conditioned by means of x-radiation and cortisone. Two days prior to cell implantation, the animals were given 328 r whole-body x-radiation; the next day they received 3 mg. of cortisone acetate. The day after transplantation a second injection of cortisone acetate was administered. Animals were killed, and the brains were removed at varying time intervals. Sections were made at five different levels anteroposteriorly, and each section was examined microscopically.

Three lines of HeLa cells, each obtained from a different laboratory, and KB cells were employed. HeLa (MBA), originally obtained from Microbiological Associates, Inc., and HeLa (D), furnished by Dr. E. H. Lennette, were used shortly after they were received. HeLa (T), supplied by Tuskegee Institute, was used after many passages in this laboratory, the earliest passage employed being No. 50. Near the end of the investigation, it was necessary to check certain results, so that new HeLa (T) cells were obtained from Tuskegee Institute and were used shortly after their arrival.

The cells were grown in Eagle’s medium containing 10 per cent serum with 200 units of peni-
cillin and 100 µg streptomycin/ml. Both human and calf sera were employed. The cells were passaged at approximately weekly intervals. For transplantation purposes, the cells were dislodged from the surface of the glass by treatment with trypsin, 0.06 per cent, at 37° C. The treatment usually required 15-20 minutes. The process of cell separation sometimes required gentle scraping with rubber policemen. The cell suspension was centrifuged at 600 r.p.m. for 10 minutes and resuspended in growth medium with 5 per cent serum, but containing no antibiotics, so as to yield 1.5 × 10^7 cells/ml. The cells were injected intracerebrally in 0.1-ml. amounts. This was done by means of a 25-gauge needle, inserted directly through the skin and skull, usually in the left parietal region, while the animals were anesthetized lightly.

RESULTS

Growth capacity of the malignant cells.—The several lines of established cultures of epidermoid carcinoma cells used in this investigation were observed to produce tumors after inoculation into brains of rats. The growths were found in the brain tissue, in the ventricles, and in the meninges, not only at the site of inoculation but at various levels of the brain. In most animals, the tumor was found in a majority of the sections studied, but sometimes it was limited to one section. The amount of tumor varied with the duration of time after inoculation and in accordance with the type of cells used.

The degree of growth was decided upon arbitrarily. It was considered minimal when only small nests of cells were identified microscopically in the various sites of the brain, and abundant when a fairly large area was replaced by tumor in one or more sections (Fig. 1). The designations slight and moderate were applied when the amount of tumor was considered to be between these two degrees.

Very little was identified on gross examination of the brains. Only rarely was a tiny piece of tumor evident on the surface of the brain. Occasionally, hydrocephalus of moderate degree was observed, even though no tumor was recognized grossly. In four instances, tumor masses were found in the subdural or epidural area, attached to the dura but not adherent to the brain itself.

The degree of growth of the different lines of cells, as observed microscopically, is indicated in Table 1. It will be observed that the KB line of cells and HeLa (MBA) had a tendency to develop abundant growth of tumor, for which reason they have been designated "high malignancy" lines. On the other hand, HeLa (T) produced only slight growth, behaving as a "low malignancy" line of abundant growths, the cytoplasm was clear in certain portions, giving the appearance of so-called "clear-cell" carcinoma. Sometimes along the periphery of the masses the cells were elongated and arranged in the form of a palisade, a feature which was seen more often in the KB tumors.

The nuclei, usually round or ovoid, showed considerable variation in size and staining quality. Mitotic figures, typical and atypical, were readily identified. Nuclear variability and enlarged, hyperchromatic nuclei were more prominent in the KB and HeLa (MBA) tumors than in the HeLa (T). However, even more pronounced nuclear pleomorphism, with gigantic and multinucleated forms, was strikingly evident in the HeLa (D) growths.

Necrosis of tumor tissue was noted, especially in some of the more abundant growths; thus it was seen more frequently in the KB and HeLa (MBA) tumors. As a rule, the necrosis was of the focal

<table>
<thead>
<tr>
<th>Line of cells</th>
<th>Days after inoculation</th>
<th>Degree of growth</th>
<th>No. rats studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>KB</td>
<td>4</td>
<td>Slight</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>6-8</td>
<td>Slight</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>Moderate to abundant</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>15-18</td>
<td>Abundant</td>
<td>10</td>
</tr>
<tr>
<td>HeLa (MBA)</td>
<td>4</td>
<td>Minimal</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>6-8</td>
<td>Moderate</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>Abundant</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>Abundant</td>
<td>15</td>
</tr>
<tr>
<td>HeLa (T)</td>
<td>4</td>
<td>Minimal</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>6-8</td>
<td>Minimal</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>Slight</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>Slight</td>
<td>56</td>
</tr>
</tbody>
</table>
type, with the individual, closely grouped cells of
the involved areas showing chiefly karyorrhexis
and pyknosis of nuclei and lysis of the cytoplasm,
the latter often being transformed into a hyalin
mass. Generally, there was no significant cellular
response to the necrotic tissue even in the larger
areas of involvement. Only seldom was an occa-
sional neutrophil observed among the necrotic
cells.

**Mucin production.**—In the more abundant tu-
mors small cystic foci resembling glands were com-
monly noted (Fig. 3). These cystic areas contained
a pale eosinophilic or basophilic material, which
sometimes gave a staining reaction that is charac-
teristic of mucin (namely, red with Mayer's
mucicarmine, purple-red with periodic acid-Schiff
method, and metachromatic with toluidine blue).
Strikingly, also, was the presence of groups of cells
in some of these tumors, not necessarily associated
with the cystic areas, which were enlarged and had
pale, finely vacuolated cytoplasm and often
peripherally situated nuclei (Fig. 4). Mucin was
demonstrated in these cells by the technics men-
tioned above.

**Host reaction to the tumors.**—A characteristic of
the growths resulting from each of the lines of cells
used in this study was the notable lack of response
of the brain and meninges to them (Figs. 1–3). In
only very few instances was there the slightest evi-
dence of any tissue reaction. In these cases, there
was an insignificant glial proliferation about some
of the tumor masses in the brain tissue with only
an occasional neutrophil, and in the vicinity of the
meningeal tumors a few histiocytes and lympho-
cytes were observed. An exception to this was at
the site of inoculation in the brain, where, regard-
less of whether tumor was present or not, there was
a reaction that could be attributed to mechanical
trauma produced by the needle; namely, necrosis
and glial proliferation with typical "gitter" cell
response. Varying degrees of hemorrhage and
hemosiderin deposition were also evident at this
site. In contrast, there was a pronounced desmo-
plastic reaction in the four tumors found in the
subdural and epidural areas.

**DISCUSSION**

Heterologous and homologous tumors have
been successfully transplanted into brains of ani-
mals (4–6, 8). Also, the rat brain has been em-
ployed as a site for the transplantation of normal
monkey kidney cells previously grown in vitro (9).
In the present study, it has been shown that this
site is useful for the transplantation of human,
malignant cells obtained from in vitro cultures.

Although others have been able to produce good
growths in brains of animals not conditioned by
such means as x-radiation or cortisone, we were
unsuccessful in our earlier attempts to produce
tumors after intracerebral inoculation of malig-
nant cells from tissue culture in the noncondi-
tioned host. A detailed investigation, however, was
not made to determine whether other factors con-
tributed to the failure in these experiments. More
work along this line will be done.

As pointed out by Greene (4), the major disad-
vantge of employing the brain as a site for
heterologous tumor transplantation is the inability
of the investigator to see or palpate the growing
transplant. However, a significant advantage is
the notable lack of host reaction in and about the
developing tumors, except at the site where trauma
was produced by insertion of the needle. The lack
of reaction in the brain tumors is in contrast to the
marked desmoplasia observed in the growths
which developed in the subdural and epidural
areas in a few of the animals in these experiments.

Multiple tumors were found in various areas
and at different levels of the rat brains (in the
meninges on the surface and in the sulci, in the
ventricles, and in the brain tissue itself) because
of the ready transportation of the malignant cells
by the fluid of the ventricular system and of the
subarachnoid space.

It has not been determined what factors are re-
sponsible for the different capacity of growth of
the various lines of HeLa cells. At first, it was
thought that the low-malignancy line, HeLa (T),
had been modified during the many passages to
which it had been subjected in this laboratory
prior to its transplantation to rat brains. However,
The production of mucin in the HeLa and KB tumors was an unexpected finding, since, apparently, this change was not observed in the biopsy specimens of the original epidermoid carcinomas. Mucin was not demonstrable in the malignant cells in vitro. This mixture of epidermoid and mucin-producing cells is reminiscent of that noted in the so-called mucoepidermoid tumors. It would be desirable to attempt to correlate this particular finding with various factors in the animal hosts. Of interest, in this regard, is the observation by Glücksman (3) that there is a relationship between hormonal changes of pregnancy and the development of a type of cervical carcinoma with epidermoid and mucinous features which he calls “mixed carcinoma.”

In a preliminary study in this laboratory (10), it was found that poliomyelitis virus multiplied in brains bearing tumors derived from HeLa and KB cells, but not in brains receiving no tumor cells. A relative increase of necrosis and partial inhibition of growth have been observed in some of the virus-infected tumors, but considerable portions of tumor remained unaffected. In some experiments, there was no increase of necrosis, compared with the controls, even though the virus reached adequate levels of multiplication. This is in contrast to the pronounced destructive changes produced by the virus in the tumor cells grown in vitro. Further investigation of this problem is under way.

ACKNOWLEDGMENTS

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