Studies on Connective Tissue Tumors in the Hamster Produced by Bovine Papilloma Virus

N. F. CHEVILLE

National Animal Disease Laboratory, Ames, Iowa

Summary

Bovine papilloma virus was injected s.c. and intracerebrally in newborn and weanling hamsters. Connective tissue tumors (fibromas, chondromas, and meningiomas) were produced in the subcutaneous regions of the back, pinna of the ear, and the meninges. The tumors did not contain evidence of viral infection when examined by histologic, immunofluorescent, and electron microscopic means. The tumors grew rapidly but remained localized and encapsulated. Large tumors consisted of masses of collagen fibers with a zone of active fibroblasts on the periphery.

Introduction

The cutaneous bovine papilloma contains a considerable subcutaneous fibroblastic reaction in addition to the epithelial hyperplasia which characterizes most infectious papillomas (2). In the reacting fibroblasts of the naturally occurring papilloma, there has been no detectable evidence of the virus by immunofluorescence (8). Bovine papilloma virus (BPV) has also been shown to evoke a connective tissue response when injected s.c. into horses (7) and hamsters (1, 4, 10). The proliferating fibroblasts of these experimentally induced lesions have not been examined for evidence of viral infection. The relationship of the papovavirus BPV with the proliferating fibroblasts of the experimental and naturally occurring lesions therefore remains obscure.

It was the purpose of this study to examine the oncogenic effect of BPV on some connective tissue elements of the Syrian hamsters. The proliferating connective tissue cells were studied by morphologic and immunologic means for the detection of viral antigens or particles.

Materials and Methods

A viral isolate obtained from naturally occurring bovine warts and stored in 50% glycerin-saline at 4°C was used. Wart tissue was ground in a mortar with saline to make a 10% tissue suspension. This was clarified by slow centrifugation. An aliquot of this supernatant was used as the crude inoculum. The remaining of this supernatant was centrifuged at 12,000 × g for 30 min and 114,000 × g for 1 hr. The resulting pellet was then resuspended in 1 ml of sterile saline and used as the partially purified inoculum (Fig. 1).

Hamsters were obtained commercially and were given injections as presented in Table 1. A 27-gauge needle was used to inject 0.25 ml of the inoculum. Females were bred and carried through gestation to provide a source of newborn animals.

A young adult cow was hyperimmunized with a partially purified BPV-Freund’s complete adjuvant emulsion. Two injections of 1 ml each were given i.m. with a 2-week interval between injections. Two weeks following the last injection, serum was collected and the γ-globulin precipitated with ammonium sulfate (3). γ-Globulin was conjugated with fluorescein isothiocyanate, dialyzed, and stored in 1-ml aliquots at −65°C. Immediately before use, it was absorbed twice with acetone-extracted canine liver powder and centrifuged. Frozen sections of tumor were fixed in acetone and incubated with conjugated globulin at 37°C for 30 min. The conjugated globulin preparation was specific for bovine papilloma virus. It produced nuclear fluorescence in bovine cutaneous papilloma sections but not in sections of canine oral papilloma or bovine papilloma sections blocked with unconjugated antisem.

Tumors were surgically removed at various stages during their development. The hamsters were killed at approximately 18 months of age, and viscera examined histologically. The lungs, liver, spleen, and adrenals were carefully examined for possible metastatic lesions. Formalin-fixed tissues were processed by conventional paraffin embedding and stained by the following methods: hematoxylin-eosin, alcian blue-periodic acid-Schiff (with diastase controls for glycogen), Feulgen, Mallory trichrome, and Wilder reticulum technics. Small portions of 5 fibromas and 2 chondromas were fixed in glutaraldehyde, post-fixed in osmium tetroxide, processed in methacrylate and stained with lead hydroxide. Ultrathin sections were cut on glass knives and examined with a Philips 200 electron microscope.

Portions of 2 large tumors were ground in a mortar, centrifuged for clarification, and the supernatant inoculated s.c. into the ear of newborn hamsters (Table 1) and 2 young calves.

Results

A summary of the results of the inoculation of BPV into hamsters is presented in Table 1. The crude viral inoculum did not induce tumor formation as consistently as did the partially purified viral preparation (Fig. 1). Inoculation of crude extracts of hamster tumor material did not produce growth when inoculated into the skin of young calves or newborn hamsters.

The rapidly growing fibromas induced by s.c. inoculation (Fig. 2) were encapsulated and noninvasive. Most tumors consisted of a single discrete mass. The center was composed of dense
TABLE 1
A SUMMARY OF THE RESULTS OF INJECTION OF BOVINE PAPILLOMA VIRUS INTO HAMSTERS

<table>
<thead>
<tr>
<th>BPV(^a) preparation</th>
<th>Site of injection</th>
<th>No. with tumors/No. injected</th>
<th>Age at injection (days)</th>
<th>Appearance av. time (wk.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude</td>
<td>s.c. (back)</td>
<td>7/15</td>
<td>1</td>
<td>38</td>
</tr>
<tr>
<td>Crude</td>
<td>s.c. (back)</td>
<td>5/15</td>
<td>21</td>
<td>61</td>
</tr>
<tr>
<td>Crude</td>
<td>i.v.</td>
<td>0/5</td>
<td>21</td>
<td>48</td>
</tr>
<tr>
<td>Partially purified</td>
<td>s.c. (ear)</td>
<td>20/20</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>Partially purified</td>
<td>s.c. (ear)</td>
<td>14/15</td>
<td>21</td>
<td>48</td>
</tr>
<tr>
<td>Partially purified</td>
<td>s.c. (back)</td>
<td>9/15</td>
<td>21</td>
<td>57</td>
</tr>
<tr>
<td>Partially purified</td>
<td>Intracerebral</td>
<td>6/10</td>
<td>1</td>
<td>?</td>
</tr>
<tr>
<td>Extract of hamster</td>
<td>s.c.</td>
<td>0/10</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>tumor cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Abbreviation: BPV, bovine papilloma virus.
\(^b\) Of these, 4 were chondromas; 1 other contained pulmonary lesions.
\(^c\) Since inoculation, 10 months have elapsed.

masses of collagen with a rim of young-appearing fibroblasts at the periphery. Occasionally, multiple tumors occurred near the inoculation site but were not seen elsewhere. In all cases, growth was progressive and eventually resulted in necrosis in the center of the tumor and ulceration of the overlying skin. Normal tissue surrounding the tumor growth was not infiltrated by lymphocytes or plasma cells.

Tumor cells consisted of well-differentiated fibroblasts which produced many collagen fibers (Fig. 3). Growth of the fibroblasts was associated with the vascular structures of the tumor (Fig. 4). Periarteriolar growth in some areas formed large concentric whorls resembling a hemangiopericytoma. Very little reticulin was present. No nuclear abnormalities were detected with Feulgen staining, and mitotic figures were rare. Specific bovine papilloma virus immunofluorescence did not occur in the nucleus of cytoplasm of the fibroblasts nor was it present extra-cellularly.

Normal and tumor-bearing hamsters examined postmortem at the age of 18 months had a high incidence of glomerular sclerosis and glomerular amyloid. Of the 61 tumor-bearing hamsters, only 1 contained lesions suggestive of metastases. These were 5 dense white nodules in the lung of a hamster inoculated s.c. in the ear with purified viral preparation. The nodules were composed of dense bundles of collagen (Fig. 5). They were encapsulated and did not appear to contain active fibroblasts. The lymph nodes draining the area of the ear tumor of this hamster did not contain such foci.

Electron micrographs of tumor cells contained fibroblastic cells in various stages of collagen production. Many cells contained a well-developed, dilated endoplasmic reticulum and cytoplasmic vacuoles. These structures were filled with an amorphous substance identical in appearance with similar substances in developing fibroblasts and chondroblasts. The vacuoles occurred at the cell surface where the amorphous material appeared to be liberated into the extracellular spaces. Nuclei of the fibroblastic tumor cells did not contain evidence of the presence of papovavirus particles (Fig. 6). Chromatin was present diffusely throughout the nucleus and small aggregates of nucleolar material were usually present.

Subcutaneous inoculation in the pinna of the external ear produced chondromas, as well as fibromas in 4 of the 15 hamsters inoculated at birth with the partially purified preparation. In cases inoculated in the ear and where the primary tumor was removed surgically by amputation, local recurrence always resulted. The proliferating cartilage cells contained large vacuoles (Fig. 7) which stained intensely for acid mucopolysaccharide (Fig. 8). A marked increase in cytoplasmic glycogen was present. No morphologic evidence of viral infection was present in these cells when examined for immunofluorescence or by electron microscopy. The large cytoplasmic vacuoles contained amorphous substance seen in normal chondroblasts. The cartilage cells were separated by areas of fibrous and amorphous elements.

Proliferation of mesenchymal elements in the pia mater occurred as the result of intracerebral inoculation of partially purified BPV preparations. Connective tissue cells were closely associated with the vascular supply and appeared to have followed the pial vessels into the brain where they were evident in the Virchow-Robin spaces. They were not seen invading the brain substance proper (Fig. 9). The cells were similar in appearance to those in the subcutaneous fibromas, although they were not examined by immunofluorescence or electron microscopy.

Discussion

Tumor cells induced by bovine papilloma virus did not differ morphologically from rapidly growing fibroblasts or chondroblasts. The diffuse pattern of nuclear chromatin and small nucleolus characteristic of collagen-producing cells was also seen in the BPV-induced tumors (5). Nuclear viral particles which occur in all papilloma viral-infected cells were absent.

Although infective virus cannot be recovered from these tumors (10), viral nucleic acid may be present in the tumor cells. Specific antigens in BPV-induced hamster tumors have not been demonstrated using virus-specific complement-fixing antigens (6). Whether tumor cell antigens can be demonstrated by immunofluorescent methods or transplantation rejection, as has been shown for hamsters bearing SV40-induced tumors (6, 9), remains to be demonstrated.

Only 1 of 61 tumor-bearing hamsters contained pulmonary lesions. Since these were not actively growing, the relationship with the subcutaneous tumor in the ear is uncertain. Pulmonary fibromas have been previously described in hamsters treated by s.c. injection with BPV (10). Whether these tumors were metastases or of multicentric origin was not determined.

It is obvious that BPV is oncogenic for mesenchymal tissues of the hamster. Its effectiveness in producing tumors seems to be related not only to the age of the target tissues but to the vascularity of those tissues as well. Although the tumors are not malignant and are noninvasive, they will recur at the site of injection up to 3 times following initial removal. The large recurring tumors are encapsulated and have no evidence of infiltration with lymphoid, plasma cells, or other immunologically competent cells.

Since no cellular evidence of papilloma viral particles was detected by immunofluorescent or electron microscopic methods,
the association of the virus with the proliferating fibroblastic cells remains obscure.

Acknowledgments

The author acknowledges the technical assistance of Mr. David Wilson, Mr. Loren Elliott, and Mr. James Heminover. The negative staining procedures used in electron microscopy were done by Mr. A. E. Ritchie.

References

FIG. 1. Electron micrograph of bovine papilloma virus particles in a negative stained preparation of the partially purified inoculum. X 48,000 (courtesy of Mr. A. E. Ritchie).

FIG. 2. Multiple fibromas on the ear of a 4-month-old hamster inoculated at 1 day of age (A). The tumor was removed surgically, and the recurring tumor is shown 35 days later (B).

FIG. 3. Fibroblasts present in a subcutaneous fibroma in a hamster. Collagen fibers can be seen in different stages of production. H & E, X 400.
FIG. 4. Fibroblasts which have proliferated around the sheath of small arterioles. This tumor resembles the hemangiopericytoma described in other species. H & E, × 40.

FIG. 5. A section of a dense white nodule seen in the lung of 1 hamster at postmortem examination. Dense collagen comprises the majority of the lesion. H & E, × 35.

FIG. 6. Electron micrograph of a tumor fibroblast surrounded by collagen fibrils. The many cytoplasmic vesicles contain an amorphous substance which appears to be liberated into the extracellular spaces. × 11,000.
Fig. 7. Chondroma originating from cartilage cells in the ear. Normal ear cartilage is seen on the left. The tumor tissue on the right is invading the subcutaneous adipose tissue. H & E, X 250.

Fig. 8. A chondroma similar to that in Fig. 5. The darkly stained cartilage indicates the presence of acid mucopolysaccharides. Alcian blue-periodic acid-Schiff, X 25.

Fig. 9. Cerebral cortex of a hamster treated by intracerebral injection. Fibroblastic proliferation of the meninges has occurred which followed the associated vascular structures into the Virchow-Robin spaces. H & E, X 100.
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