Induction of Neoplasia *in vitro* in Hamster Kidney Tissue by Adenovirus 7-SV40 "Hybrid" Strain (LLE46). (30811)

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When explants of newborn hamster kidney were infected with simian virus 40 (SV40) *in vitro,* cell lines composed of rapidly growing cuboidal and polygonal cells were derived(1). Injection of cell suspensions from these lines into newborn hamsters resulted in rapid appearance of tumors at the injection sites. Histologically, the tumors had areas of well-differentiated adenocarcinoma with tubule formation and areas of undifferentiated carcinoma(1). Reports of transformation of hamster kidney cells by SV40 in other laboratories where different cultural conditions were used, have also described cell lines which produce carcinomas with tubular elements when transformed cells were injected into newborn and adult hamsters(2,3).

Although induction of neoplasia *in vitro* by polyoma virus in hamster embryo and newborn hamster kidney cell cultures has been described(4,5,6), the tumors produced by the transformed cells have been sarcomas with no evidence of neoplastic transformation of epithelial cells. There have been only 2 reports of *in vitro* transformation of hamster kidney cells by adenovirus 12, but tumor production after implantation of the transformed cells was not described in either(7,8). We have attempted to use adenovirus 12 instead of SV40 with our newborn hamster kidney explant techniques(1), but we have been unable to obtain any evidence of transformation or *in vitro* induction of neoplasia. The ability to induce neoplastic transformation of hamster renal epithelium *in vitro* therefore seems to be a unique property of SV40.

Recent studies with LLE46, a substrain of adenovirus 7 which had been passed in rhesus monkey kidney cells with SV40 and subsquently freed of SV40 by passage in the presence of SV40 antiserum, showed that this virus was oncogenic in hamsters(9). The subcutaneous tumors which were produced contained T antigens of SV40 even though no infectious SV40 could be detected in the inocula or in the tumors. On the basis of this observation and studies of antigens produced in human and monkey kidney cells infected with LLE46 *in vitro,* it has been postulated that LLE46 preparations contain particles with a portion of the genome of SV40 inside an adenovirus 7 capsid(9,10,11).

Black and Todaro have recently reported that LLE46 produces transformation of monolayer cultures of weanling hamster kidney cells(12). They stated that the transformed cells resembled those produced in similar cultures by SV40, although they noted that the cells of LLE46 transformed cultures were smaller and had less "piling up." We were interested in determining whether the portion of the SV40 genome within the adenovirus 7 capsid in LLE46 could induce neoplastic transformation of hamster renal epithelium. We therefore have studied the histopathology of tumors produced when LLE46 transformed kidney cells were implanted into hamsters.

Materials and methods. Viruses. LLE46, the adenovirus 7-SV40 hybrid, obtained from Dr. Wallace P. Rowe, National Institute of Allergy and Infectious Diseases, N.I.H., was passed in primary African green monkey kidney (GMK) cell cultures maintained in 2% fetal bovine serum (FBS) and 98% mixture 199. It was titrated by serial 10-fold dilutions in tube cultures of GMK cells and primary human embryo kidney (HEK) cells maintained in 2% FBS and mixture 199. Three to five tubes per dilution were used, and tubes were incubated at 37° on roller drums and observed for 21 days. The pool used in the present experiments contained $10^{8.2}$ $TCID_{50}$ measured in HEK cells and $10^{6.9}$ $TCID_{50}$ when titrated in GMK cells.

Adenovirus 7, Gomen strain, obtained from Dr. J. Kasel, National Institute of Allergy and Infectious Diseases, N.I.H., was grown in HEp2 cells and titrated in HEK and GMK

cultures. The pool we used contained $10^{9.44}$ $TCID_{50}$ per ml for HEK cells and $10^{3.2}$ $TCID_{50}$ per ml for GMK cells.

SV40, strain Vac777 L1, obtained from Dr. Paul Gerber, Division of Biologics Standards, N.I.H., was grown and titrated in GMK cell cultures. The pool we used titered $10^{7.9}$ $TCID_{50}$ per ml. *Culture methods.* Newborn Syrian hamsters were obtained from the Animal Production Section, N.I.H. They were killed on the day of birth and the kidneys were removed aseptically, minced into explants 1 mm in diameter, and grown in 2 ounce prescription bottles (Brockway Saniglas) in a medium composed of 20% FBS and 80% mixture 199, with methods previously described(1).

Injection of cell suspensions in animals. Methods for preparation of cell suspensions and injection of newborn hamsters have been previously described(1). Adult hamsters were given 400 R of total body X-ray and on the following day were injected subcutaneously with 0.5 ml of cell suspension. *Immunofluorescent methods.* Antisera against T antigens were obtained from hamsters bearing large transplanted tumors induced by adenovirus 12, SV40 and the Gomen strain of adenovirus 7 respectively. Fluorescent antibody techniques and tests for reactivity and specificity of the sera have been previously described (13).

Experiments and results. Four prescription bottle cultures each containing approximately 25 explants of newborn hamster kidney were initiated. After 24 hours, groups of cuboidal and elongated cells were seen migrating from the edges of some of the explants in all of the bottles. After 48 hours, one bottle was infected with $10^{7.9}$ $TCID_{50}$ (titrated in HEK cells) of LLE46, the adenovirus 7-SV40 hybrid; one bottle was infected with $10^{9.1}$ $TCID_{50}$ (titrated in HEK cells) of the Gomen strain of adenovirus 7; one bottle was infected with $10^{7.6}$ $TCID_{50}$ (titrated in GMK cells) of SV40; and one bottle was kept as a virus-free control.

Two days after infection, adenovirus type cytopathic effects (CPE) were noted in cultures infected with LLE46 and the Gomen strain of adenovirus 7 but no CPE was present in the SV40 culture or the control. Eight days after infection, the SV40 infected culture and the control contained a moderate growth of elongated cells. The LLE46 culture also contained a moderate growth of elongated cells with scattered areas of adenovirus-type CPE. The culture infected with the Gomen strain had very sparse cellular growth and most of the cells showed adenovirus CPE.

During the subsequent month, the cultures were fed 3 times weekly. The cells in the control culture grew poorly and there was no growth of cells in the culture infected with the Gomen strain. In the cultures infected with LLE46 and SV40 there was progressive growth of both cuboidal and elongated cells with predominance of the cuboidal cells. One month after initiation of the cultures, the LLE46, the SV40 and the control cultures were trypsinized with previously described methods(1) and 2 new bottles seeded with the cells from each of the 3 bottles. After trypsinization, the control cells grew poorly and degenerated, while the cells from the LLE46 and SV40 cultures grew rapidly and formed confluent monolayers of cuboidal and polygonal cells. The cells of the SV40 culture were generally larger than those of the LLE46 culture and had a greater tendency to grow in clumps. Both the LLE46 and SV40 cultures have been trypsinized each week for 6 months and have continued to grow rapidly.

After 2 months, attempts were made to isolate infectious adenovirus 7 and SV40 from the LLE46 and SV40 transformed cells by growing the cells in contact with monolayers of HEK cells and GMK cells(14). The HEK and GMK cultures were observed for 21 to 30 days but no infectious adenovirus 7 or SV40 could be demonstrated.

Immunofluorescent studies of cells from the LLE46 and SV40 transformed cultures grown on coverslips were made with hamster antisera against adenovirus 12 T antigens, adenovirus 7 T antigens, and SV40 T antigens. More than 90% of the cells in both the LLE46 and the SV40 cultures contained SV40 T antigens in their nuclei. No adenovirus 12 or adenovirus 7 T antigens could be demonstrated in either culture.

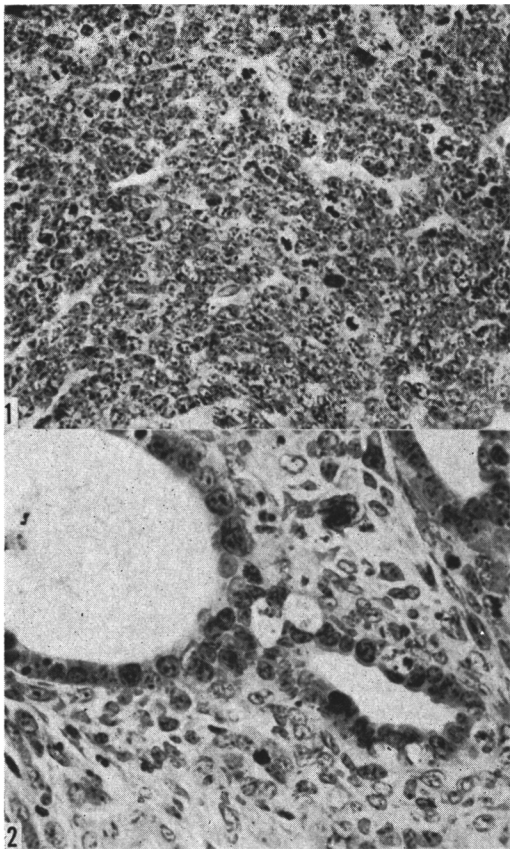

FIG. 1. Subcutaneous tumor in irradiated adult hamster after injection of LLE46 transformed newborn hamster kidney cells. The tumor was undifferentiated and composed of closely arranged cuboidal cells with poorly defined cytoplasm and numerous mitoses. Hematoxylin and eosin 274 ×.

FIG. 2. Subcutaneous tumor in irradiated adult hamster after injection of SV40 transformed newborn hamster kidney cells. The tumor contained well differentiated tubules lined by epithelial cells with large pleomorphic hyperchromatic nuclei. Hematoxylin and eosin 274 ×.

Thirty-nine days after initiation of the cultures, the cells from one 2 ounce bottle of the LLE46 culture were scraped from the glass, suspended in 0.5 ml of their media, and injected subcutaneously into a weanling male hamster that had received 400 R of total body X-ray on the preceding day. Another irradiated hamster was injected with similarly prepared cells from an SV40 culture. Tumors were palpable at the injection sites 26 days after injection of the LLE46 cells and 35 days after injection of the SV40 cells. Histologically the tumor arising at site of injection of the LLE46 transformed cells was composed predominantly of sheets of small cuboidal cells with no evidence of any type of differentiation (Fig. 1). It resembled tumors produced by inoculation of adenovirus 12 in newborn hamsters(15). There were, however, small areas resembling sarcomas produced by SV40. The tumor produced by injection of the SV40 transformed cells, on the other hand, was predominantly carcinoma with areas of well-formed tubular and glandular structures (Fig. 2). Both types of tumors were serially transplanted in non-irradiated adult hamsters and have grown progressively and maintained their distinctive morphology. Tumors were also produced by injection of cell suspensions of LLE46 and SV40 transformed cells into newborn hamsters(1). Again, those produced by the LLE46 transformed cells were undifferentiated and morphologically similar to tumors produced by adenovirus 12, while those produced by the SV40 transformed cells were carcinomas with areas of tubular differentiation.

Comment. Our findings confirm the observations of Black and Todaro that LLE46 can transform hamster kidney cells and that the transformed cells contain the T antigens of SV40 but not the T antigens of adenovirus 7 or 12(12). Our study further proves that the transformed cells can produce tumors when injected into newborn and irradiated adult hamsters indicating that the transformation represents *in vitro* induction of neoplasia. Histologically, however, the tumors produced by LLE46 transformed cells were predominantly undifferentiated and typical of tumors produced by adenoviruses with none of the glandular and tubular epithelial elements associated with SV40 transformation of newborn hamster kidney.

Although there is evidence that LLE46 contains particles with an adenovirus 7 capsid enclosing the portions of the SV40 genome responsible for T antigen production(9-11), enhancement of growth in monkey cells(10), and production of ependymomas in newborn hamsters(16), our present studies suggest that the portions of SV40 involved in induction of neoplasia in hamster renal epithelial cells are absent or ineffective. They further

suggest that the morphological features of tumors induced by adenoviruses and SV40 are not determined by T antigens. Additional studies of *in vitro* induction of neoplasia of hamster kidney with LLE46, other adenovirus-SV40 "hybrids," and mixtures of SV40 and adenoviruses will be necessary to clarify these points.

Summary. Newborn hamster kidney cell cultures were transformed *in vitro* by LLE46 virus, an adenovirus 7-SV40 hybrid, and by SV40. The cultures transformed by both viruses consisted of rapidly growing cuboidal and polygonal cells, although those in the LLE46 cultures were smaller and had less tendency to grow in clumps. Neither infectious adenovirus 7 nor SV40 could be recovered from the transformed cells. More than 90% of the cells in both the LLE46 and the SV40 cultures contained persistent intranuclear SV40 T antigen while no adenovirus 12 or 7 T antigens were demonstrable. Tumors were rapidly produced when both types of transformed cells were injected into newborn hamsters, and into irradiated young adult hamsters. As previously described, the tumors produced by the SV40 transformed cells were predominantly carcinomas with areas of tubular differentiation. Those produced by the LLE46 transformed cells, however, were predominantly undifferentiated and histologically similar to adenovirus 12 tumors. Although they did contain a few small foci resembling SV40 sarcomas, no tubular epithelial structures were seen.

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A Study of Antibodies Produced by Spleen Fragments *in vitro*.* (30812)

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The production of antibodies by spleen, lymph nodes and other tissues has been demonstrated in a variety of *in vitro* systems(1, 2). However, experimental demonstration of antibody production by cultured spleen has been less successful than by lymph node cultures even when the donor animal had been immunized by the intravenous injection of antigen(3,4). A comparison of the chemical and immunologic properties of antibodies produced *in vitro* with those produced *in vivo* has been difficult, mainly because the amounts produced *in vitro* are extremely minute. Such comparisons are important in order to de-

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