Intracranial Fibroblastic Neoplasms in the Hamster from Bovine Papilloma Virus

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SUMMARY

Intracranial fibroblastic neoplasms developed in 16 of 19 hamsters inoculated intracerebrally with bovine papilloma virus. The tumors presumably arose in the meninges and infiltrated the adjacent brain along the vascular channels. Tumor nodules that developed in the subcutis of the extremities, ears, and body were probably due to viremia following intracerebral inoculation. Fibroblasts of the cranial cavity and subcutis appeared to be more susceptible to the oncogenic action of bovine papilloma virus than fibroblasts elsewhere in the hamster.

INTRODUCTION

Intracranial meningiofibromas can be produced in calves with BPV (5). Two reports have indicated that the BPV can induce intracranial tumors (7) or the proliferation of mesenchymal elements in the pia mater (2) at the site of injection when it is injected i.c. into newborn hamsters.

This report is concerned with the intracranial fibroblastic neoplasms produced in hamsters by BPV and the disseminated tumors resulting from i.c. inoculation.

MATERIAL AND METHODS

BPV was extracted from frozen cutaneous bovine fibropapillomas (Isolate 311) as previously described (5). Samples from the viral preparations were negatively stained and examined with the electron microscope to verify virus content (Fig. 2). Antibiotics (100 units of penicillin and 50 μg of streptomycin per ml, Squibb Pharmaceutical Company, New York, N. Y.) were added to the BPV preparation which was then frozen and stored at -18° until use for i.c. inoculation. Prior to inoculation, the viral preparations were cultured on tryptose broth and thioglycollate media. No bacterial growth was evident after 4 days incubation at 37°. A portion of this viral preparation was treated by heating at 92° for 30 min and served as a control inoculum.

Approximately 0.02 ml of the virus suspension was injected through a 26-gauge needle into the right cerebral hemisphere of each hamster. A small amount of carbon was added to the BPV suspension to mark the site of injection. Twenty-four hamsters, approximately 10 days old, were inoculated with the active BPV suspension, and 10 hamsters were inoculated with the heat-treated BPV preparation. The hamsters were weaned at approximately 25 days of age and placed in individual cages for the duration of the experiment.

Hamsters were necropsied at intervals between 30 to 315 days after inoculation. The entire brain was fixed in formalin for 24 hr before sectioning. Portions of various other tissues and organs were fixed in formalin for subsequent histological examination. Tissue sections were routinely stained with hematoxylin and eosin, and representative intracranial tumor sections were also stained with the Masson, Bodian, Holzer, and reticulum procedures. Tissues for electron microscopy were fixed in glutaraldehyde and embedded in Epon 812 for sectioning. Ultrathin sections were stained with uranyl acetate.

RESULTS

Nineteen of 24 hamsters inoculated with BPV and 7 of 10 hamsters inoculated with heat-treated BPV survived the immediate trauma of i.c. inoculation. Intracranial tumors were observed either grossly or microscopically in 16 of the 19 hamsters that survived 30 days or more after receiving active BPV. Macroscopic tumors were often localized, firm, white nodules (Fig. 5) while others were less distinctly evident (Fig. 4). Moderate to marked dilation of the lateral and 3rd ventricles and slight dilation of the 4th ventricle were observed in 9 of the hamsters with intracranial tumors (Fig. 4). Clinical signs of incoordination, lethargy, and depression were observed 30 to 60 days prior to death in 10 hamsters with intracranial tumors that survived for 6 or more months after inoculation. Several of these animals exhibited an abnormal behavior in that they would bite the handler's glove and not release it until forced to do so.

No abnormal clinical signs or tumors were observed in the 7 hamsters receiving heat-treated BPV. Most of these control hamsters lived for approximately 450 to 500 days after inoculation.

Neoplasia of the meninges was evident by microscopic examination in 2 hamsters as early as 30 days postinoculation.

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At 60 days postinoculation, tumor cells up to 20 cell layers thick were observed in the meninges (Fig. 8). Carbon particles were noted microscopically in most of the tumors. The carbon particles served as a marker for the inoculum and were observed most often in the tumors examined during the early developmental stages. Tumor cells grew in close association with the adventitia of meningeal blood vessels, cerebral, and cerebellar capillaries (Figs. 6, 7, and 10). The tumors were composed of neoplastic spindle-shaped cells that had elongate to pleomorphic nuclei and poorly defined cytoplasmic borders. The differential staining features of the tumor cells with Masson (Fig. 9), reticulum, Bodian, and Holzer stains indicated that these cells were of mesenchymal origin. A moderate glial cell reaction adjacent to the tumors was sometimes noted.

Electron microscopic studies of intracranial tumor cells failed to reveal intracellular or extracellular viral particles. The tumor cells were immature, active fibroblasts, which contained large, elongate, or pleomorphic nuclei with margination of the chromatin and enlarged nucleoli. There was a well-developed Golgi apparatus and a prominent rough endoplasmic reticulum with numerous dilated cisternae. Extracellular aggregates of collagen adjacent to the cytoplasmic membrane of tumor cells were commonly observed.

Individual tumors varied in their pattern of growth and cellularity. One large, firm tumor (1 cm wide) extended the full length of the right cerebral hemisphere and appeared to be encapsulated (Fig. 5). The center of this tumor was composed of fibroblastic tumor cells arranged in irregular bundles (Fig. 9) whereas, at the periphery, tumor cells radiated from the central mass and diffusely infiltrated the brain parenchyma in close proximity to the blood vessels (Fig. 7). In another tumor, the neoplastic cells were principally arranged in circular masses around small blood vessels (Fig. 6). One tumor which evidently arose from the lateral meninges of the right cerebral hemisphere invaded the brain, extended posteriorly, and infiltrated the brain tissue of the pons (Fig. 4). In the area of the pons, the tumor was very collagenous, whereas the meningeal part of the tumor contained only a small amount of collagen. There was edema of the brain, degeneration of neurons, and moderate glialis at the periphery of the sclerotic area of the tumor in the pons. The collagen content of the intracranial tumors was always less than that observed in BPV-induced s.c. fibromatous tumors.

In addition to intracranial tumors, disseminated tumor nodules were found in the s.c. tissue of 14 hamsters at various sites over the body including the lips, ears, neck, back, leg, and the lateral abdominal wall (Figs. 1 and 3). These disseminated tumor nodules were evident 144 to 305 days (average, 217 days) after i.c. inoculation with BPV. Five animals developed tumors in the subcutis near the external auditory meatus of the right ear. There was neither gross nor microscopic connection between these tumors and those in the brain. Tumor nodules located s.c. also developed in 2 hamsters, where the skin was punctured during i.c. inoculation.

The histological appearance of the disseminated tumors was similar to the fibrosarcomas induced by s.c. inoculation with BPV in other studies (2, 7, 12). Tumor nodules were not observed in the thoracic or abdominal cavities.

**DISCUSSION**

The mesenchymal elements of the cranial cavity and subcutis of the hamster are highly susceptible to the oncogenic action of BPV. Intracranial tumors induced by BPV in the hamster can be regarded as meningeal sarcomas (7), meningiomas (1), or meningiofibromas (2). The tumor cells appeared to arise in the meninges, but whether the cells are derived from pericytes, advential cells of blood vessels, or the connective tissue cells of the meninges is not known. Malignancy of the meningeal hamster tumors is suggested because of their anaplastic character and the progressive infiltration of the brain parenchyma, especially along the vascular channels. Although the cells of the intracranial tumors appeared to be more anaplastic than those of the s.c. tumors, connective tissue cells were transformed in both locations.

Disseminated tumor nodules in the subcutis were observed in hamsters following i.c. inoculation with BPV in this study. Lasneret et al. (7) observed one tumor nodule on the leg after i.c. and i.p. inoculations. Disseminated tumor nodules were not reported after i.c. inoculation of hamsters by Cheville (2). Since the induction period of tumors in this study was similar to that following s.c. inoculation of BPV in other studies in our laboratory (12), it would seem that the disseminated tumors were induced by BPV circulating in the blood soon after i.c. inoculation. The amount (0.02 ml) of BPV suspension injected i.c. was a relatively large volume in relation to the volume of the hamster brain. Mims (8) reported that a volume of 0.03 ml injected into the brain of an adult mouse would be equivalent to 100 ml injected i.c. into the brain of a man. Mims (8) also reasoned that i.c. inoculation of a relatively large volume of inoculum would probably rupture the arachnoid villi allowing the injected material to enter the blood stream. Although the brain of the mouse is approximately one-half the size of a hamster brain, the volume of inoculum injected into the brain of young hamsters was still relatively large.

The single or multiple tumor nodules found in the lungs in the studies of others were probably due to metastasis of tumor cells since they were much smaller than the primary intracranial and s.c. tumors. In addition, they were found only in those hamsters in which the primary tumor had been evident for a relatively long time (7, 12). In 1 instance during a related study it was observed that s.c. inoculation of BPV induced a meningeal sarcoma (Fig. 10) in addition to the primary tumor that developed at the s.c. injection site. This observation, to the authors' knowledge, is the 1st report of an intracranial tumor induced in the hamster following s.c. inoculation of BPV. Whether this meningeal tumor originated from metastasis of tumor cells or from virus following viremia subsequent to accidental i.v. inoculation could not be determined.

In a preliminary study, hamsters were inoculated by various routes with a 10% suspension of bovine fibropapilloma (Isolate 301). Groups of 5 3-week-old hamsters were used for each route of administration. Local fibroblastic tumors developed in 8 to 24 months in most of the hamsters inoculated in the skin, cheek pouch, and cerebrum. Only a few hamsters developed tumors after injection into the muscles of the thigh,
lung, or testis. No tumors were produced by i.p., intratracheal, intraocular, or intracardial injections, or following p.o. administration with a stomach tube.

Intracranial tumors have been produced in the hamster by several other oncogenic viruses following i.c. inoculation. Their histological character appears to be typical for each oncogenic virus. Ependymomas have developed from SV40 virus (4), gliomas from Rous sarcoma virus (9, 10), noninvasive primitive mesenchymal neoplasms from adenovirus type 12 (6), and leptomeningeal sarcomas from polyoma virus (11). The possibility of existence of these oncogenic viruses in the stock of hamsters used in this study was considered, but sera lacked complement-fixing antibodies for polyoma, SV40, adenovirus type 12, or Rous sarcoma viruses (12).

The cerebral ventricular dilation was believed to be the result of obstruction to the normal flow of cerebral spinal fluid by tumor growth and by displacement of brain tissue. The obstruction may have been more critical at the level of the 4th ventricle since dilation occurred in all 4 ventricles (3, 13).

REFERENCES

Intracranial Fibroblastic Neoplasms from BPV

Fig. 1. Disseminated tumors in the subcutis of the ear and back of a hamster 210 days after i.c. inoculation with BPV.

Fig. 2. Electron micrograph of BPV preparation used for inoculation. The virus particles are approximately 54 nm in diameter. Negative stain, X 278,000 (approximately).

Fig. 3. Disseminated tumor on right rear leg of a hamster 210 days after i.c. inoculation of BPV. The tumor was first evident at 180 days postinoculation.

Fig. 4. Coronal sections of hamster brain 210 days after i.c. inoculation with BPV. Histologically, tumor was evident in the 3rd (arrow) through 6th (arrow) coronal sections. Tumor was also present microscopically in the ventrolateral meninges of the right cerebral hemispheres of the 3rd through 5th coronal sections. The cerebral tumor extended posteriorly into the pons in the 6th and 7th sections. Internal hydrocephalus involved all 4 ventricles.

Fig. 5. Fourth and 5th coronal sections of brain from a hamster inoculated i.c. 200 days previously with BPV. The tumor (arrows) has partially replaced the brain tissue of the right cerebral hemisphere. The animal was lethargic and depressed 30 days prior to death.

Fig. 6. Tumor cells were observed in the cerebrum of the hamster noted in Fig. 4. The tumor cells in this area were arranged in bundles and circular masses around small blood vessels (arrows) and capillaries. H & E, X 125.

Fig. 7. Infiltration of tumor cells into the brain at the periphery of tumor nodule illustrated in Fig. 5. H & E, X 100.

Fig. 8. Fibroblastic neoplasm showing early, local, perivascular invasion (arrow) into the cerebrum. This hamster was necropsied 60 days after i.c. inoculation with BPV. H & E, X 125.

Fig. 9. Irregular arrangement of tumor cells near the center of the tumor illustrated in Fig. 5. Masson's trichrome, X 100.

Fig. 10. Perivascular infiltration of tumor cells occurred in the molecular and granular layers of the cerebellum of a hamster 320 days after s.c. inoculation of a bovine papilloma suspension in preliminary studies. Purkinje neurons can be seen (arrows). H & E, X 125.
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