Induction of Brain Tumors in Newborn Hamsters by Simian Adenovirus SA7

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SUMMARY

Intracerebral inoculation of the simian adenovirus SA7 induced brain tumors in 26 of 118 hamsters inoculated as newborns. The tumors were primarily of two histologic types. Twenty-four resembled human medulloblastomas, but two tumors were histologically similar to the perivascular sarcoma because there was reticulum in part of the tumor. Other areas contained no reticulum and were almost identical with the astrocytoma IV. The tumor cells could be grown in tissue culture only after passage on feeder layers of irradiated cells. No antibodies to the SA7 tumor antigen could be detected in the sera of tumor-bearing hamsters, although some of the brain tumors were positive for the presence of this antigen by complement fixation. One of the brain tumors has been maintained by serial subcutaneous and intracerebral passage; the resultant tumors have retained their original histologic pattern and low level of virus-induced tumor antigen. Failure to detect antibody to tumor antigen in hamsters bearing brain tumors suggests that similar results with human sera from patients with brain tumors would not rule out the possibility of viral etiology for this type of neoplasia.

INTRODUCTION

At the present time, a good model system for the study of human brain tumors is not available. Although many agents, including irradiation, chemicals, and viruses, have been used in attempts to produce such a model (see Ref. 13 for review), a system of reproducible tumors with the morphologic characteristics seen in human tumors has yet to be developed.

The simian adenovirus SA7 was originally isolated from African green monkeys (18). When injected subcutaneously into newborn hamsters, it produced rapidly growing tumors at the site of inoculation with a latent period of 28 to 48 days in newborns. The tumors were primarily of two histologic types. The tumors were positive for the presence of this antigen by complement fixation. One of the brain tumors has been maintained by serial subcutaneous and intracerebral passage; the resultant tumors have retained their original histologic pattern and low level of virus-induced tumor antigen. Failure to detect antibody to tumor antigen in hamsters bearing brain tumors suggests that similar results with human sera from patients with brain tumors would not rule out the possibility of viral etiology for this type of neoplasia.
bearing virus-induced brain tumors. Primary African green monkey kidney cells growing on coverslips were inoculated with SA7 virus and harvested 24 hours later. After suitable fixation and treatment with the sera under test \(10\), the cells were reacted with anti-hamster horse antibody that had been conjugated with fluorescein isothiocyanate. Microcomplement fixation tests \(31\) were also performed with tumor cell extracts and sera from hamsters bearing subcutaneous tumors induced by SA7. Two full units of complement were used with overnight fixation. The hemolytic system was then added, and the test was incubated at \(37^\circ\)C for two hours before endpoints were determined. All tests included serum known to react with SA7 tumor antigen, as well as negative controls.

**RESULTS**

**Production of Brain Tumors by Injection of SA7.** Newborn Syrian hamsters (within 24 hours of birth) were inoculated intracerebrally with SA7 virus. Each hamster received about \(10^6\) plaque-forming units of virus in \(0.1\) ml. The animals were weaned at 21 days and observed afterwards for clinical signs of illness. Since death usually occurred within 2–3 days of the first signs of clinical illness, which included lethargy, ataxia, and weakness, most animals in the study were sacrificed at the first signs of clinical illness.

Of the 118 inoculated animals that survived to be weaned, 26 developed tumors of the brain. An additional 15 animals developed subcutaneous tumors in the head region at the site of inoculation. The latent period for tumor development in the brain was 23 to 56 days after virus inoculation. The brain tumors were soft and often necrotic and hemorrhagic; they were grossly well demarcated from normal brain.

Histopathologic diagnosis of the tumors was difficult because there were no precedents for the designation of viral-induced experimental brain tumors. We were, however, able to utilize to some extent the diagnostic criteria and nomenclature established for human brain tumors. All of the tumors produced were placed in the general group of gliomas, although two of the tumors, which we have designated astrocytoma IV (glioblastoma multiforme), did show some reticulum formation. Kernohan and Sayre \(16\) point out that the histologic picture of these tumors in man is an inconstant and varied one.

The tumors in the hamsters were also varied.

**Histopathology of the Medulloblastomas.** Most of the medulloblastomas were tumors composed of closely packed small cells with irregular-shaped, round, or slightly elongated hyperchromatic pleomorphic nuclei and small amounts of poorly defined lightly staining eosinophilic cytoplasm (Figs. 1, 2). The nuclei of many of the cells showed a fine chromatin network with many large prominent nucleoli. Other nuclei were so hyperchromatic that intranuclear detail was obscured. In some areas mitoses were numerous and many atypical mitotic figures were seen. Although large areas of necrosis were rare, occasional small foci of necrosis as well as tumor cells showing pyknotic nuclei and karyorrhexis were noted. While there were few multinucleated giant cells, there were many giant nuclei among the tumor cells. An occasional tumor was composed of small elongated cells with little cytoplasm and hyperchromatic nuclei in which there were many pseudo-rosettes. These pseudo-rosettes usually occurred around a small blood vessel or a microarea of acidophilic material. An occasional tumor showed a palisading arrangement of the cells (Fig. 3). Such tumors were composed primarily of elongated cells with elongated nuclei and tended to be somewhat more vascular than the more closely packed round cell type. Mitotic figures were numerous, and small focal areas of necrosis were common. All tumors were highly cellular with little stroma and were poorly vascularized. No reticulum was produced by the tumor cells. The line of demarcation between the medulloblastoma and the brain was fairly sharp. Adjacent brain tissue either was normal or showed mild edema. The tumor cells did not appear to invade the surrounding brain tissue.

**Histopathology of the Astrocytoma IV (Glioblastoma Multiforme).** These tumors were composed of cells characterized by pleomorphism. Many of the cells had giant nuclei, and numerous large multinucleated giant cells were present (Fig. 5). Few or no astrocytes were recognized. Many cells contained hyperchromatic nuclei, while some contained nuclei with a definite fine fibrillar chromatin network. There were both large and small areas of necrosis throughout the tumor. Many tumor cells showed pyknotic nuclei and karyorrhexis. The lumina of many of the blood vessels were obliterated by proliferation of the lining of the endothelial cells (Fig. 6). Bizarre mitotic figures were frequently observed in the tumor cells, and mitotic figures were also seen within the proliferating endothelial cells of the blood vessels. Reticulum was seen in some areas of the tumor, but was absent in others (Fig. 7). There was no apparent difference in the cellular pattern between the areas showing reticulum and those not producing reticulum. The brain tissue surrounding the tumors was edematous, and there was no sharp line of demarcation between the brain and the tumor (Fig. 8).

**Growth of the Tumor Cells in Vitro.** Eight of the brain tumors were removed, trypsinized, and attempts were made to grow the cells in vitro. All attempts were unsuccessful. Cells from the brain tumors were then grown by plating the tumor cells onto a feeder layer of normal cells. Newborn hamster brain cells were grown in Petri dishes to form a monolayer. These cells were then irradiated with \(2000\) R. When the tumor cells from the brain were plated onto these cells, growth of the tumor cells occurred.

After a number of passages on feeder layers of cells, it was possible to propagate the cells in vitro. Occasionally it was necessary to replate the cells on feeder layers when cell growth appeared to decline.

**Testing of Tumor Cells and Sera from Tumor-bearing Animals for the Presence of Virus-specific Antigens and Antibodies.** Sera from 11 hamsters with virus-induced brain tumors were tested for the presence of antibodies to the SA7-specific tumor antigen by the indirect immunofluorescence test and the complement-fixation test. The results were negative. Similarly, no virus-specific (neutralizing) antibodies were detected. One tumor cell line that had been established by plating the cells onto feeder layers was found to react with hamster sera positive for SA7 tumor antigen by the complement-fixation test; in the immunofluorescence test, these cells demonstrated filaments of antigen in the cytoplasm when reacted with serum from a hamster bearing a subcutaneous tumor induced by SA7.
Transformed brain cells (see below).

Transplantability of the Tumor Cells. Four of the primary brain tumors were inoculated intracerebrally and subcutaneously into weanling hamsters. Twelve brain tumors have been examined following intracerebral inoculation of tumor cells. One brain tumor has produced tumors at the site of inoculation when the cells were injected subcutaneously, and subcutaneous transplants of the tumor have been successfully carried out. These brain and subcutaneous tumors resemble the medulloblastomas histologically and can be maintained by serial trocar passage subcutaneously in weanling hamsters (Fig. 4). Although SA7 tumor antigen was not detected in cultured cells from the tumors by immunofluorescence, extraction of antigen from concentrated cell packs has yielded preparations capable of fixing complement in the presence of SA7 tumor antibody.

DISCUSSION

A number of viruses can produce brain tumors in experimental animals. Polyoma virus induces meningeal and intracranial sarcomas in newborn hamsters injected intracerebrally (6, 26, 30) and hemangiomlas in rats (11). SV40 induces ependymomas and tumors of the choroid plexus in hamsters (7–9, 12, 17, 33) and papillary ependymomas in mastomys (28). Both SV40 and polyoma viruses can transform astrocytes growing in vitro (32); when these cells are injected subcutaneously or intracerebrally, astrocytomas are produced at the site of inoculation, although intracerebral injection of the virus did not produce astrocytomas. Human adenovirus type 12 has produced undifferentiated or unclassified brain tumors after intracerebral inoculation of hamsters (14, 27). Various strains of Rous sarcoma virus, including the Schmidt-Ruppin, Harris, and Bryan strains, have induced gliomas and choroid plexus papillomas in hamsters (3, 5, 19, 23, 24), meningeal sarcomas and gliomas in rabbits (25), leptomeningeal sarcomas, gliomas, and astrocytomas in dogs (2, 13, 20, 21), and cells transformed in vitro have produced tumors in rhesus monkeys (22).

We have demonstrated that intracerebral inoculation of the simian adenovirus SA7 will occasionally produce brain tumors in hamsters that morphologically resemble the astrocytoma Grade IV (glioblastoma multiforme) in man, although a portion of one of the tumors did produce reticulum.

Inoculation of SA7 virus into hamsters generally produced medulloblastomas. Kernohan and Sayre (16) point out that the histologic picture presented by the medulloblastoma of man is an inconstant and varied one, and the tumors produced in hamsters follow this pattern. No reticulum was produced by the tumor cells.

The failure to detect virus-specific antibodies in the sera of animals bearing SA7-induced tumors is interesting. It is possible that since the brain tumors are relatively small, there was not enough antigenic stimulation for antibody production before the animal was sacrificed. It is also possible that the brain as an immunologically privileged site prevented good antigenic expression. The SA7 tumor antigen was detected in some tumor cells (following concentration) by the complement-fixation test and by immunofluorescence tests using sera from hamsters bearing subcutaneous tumors.

The experimental model described in this report should prove useful for the study of the pathogenesis of brain tumors. The system has already been utilized to determine the degree of localization in tumor target cells of a series of indium-tagged chelates (4), and preliminary experiments have yielded information useful for brain imaging. The results are presently being applied to the study of human tumors.

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REFERENCES


Fig. 1. Photomicrograph of a typical medulloblastoma. Cells are closely packed. Many of the nuclei are so hyperchromatic that the nuclear detail is obscured. H & E, original magnification × 192.

Fig. 2. High-power photomicrograph of a typical medulloblastoma. The cells show a small amount of cytoplasm. Some of the nuclei are hyperchromatic, while others show a fine chromatin network. H & E, original magnification × 380.

Fig. 3. Photomicrograph of a medulloblastoma showing the typical palisading arrangement of the cells. Brain tissue below, tumor above. H & E, original magnification × 192.

Fig. 4. Subcutaneous transplant of medulloblastoma from hamster brain. The histologic pattern is similar to the tumor seen in Fig. 1. H & E, original magnification × 192.

Fig. 5. Photomicrograph of typical astrocytoma IV. There are many multinucleated giant cells. Many of the cells contain small single nuclei surrounded by abundant cytoplasm. H & E, original magnification × 192.

Fig. 6. A blood vessel within an astrocytoma. Note the proliferation of endothelial cells which occlude the lumen of the blood vessel. Pyknotic tumor nuclei and a small area of necrosis is also seen. H & E, original magnification × 192.

Fig. 7. A photomicrograph of an area of astrocytoma with reticulum. Wilder's silver stain, original magnification × 380.

Fig. 8. Typical boundary between astrocytoma (T) and brain tissue (B). The brain tissue is edematous. Pleomorphic tumor cells invade the normal brain tissue. The boundary between the brain tissue and tumor is not sharp. H & E, original magnification × 192.
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