A Comparative Study of Childhood Rhabdomyosarcoma and Virus-induced Rhabdomyosarcoma in Mice

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SUMMARY

Childhood rhabdomyosarcoma was compared with Moloney sarcoma virus (MSV)-induced rhabdomyosarcoma of mice. By light microscopy, electron microscopy, and immunofluorescence for myosin the tumors were similar. In man the ultrastructure is distinctive enough to make electron microscopy an important technic in the diagnosis of this tumor. Virus particles typical of MSV and murine leukemia viruses were seen in the 12-day-old mouse tumor. Viral particles were not found in the human tumor. Particles measuring 110 to 120 μm in diameter were observed in one specimen of human tumor. We believe that these are electron-dense fragments of endoplasmic reticulum, not virus.

INTRODUCTION

Recently a murine sarcoma virus (MSV) has been identified. It induces a tumor in mice (7, 11) which upon light microscopy examination resembles the embryonal rhabdomyosarcoma of childhood (4, 12). Electron microscopic studies of this tumor showed virus particles budding into the endoplasmic cisternae and from cell membranes (12).

In this study we examined the ultrastructure of childhood embryonal rhabdomyosarcoma and compared it with that of the MSV tumor in order to determine: (a) whether the childhood and mouse sarcomas are similar by ultrastructural criteria; (b) whether ultrastructural details of the childhood sarcoma can be used in defining its cytology because this tumor is frequently difficult to diagnose by light microscopy; (c) whether the childhood sarcoma demonstrates evidence of virus formation similar to that found in the mouse sarcoma.

In addition, we utilized antimyosin immunofluorescence (8, 9) to determine whether the childhood and mouse sarcomas both contained myosin, which would be further evidence of their similarity.

MATERIALS AND METHODS

MSV-induced Rhabdomyosarcoma. Ten percent (w/v) extracts of MSV-induced rhabdomyosarcoma (11) were prepared by homogenizing the tumor in phosphate-buffered saline (pH 7.2). The homogenate was centrifuged at low speed (2,000 × g/60 min) and the supernatant was inoculated into the thigh of newborn BALB/c mice (0.1 ml per mouse). After 12 days the animals were sacrificed and the tumors were examined.

Childhood Embryonal Rhabdomyosarcoma. Eight biopsy specimens and one necropsy sample from six children with rhabdomyosarcoma were examined. The children were aged 4, 4, 6½, 10, 12, and 16 years respectively at the time of diagnosis. The primary tumors arose in the orbit in two patients, on the face in two, in the gluteal region in one, and on the hand in one.

Electron Microscopy. For electron microscopy, the tissue was fixed in glutaraldehyde, postfixed in osmium tetroxide, dehydrated in ethyl alcohol, and embedded in Maraglas according to standard methods. Sections were cut on an LKB III ultramicrotome, double stained with uranyl acetate (6) and lead citrate (14), and examined in a Seimens Elmiskop I.

Indirect Immunofluorescence for Myosin. The indirect immunofluorescence test as described by Johnson et al. (9) and Hiramoto et al. (8) was used to detect the presence of myosin. This consists of adding rabbit antimyosin globulin or serum to frozen sections of tumor followed by fluorescein isothiocyanate..labeled goat anti-rabbit globulin globulin (FITC-GRGG). Frozen sections of kidney, liver, and skeletal muscles were used as controls.

RESULTS

Light Microscopy. The histology of the childhood rhabdomyosarcoma was in keeping with Stout's description (15) and was characterized by pleomorphism with many small, round and spindle cells and occasional elongated cells and giant cells. The mouse tumor was similar to the human rhabdomyosarcoma, with elongated strap and tadpole cells (12). Many cells in the mouse stained acidophilic and there were occasional giant cells. Cross striations were not noted in the mouse tumor, and in contrast to the human sections many neutrophils were seen.

Electron Microscopy. The ultrastructure of the human tumors revealed considerable pleomorphism. Most frequent were small and medium-sized cells, round to oval in shape, and measuring approximately 10-20 μm in diameter (Figs. 1, 3). There were also elongated and tadpole cells (Fig. 7) measuring approximately 10 × 30–40 μm and occasional giant cells. Most
of these cells were characterized by an irregular, eccentrically-placed nucleus, by an abundant cytoplasm containing filaments, and by large numbers of mitochondria (Figs. 1, 3, 5, 7). The nuclei were highly irregular, reflected the shape of the cell, and had a thin layer of chromatin lining their membranes. Nucleoli were frequently present and were often large and honeycombed.

The cytoplasm of the commonly seen round to oval cells was abundant, containing numerous vacuoles and many mitochondria and filaments. There were occasional small, round cells with relatively large nuclei as well, and only a thin rim of cytoplasm which contained abundant ribosomes, a moderate number of mitochondria, and a few scattered filaments. Occasionally the Golgi apparatus appeared moderately well developed in these cells. The cytoplasm of the tadpole-shaped cells appeared much the same as in the commonly seen round to oval cells. The cytoplasm of the giant cells was very extensive, containing large numbers of mitochondria. The mitochondria were quite variable. At times they were small and darkly stained, whereas at other times they were large and round or tubular shaped. Sometimes they lacked cristae and appeared empty and at times they were peripherally displaced. In close proximity to the mitochondria were bundles of filaments (Figs. 3, 5). The specimen from one patient revealed thick (150 Å) and thin (60 Å) filaments (Figs. 5, 6).

Scattered among the filament bundles were electron-dense regions suggesting primitive Z band formation. Specimens from the other patients revealed cells of all three types similar to the above, containing bundles of filaments but without definite thick and thin filaments.

The necropsy specimen was obtained from a tumor which had invaded the brain. In some cells numerous cytoplasmic particles measuring 110 mµ in diameter surrounded by a limiting membrane were seen. On these membranes 14 to 15 evenly spaced, dense, round structures resembling ribosomes were observed (Fig. 8). These particles appeared to be fragments of rough endoplasmic reticulum containing an electron-dense core and when seen in cross section there was some superficial resemblance to virus particles. However, longitudinal sections of these fragments were also seen (Fig. 8).

Electron microscopic examination of the virus-induced mouse rhabdomyosarcoma in BALB/c mice revealed numerous virus particles indistinguishable from the viruses of the murine leukemias (Fig. 9). These particles were approximately 100 mµ in diameter with nucleoids of 50–60 mµ in diameter. They were found both at the plasma membrane and in the cytoplasmic cisternae. Occasional membrane budding was observed principally at the plasma membrane.

In general, the ultrastructure of the mouse tumors we examined was in keeping with that described by Dalton (4). There were small, round to oval, and elongated cells present (Fig. 10). Giant cells were also seen. All cell types contained an eccentric nucleus, which generally reflected the shape of the cell, and abundant cytoplasm containing numerous fibrils and mitochondria. However, the cytoplasm of the smaller oval or elongated cells contained more rough endoplasmic reticulum and fewer filaments than the giant cells. In contrast to Dalton's observations in the mouse rhabdomyosarcoma, no definite periodicity in the filament bundles could be discerned, although we did see many bundles of filaments.

The ultrastructure of the mouse rhabdomyosarcoma was similar to that of the human rhabdomyosarcoma. However, it was found that the nuclei of the mouse tumor cells generally were not as irregular as those seen in the human cells and that their cytoplasm contained more rough endoplasmic reticulum. The giant cells of the mouse tumor were similar to those in the human and it was here again that the filaments were most prominent and were associated with many mitochondria (Fig. 11). However, in the mouse rhabdomyosarcoma cells, neither thick and thin filaments, nor Z bands, were seen. Abundant neutrophils were scattered throughout the mouse tumor.

Immunofluorescence. The immunofluorescence test for myosin was performed on specimens from four patients and was positive in all four cases (Fig. 12). There were scattered cells which demonstrated a bright green cytoplasmic fluorescence. In the mouse tumor, specific immunofluorescence confined to the cytoplasm was also present (Fig. 14). In both human and mouse material the immunofluorescent cells were often elongated or giant cells. Liver and spleen controls were negative, whereas skeletal muscle controls were positive (Fig. 13) with bright green cytoplasmic fluorescence.

DISCUSSION

The diagnosis of childhood embryonal rhabdomyosarcoma can often be difficult to establish on light microscopy. Indeed, Porterfield and Zimmerman (13) found convincing cross striations in only 60% of these tumors. We found no cross striations in repeat specimens from our six patients. Immunofluorescence to detect myosin is of value in the diagnosis (9).

The ultrastructure of both embryonal and differentiated rhabdomyosarcoma has been described (1, 3, 5, 10). In the differentiated type, diagnostic actin (thin) and myosin (thick) filaments were obvious, thus confirming the diagnosis. However, it is the embryonal, not the differentiated, type that poses the diagnostic problem with light microscopy. This problem again arises with electron microscopy because thick and thin filaments and Z bands are not commonly seen in the embryonal rhabdomyosarcoma.

Nonetheless, the ultrastructure of the embryonal rhabdomyosarcoma in man reveals several distinctive features. First, there are two cells which typify this tumor, the elongated or tadpole cell, and the giant cell. Both have irregular, eccentrically placed nuclei with a thin layer of chromatin lining the nuclear membrane. Second, in the cytoplasm, particularly of the giant cells, there are large numbers of thin filaments which tend to form bands. The filaments are associated with numerous mitochondria. Only rarely were thick and thin filaments and primitive Z bands discernible, but when present they confirmed the fact that these were indeed myofilaments, and thus established the diagnosis. These elongated and giant cells containing bands of filaments, even without diagnostic thick and thin filaments or Z bands, are probably pathognomonic for this tumor.

Immunofluorescence for myosin in all the human rhabdomyo-
sarcomas examined was positive. This is direct evidence that these cells were producing myosin and circumstantial evidence that the filaments are indeed myofilaments. Therefore, we believe that the ultrastructural analysis of childhood rhabdomyosarcoma is of definite value in establishing this diagnosis.

Interestingly, both the tadpole and giant cells contained nuclei which were displaced to one side, and the extensive cytoplasm probably contained myofilaments. This may indicate an abortive attempt by the malignant rhabdomyoblasts to form muscle cells. The earliest cell appears to be a small, round cell. This cell contains a centrally placed nucleus and a relatively small amount of cytoplasm with only a few mitochondria. Cells intermediate in size and ultrastructure between the small, round cell and the tadpole and giant cells were seen. This leads us to suspect that the small, round cell may give rise to both the tadpole or elongated cell, and the giant cell.

It was apparent from the ultrastructure and immunofluorescence for myosin that the tumors in both mouse and man were rhabdomyosarcomas. In the mouse, however, we saw numerous viral particles typical of the murine leukemia and sarcoma viruses at the cell surface and in the cisternae. We did not see these particles in the human tumor, but this does not rule out the possibility of a viral etiology. Although one human necropsy specimen revealed particles the size of viruses, it is probable that these are fragments of rough endoplasmic reticulum since elongated fragments were also seen.

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REFERENCES

Fig. 7. Human rhabdomyosarcoma cell. This “tadpole” cell has an elongated, irregular, eccentric nucleus lined by a thin layer of chromatin. The cytoplasm contains numerous ribosomes. This cell appears more primitive than the cell in Fig. 1. × 16,500.

Fig. 8. Human rhabdomyosarcoma cell. Particles measuring 110 m in diameter with an electron-dense core are seen (arrow). These particles appear to be fragments of rough endoplasmic reticulum. They are occasionally seen in longitudinal section (arrows). × 97,200.

Fig. 9. Mouse rhabdomyosarcoma cell. Virus particles measuring 100 m are seen at the plasma membranes. These particles are indistinguishable from the particles of murine leukemia. × 92,000.

Fig. 10. Mouse rhabdomyosarcoma cell. The cell has an eccentrically placed nucleus. Most likely there is only one highly irregular nucleus which has been cut at a tangential section. The cytoplasm is abundant, with many ribosomes, a moderate number of mitochondria, and some rough endoplasmic reticulum. There is similarity to the human rhabdomyosarcoma cells in Figs. 1, 7. × 14,000.

Fig. 11. Mouse rhabdomyosarcoma cell. Numerous filaments are seen closely related to mitochondria. However, no definite Z bands or myofilaments are seen. Notice the similarity to human rhabdomyoblast (Figs. 2, 4). × 25,200.

Fig. 12. Section of human rhabdomyosarcoma stained for myosin immunofluorescence showing cytoplasmic fluorescence in elongated tumor cells. × 250.

Fig. 13. Section of human skeletal muscle, stained for myosin immunofluorescence, demonstrating cytoplasmic fluorescence in cells of a muscle fascicle. × 150.

Fig. 14. Mouse rhabdomyosarcoma cell stained for myosin, showing immunofluorescence confined to the cytoplasm. × 600.
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