Heterologous Transplantation of Tissue Culture Lines*

ELIAS E. MANUELIIDIS

(Department of Pathology, Yale University School of Medicine, New Haven, Conn.)

SUMMARY

Attempts were made to transplant neoplastic and non-neoplastic cell lines from tissue culture to animals. The subcutaneous space of hamsters and mice conditioned with x-radiation and cortisone as well as the brains and eyes of normal, unconditioned guinea pigs were used as transplantation sites. No growth of non-neoplastic cell lines was obtained in any site. Neoplastic cell lines grew poorly to produce in most instances small tumors without differentiation or organization in conditioned hosts. However, transfer of such tumors to the brains or eyes of normal guinea pigs resulted in large growths capable of serial transplantation and characterized by an organized structure and an abundant vasculature. The latter behavior contrasted sharply with the complete failure to obtain growth in normal guinea pigs with the same cell lines taken directly from tissue culture.

The morphological differentiation between normal and neoplastic cells in monolayer tissue cultures is extremely difficult (13, 15), and attempts have been made to utilize the biological criterion of transplantability as a means of distinction. To this end, Moore et al. (15) originally isolated by Chang from normal persons (1) by transfer to the subcutaneous space of human volunteers and irradiated rats. Tumors developed in both hosts, but their architecture was not characteristic for the injected tissue culture lines. In contrast, human adult and embryonic fibroblasts growing in vitro failed to produce tumors in treated rats. Foley and Handler devised a method of biological test based on transplantation to the hamster’s cheek pouch and found that the number of tissue culture cells required to induce growth was much greater in the case of normal than in the case of neoplastic cell lines (3).

Recently, Scotti et al. (18) investigated the transplantability of two tissue culture lines arising from epidermoid carcinomas, the HeLa line (5) and the KB line (2), in the brains of irradiated and cortisone-treated rats. The amount of growths varied considerably, and only rarely was tumor evident on gross examination. Microscopically, the tumor consisted of masses or sheets of cells with no differentiation toward keratinizing squamous epithelium.

The present communication is also concerned with the problem of the heterologous transplantation of tissue culture cells. A method will be described whereby neoplastic tissue culture cells were transplanted to the anterior chamber of the eye and to the brain of heterologous hosts with the development of well vascularized tumors.

MATERIALS AND METHODS

The following types of tissue culture cells were used for transplantation purposes:

1. Glioma tissue culture 178 (TC 178).
2. The S-3 strain of the HeLa tissue culture cells.
3. A permanent line of tissue culture fibroblasts.
4. A tissue culture of kidney epithelial cells.

The glioma line was established in this laboratory from a human case of glioblastoma multiforme (14). These cells have been growing in continuous cultivation since September 4, 1957. The generation time of this line is similar to the generation time of the S-3 strain (17) of the HeLa cells—namely, about 26 hours (14). The fibroblast tissue culture cells arose from monkey testicular tissue in our laboratory; their generation time is approximately 39 hours (12). The kidney epithelial cells represent short-term tissue cultures, which are used routinely for virological purposes.

For the cultivation of cells, 8-oz. wide-mouth
Blake bottles were used. The same medium was used in all cases and consisted of 20 cc. of Eagle's medium; glutamine, 1 per cent; penicillin, 100 units/cc; and streptomycin, 100 μg/cc. When a luxuriant and abundant growth of healthy cells was present, the tissue culture bottles were scraped with a rubber policeman. The dispersed cells were centrifuged at 500 r.p.m., after which they were washed with fresh medium. After cell counts with the hemocytometer the desired dilutions of cells for implantation into the heterologous hosts were prepared in complete Eagle’s medium. The enumeration of cells was less accurate in the fibroblast line and kidney epithelial tissue culture cells because of clumping. In the neoplastic lines gentle repeated aspirations in a pipette achieved a more uniform separation of the tissue culture cells into individual units or small aggregates of two to four cells. It was felt more desirable to have less accurate cell counts than to damage the cellular population by the addition of trypsin.

RESULTS

Group I: Subcutaneous transplantation of tissue cultures into conditioned hosts.—Approximately $5 \times 10^5$ cells in 0.5 ml. of the various cultures were injected into the right flanks of mice and hamsters, and these animals were treated with cortisone acetate, total-body radiation, or both, by a modified method recommended by Toolan (20). The mice were given 1.5 mg. cortisone acetate, and the hamsters 4.5 mg., at the time of inoculation on alternate days for three doses. In addition, the hamsters were given 200 r of total-body radiation shortly before inoculation. One hundred fifty mice and 200 hamsters received S-3 cells, and 50 mice and 400 hamsters glioma TC 178 cells. Monkey kidney epithelial tissue culture cells were implanted into eight mice and eight hamsters, and tissue culture fibroblasts were injected into five hamsters.

The experimental animals were killed 8-15 days after transfer, and, in the majority of cases, the transplanted material was found on examination of the subcutaneous space. The transplants of kidney epithelial cells and of fibroblasts presented as flat yellow plaques and microscopically were made of necrotic debris surrounded by a zone of granulation tissue and inflammatory infiltrates. In contrast, the majority of transplants of HeLa cells and glioma TC 178 cells had increased in size, and growth was evident. In the latter cases, the usual tumor mass measured approximately 7 mm. in diameter (Fig. 1) and consisted of a necrotic center surrounded by a ring of well preserved tumor cells with little or no vascularization. Histologically, these tumors consisted of pleomorphic cells without organization, and no distinct pattern of growth distinguished the different cell lines (Fig. 2). Rarely, larger tumors measuring up to 1 cm. in diameter were found, and these were made up of healthy, well vascularized tumor tissue without necrosis. In these growths, however, organization was present, and in the case of HeLa cell tumors differentiation toward squamous epithelium was occasionally found.

The incidence of larger tumors (over 7 mm. in diameter) was not different in the two neoplastic cell strains, and their growth characteristics were generally similar. It was our impression, however, that the HeLa cells grew better in mice and that the glioma TC 178 cells produced larger tumors in hamsters.

Serial homologous transplantation of the tumors to both conditioned and unconditioned animals was attempted in a number of cases. The latter was not successful, but transfer to other conditioned hosts was achieved. In one instance the glioma TC 178 was carried for six passages in hamsters, and in another the HeLa tumor was maintained in mice for five generations.

Group II: Intracerebral inoculation of tissue cultures into unconditioned animals.—The methods of heterologous transplantation introduced by Greene were utilized for this purpose (6, 11). The animals did not receive either cortisone or x-radiation. Twenty-four mice were given injections intracerebrally of 0.03 ml. of a suspension of $5 \times 10^6$ cells/ml of the S-3 strain of the HeLa cells and a similar number with the same amount of glioma TC 178 cells. Implantation of the cells was performed with a 22-gauge needle, which was inserted directly through the skin and skull into the right basal nuclei. The animals that survived the operation recovered within 24 hours and were observed for varying periods up to 3 months.

In addition, both cultures were injected intracerebrally into guinea pigs, with eighteen animals used in each group. For this purpose a 22-gauge needle and 0.1 ml. of a suspension of $5 \times 10^6$ cells/ml was employed. The animals were sacrificed at intervals during the next 3 months.

None of the animals in this experiment developed intracranial growths. The brains were sectioned, and tissue along the persisting needle tract was examined histologically; but no remnants of the transplanted material could be found. In view of the possibility that phagocytosis of the injected cells occurred prior to stroma induction, a second experiment was carried out in which the neoplastic cells were imbedded in Gelfoam, but again no growth occurred.
Group III: Transplantation into the brain and the anterior chamber of the eye of healthy, unconditioned guinea pigs with the subcutaneous tumor developed in conditioned hosts.—Transfer to the brain and eye of normal, unconditioned guinea pigs was attempted with tissue derived from transplants of glioma cells and of non-neoplastic cells in conditioned hamsters and from transplants of HeLa cells in conditioned mice.

Growth of both the glioma TC 178 cells and the HeLa cells were obtained in guinea pig brains or in the anterior chamber of the eyes (Figs. 3, 4), but in no instance did a tumor follow transfer of fibroblasts or kidney epithelial cells. Vascularized brain growths were well demarcated, and, except for some swelling of astrocytes, the surrounding brain tissue was normal in appearance and contained no cellular infiltrates or microglial proliferation.

A point of major interest concerns the histology of these tumors. This will be reported in detail in a separate publication, but in the present context it may be noted that the cellular pattern after transplantation resembled an epithelial-like tumor (Figs. 5, 6) and not the original glioblastoma. The importance of this point lies in the fact that the glioblastomas transferred directly to guinea pigs from man by Greene (8) retain their typical architecture (Fig. 7), thus raising the question as to the part played in the apparent architectural alteration by growth in tissue cultures.

The subcutaneous nodules of HeLa cells derived from conditioned mice also grew in the eyes and brains of normal guinea pigs. This tumor has also been carried serially and is at present in its eighth brain and third eye generation. In both sites the histology of the growths was that of an epidermoid carcinoma (Fig. 8).

Attempts have been made to transfer the two tumors back into the subcutaneous space of hamsters and mice. No growths whatsoever were obtained in normal animals, but both tumors grew in conditioned hosts. In one instance, the glioma was carried for six passages in conditioned hamsters and the HeLa cell tumor for five passages in mice. Such serial transfer usually resulted in loss of histological resemblance to the original tumor and increasing necrosis.

In both the tumors produced by the glioma

---

**Table 1**

<table>
<thead>
<tr>
<th>Types of tissue culture</th>
<th>Subcutaneous transplantation of tissue culture cells into conditioned animals</th>
<th>Intracerebral transplantation of tissue culture cells into unconditioned animals</th>
<th>Intracerebral and intracocular transplantation into unconditioned animals of subcutaneous nodules developed in conditioned animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Glioma TC 178 cells</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>HeLa cells</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fibroblast cells</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Kidney epithelial cells</td>
<td>++</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Key: Types of lesion: 1. Small focus of cells without organization and much necrosis. 2. Growth with slight organization but with no resemblance to parent tumor. 3. Vascularized, in contrast to (1) and (2), with cellular differentiation and organization. 0 Lesion not seen. + Lesion seen rarely. ++ Lesion seen in most instances. -- No experiment done.

Animals bearing the glioma TC 178 and the HeLa cell tumors intracerebrally developed neurological signs in from 1½ to 2 months. These consisted of a weakening of the extremities on the contralateral side and a tendency to move in a circle in a direction away from the affected cerebral hemisphere. Paralysis and death usually followed within 24 hours.

The first passage of the glioma TC 178 resulted in three takes among eighteen guinea pigs, and, in each instance, the tumors were large firm growths occupying almost the entire cerebral hemisphere. Serial transfer of tumor tissue derived from these animals has been successful, and at the present time the growth is in its sixteenth generation. Transfer from the brain into the anterior chamber was attempted with material from the sixth brain passage. Good growth was obtained, and the tumor is also being maintained by serial passage in this site.

The tumors in the brain and the eye were well vascularized. Brain growths were well demarcated, and, except for some swelling of astrocytes, the surrounding brain tissue was normal in appearance and contained no cellular infiltrates or microglial proliferation.
TC 178 cells and those produced by the HeLa cells some foci of necrosis were seen; these stained positively with Alcian green, which is reportedly characteristic for mucin. Since both neoplasms are known as nonmucin-producing, it was felt that the positive reaction was probably due to some unknown substances associated with cellular degeneration.

DISCUSSION

For many years heterologous transplantation of neoplasms from man into healthy hosts has been carried out by Greene (7, 9, 10). The introduction of his methods into the field of tissue culture required an additional step, since injection of neoplastic tissue culture cells into the brains of unconditioned animals failed to produce any growth whatsoever. This step consisted in a preliminary subcutaneous transfer to conditioned hamsters and mice, and the use of the three-dimensional growth thus obtained for transplantation to normal guinea pigs.

The results obtained on the transfer of the various tissue culture lines are summarized in Table 1. It should be noted that true, vascularized neoplasms were rarely found in the subcutaneous space of treated hosts and, in sharp contrast, represented the only type of growth found in the normal guinea pig. In effect, the subcutaneous growths resembled three-dimensional tissue cultures rather than true neoplasms. Such growths were similar to those obtained by Moore et al. in conditioned animals (15). Our intracerebral growths, in contrast to those of Scotti et al. (18), showed both organization and differentiation.

In our hands, no neoplasms were obtained on transfer of non-neoplastic tissue culture cells to either normal or conditioned hosts. This is in agreement with the findings of Moore et al. (15). Foley and Handler (3) recorded growth of neoplastic and normal tissue culture cells in the cheek pouch of hamsters; however, malignant cell lines produced tumors after implantation of approximately ten cells in the cheek pouch of conditioned hamsters, whereas those of normal origin failed to survive and grow if the inoculum contained fewer than 1000 cells. This difference was even more pronounced when normal unconditioned hamsters were used. Foley and Handler (4) reported that, unlike the frankly invasive, transplantable tumor produced by malignant tissue culture cells, the cell growth that resulted from inocula of cell lines of normal origin was not invasive, regressed rapidly, and could not be transplanted to other hamsters.

Sigel et al. (19) obtained growth with tubule formation on transfer of non-neoplastic monkey kidney tissue culture cells to the brains of conditioned rats.

The tumors produced in normal guinea pigs were transplantable serially and have been maintained for many passages without further modification of histological appearance. In contrast, the growths obtained in conditioned hamsters and mice could only be transferred successfully to other conditioned animals, and in our hands this was possible only for a limited number of generations. Continued transfer in such cases was not associated with an increase in vascularity or in differentiation and organization of the tumor cells.

ACKNOWLEDGMENTS

The author is greatly indebted to Mr. George L. Morann and Miss Phyllis E. Johnson for their technical help.

REFERENCES

8. ―――. The Transplantation of Human Brain Tumors to the Brains of Laboratory Animals. Ibid., 13:448-56, 1953.
14. MANUELIDIS, E. E., and POND, A. R. Continuous Cultivation of Cells Arising from Human Case of Glioblastoma...

Fig. 1.—Nodule in the subcutaneous tissue of cortisone-treated x-radiated hamster produced by injection of glioma TC 178 cells. Pleomorphie elongated round and oval cells growing in the subcutaneous tissue of conditioned hamster after injection of glioma TC 178 cells. No characteristic cytoarchitecture is present. H. & E., ×1000.

Fig. 3.—Epidermoid carcinoma produced by HeLa cells occupying a hemisphere of the brain of unconditioned guinea pig. Neop epithelial tumor produced by glioma TC 178 cells growing in the anterior chamber of the eye of unconditioned guinea pig.
FIG. 5.—Neuroepithelial tumor produced by glioma TC 178 cells invading the brain tissue of unconditioned guinea pig. The tumor has a carcinoma-like appearance, and it is well vascularized. H. & E., ×440.

FIG. 6.—Higher magnification of Fig. 5. Marked pleomorphism of cells is seen. Many mitotic figures are present. The tumor does not bear any resemblance to a glioblastoma. H. & E., ×1100.

FIG. 7.—Tumor produced after brain transplantation of human glioblastoma multiforme directly to unconditioned guinea pig. The tumor is composed of elongated, spongioblast-like cells and closely resembles the human neoplasm. H. & E., ×1100.

FIG. 8.—Anaplastic epidermoid carcinoma produced by HeLa cells invading the nervous tissue of unconditioned guinea pig. H. & E., ×440.
Heterologous Transplantation of Tissue Culture Lines

Elias E. Manuelidis