Morphological Studies on Transmissible Feline Fibrosarcoma

Stanley P. Snyder, Gordon H. Theilen, and W. P. C. Richards

SUMMARY

Inoculations s.c. of cell-free Millipore filtrates of the S-T feline sarcoma virus resulted in the rapid induction of fibrosarcomas in kittens. Metastatic tumors, seen in a large percentage of the inoculated kittens, were of two distinct types. The first was a solid tumor, similar to the tumors located in the subcutaneous tissue. The second type, seen primarily in the liver and brain, was formed by numerous, cystic, blood-filled cavities surrounded by a thin shell of neoplastic cells. Ultrastructural studies confirmed light microscopic findings of three cell types in all solid tumors: fibroblasts, macrophage-like cells, and mast cells. C-type viral particles seen in tumor tissue of the original source cat with spontaneous fibrosarcomas from all inoculated experimental kittens were uniform in morphology and apparent mode of production and were similar to those of feline leukemia. The findings help to substantiate the viral etiology of this feline fibrosarcoma.

INTRODUCTION

Lymphosarcoma is one of the most common spontaneous neoplasms of cats (31). Recently, the association of this neoplasm with a virus, which is evidently causative, has been shown by several groups of research workers (16, 19, 28, 35) whose findings have stimulated wider interest in feline tumors.

Spontaneously occurring feline fibrosarcomas are found much less commonly than lymphosarcomas and occur mainly in adult or aged animals (23). They are usually found as solitary masses and often recur following surgical excision (15). Due to infrequency of their occurrence and to the lack of particular interest in them, little morphological or biological data are available for review. The paucity of knowledge concerning spontaneous fibrosarcomas in cats is reflected in surveys of feline neoplasms (7, 25, 31).

Kasza et al. (17) demonstrated the presence of a cytopathogenic agent from an unclassified cat sarcoma in an established canine melanoma cell line. They suggested that the virus was similar to a myxovirus. Fairly large doses of tissue culture fluid were ineffective in inducing tumors in kittens within the experimental period of 5 months following inoculation.

Recently, we reported the first successful transmission of fibrosarcomas in cats (32). Puppies and rabbits also developed fibrosarcomas following s.c. inoculation of either cellular homogenates or cell-free Millipore filtrates of the feline fibrosarcoma material. Associated with the original and induced tumors are large numbers of C-type viral particles which were indistinguishable morphologically from feline leukemia virus (18). The relationship of the S-T feline sarcoma virus to the K-T feline leukemia virus is being investigated, and it may prove similar to the relationships of the avian sarcoma-leukosis (13) and the murine sarcoma-leukemia systems (9). The present communication is an account of the morphological characteristics of experimentally induced sarcomas in cats.

MATERIALS AND METHODS

Experimental Animals. In this experiment, 4 litters of domestic shorthaired kittens were used, 1 for each of 4 successive serial passages. Adult females were fed commercial cat food supplemented with pasteurized bovine milk, and their kittens were inoculated s.c. in the dorsal thoracic region when they were 1 to 6 days old.

Inoculum. Tumor tissue which was frozen at −70° from 2 weeks to 6 months was used as the source of inoculum. The tumor tissue was collected aseptically from kittens under halothane anesthesia or after barbiturate euthanasia and exsanguination. Samples of the frozen tumors were thawed in a 25° water bath, minced with scissors, and ground in TenBroeck tissue grinders. Sterile 0.9% NaCl solution was added to give a final dilution of 1:1 tissue to 0.9% NaCl solution (w/v). The homogenate was then centrifuged in a refrigerated centrifuge at 2000 rpm for 15 min. The supernatant fluid was removed and passed through 450-mm Millipore filters. The filters were washed and found to be impervious to a suspension of Escherichia coli. Additional 0.9% NaCl solution was added to restore the filtrate to the volume of the original homogenate. One-ml aliquots of the filtrate were inoculated into each kitten.

Light Microscopy. For histopathology, tissues were fixed in either formalin or Zenker’s fluid and embedded in paraffin. Sections were cut at 4 or 6 μ and routinely stained with...
hematoxylin and eosin. Phosphotungstic acid-hematoxylin, Mallory's trichrome, Gordon and Sweet's reticulum, Alcian blue-periodic acid-Schiff, and Giemsa stains were also used. Impression smears of tumors were routinely stained with Giemsa stain.

Electron Microscopy. Tumor tissue was sliced into 1-mm cubes, fixed in 1.5% glutaraldehyde in 0.06 M phosphate buffer (300 to 310 milliosmols) for 3 hr (30), washed several times with phosphate buffer, postfixed in 1% OsO$_4$ in phosphate buffer, pH 7.4 (21), and embedded in Epon 812 (20). Ultrathin sections on grids were stained with uranyl acetate (36) and lead citrate (27). Specimens were examined with a Philips EM 200 electron microscope at 80 kV.

RESULTS

Transmission Studies. Results of serial transmissions in 4 litters of kittens are summarized in Table I.

Gross Pathology. Tumors in all kittens arose as nonencapsulated, multiple or multinodular, firm, white to pink nodules in the subcutaneous tissue (Fig. 1). Many tumors were firmly anchored to the dermis; others were freely movable in the subcutaneous tissue. Some tumor masses contained red focal areas of hemorrhage or brown areas of necrosis. The first tumor nodules arose at the site of s.c. inoculation; but within 10 days, many smaller ones could be palpated surrounding the original mass and often at a considerable distance from it. Gross evidence of invasion of surrounding soft tissues was often readily appreciated at the time of biopsy or necropsy. In some kittens, the combined weight of the tumors comprised more than 10% of the animal's body weight. Metastatic tumors were widely distributed in the various organs and were found in skeletal muscle, liver, central nervous system, eyes, tongue, omentum, spleen, pleura, kidneys, tonsils, heart, and lymph nodes. The metastases were of 2 basic types. The most commonly encountered type was a solid tumor nodule similar to those found in the subcutis. These occurred in the skeletal musculature of all kittens with metastases, and were also occasionally found in internal organs. The 2nd type was characterized by soft to firm red foci from which fresh or clotted blood could be readily expressed. These lesions were most frequently seen in the 3rd and 4th serial passages. They ranged from a few small red blebs in liver, brain parenchyma, or meninges to patterns in which the organ was riddled with numerous cystic, blood-filled cavities (Figs. 2 and 3).

Histopathology. All tumors produced were fibrosarcomas (33) with some individual variation. The majority of the cells were fusiform with abundant eosinophilic cytoplasm and round, oval, or cigar-shaped nuclei containing clumps of chromatin material. The basic histological pattern was produced by bundles of parallel cells traversing the tumor tissue in various planes (Fig. 4). Cells in mitosis were numerous and often displayed abnormal mitotic spindles. Hemorrhage and necrosis were a common finding, especially in the more rapidly enlarging tumors (Fig. 5). Some tumors contained no giant cells; others had numerous giant cells with single large or multiple smaller centrally clustered nuclei.

Metastatic tumors were either solid (Fig. 6) or cavernous. The solid metastases had structures like that described above. The metastases with cavities, found most often in the liver and central nervous system, consisted of free or clotted blood surrounded by a thin layer of neoplastic cells (Fig. 7). Histologically, these tumors were similar to hemangiosarcomas. The amount of collagen in the tumors was variable but generally sparse. The neoplasms were well vascularized.

Table 1

<table>
<thead>
<tr>
<th>Passage</th>
<th>Animal No.</th>
<th>Sex</th>
<th>Age when inoculated (days)</th>
<th>Latent period (days)$^a$</th>
<th>Age when killed (days)</th>
<th>Comment</th>
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<td>1</td>
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<td>20</td>
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<td>FS-51</td>
<td>F</td>
<td>C</td>
<td>34</td>
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$^a$Latent period refers to time after inoculation to 1st palpable evidence of tumor formation.

$^b$Histological evidence of early tumor formation at inoculation site.

$^c$Uninoculated contact controls.
The solid tumor masses were characterized by 3 basic cell types: fibroblasts, macrophage-like cells, and mast cells. The macrophage-like cells were pleomorphic neoplastic cells which grade into the fusiform fibroblastic cells and their characteristics were seen best in impression smears and electron micrographs. In impression smears, the macrophage-like cells had vacuolated or foamy cytoplasm, round or oval nuclei, and often contained phagocytized debris (Fig. 8). The mast cells, evidently nonneoplastic, were most numerous along small blood vessels, but were also scattered throughout the neoplastic tissue (Fig. 9). Impression smears made from the cut surface of tumors revealed that the fibroblasts were the most numerous; macrophage-like cells and mast cells were respectively fewer. We were unable to determine if these 3 cell types also formed the cystic tumors, since these lesions were very difficult to examine.

Lymphocytes were present in variable numbers at the periphery of tumor masses from animals that had palpable evidence of spontaneous tumor regression. In tumors of 1 kitten (FS-8), lymphocytes were found infiltrating the entire neoplasms. Neutrophils were often seen in or near necrotic foci in tumors.

**Electron Microscopy.** During the earlier stages of development the tumors tended to have more intercellular space than in later stages, when they were characterized by increased compactness and collagen fibrils in small packets between cells. The amount of collagen increased with the age of the tumors, but it was never extensive.

The three cell types seen with light microscopy were noted in electron micrographs. When sectioned longitudinally, fibroblasts were readily recognized by their elongated nuclei with infoldings of the nuclear membranes (Fig. 10). Their nuclear chromatin was coarsely granular and was distributed homogenously with isolated areas of heterochromatin. Commonly, several nucleoli were seen per fibroblast nucleus, with well-developed nucleolonema surrounding pools of pars amorphaa. As in normal fibroblasts (5), rough-surface endoplasmic reticulum was organized and mitochondria and the Golgi apparatus had no special features. Ribosomes and polysomes were numerous. Small bundles of fine filaments were sometimes seen within the cytoplasm, and tended to be peripherally located in the cell.

The macrophage-like cells had large round, oval, or indented nuclei with coarsely granular nuclear chromatin and usually a single nucleolus. Some clumping and margination of chromatin was commonly found. The Golgi apparatus and mitochondria were poorly developed. Small amounts of organized ergastoplasm were seen but ribosomes were scattered randomly through the ground substance. Characteristic features of these cells were their numerous cytoplasmic processes and dilated cytoplasmic vacuoles (Fig. 11). Lysosomes and myelin figures were commonly seen in these cells. The cells frequently contained phagocytized erythrocytes and cellular debris in areas of hemorrhage and necrosis. An occasional cell contained lipid droplets.

Typical mast cells were scattered throughout all solid tumors; their cytoplasm was packed with electron-dense granules (Fig. 12). Their round or indented nuclei were smaller than those of the other cells and usually contained a single nucleolus (Fig. 13).

Virus particles were not found in either the subcutaneous tissues or Achilles tendons (examined as a different site of fibrous tissue) of the littermates that served as uninoculated contact controls. Typical C-type virions, with outer diameter of approximately 115 m$, were found budding from cytoplasmic membranes and into membrane-bound vacuoles within the cytoplasm of both the fibroblasts and the macrophage-like cells in tumors of all inoculated kittens. Budding and immature [or enveloped A (9)] forms had inner concentric shells of about 90 and 65 m$. Mature virions contained electron-dense nucleoids measuring approximately 90 m$ in diameter (Fig. 17). Budding and immature particles were found much more frequently than were the mature forms. Intracisternal virus particles were also occasionally observed. C-type virions were found in all stages of tumor development and were also seen in tumors undergoing spontaneous regression.

**DISCUSSION**

Many similarities exist between this transmissible cat sarcoma and those of the avian and murine species. The sarcomas in all 3 species are rapidly induced, have many morphological similarities, sometimes regress spontaneously, and are associated with C-type virus.

Examination of kittens with induced fibrosarcomas revealed that tumors were invariably multiple and metastases were frequent. Metastatic tumors were either solid or cystic, closely resembling those described for both avian (10, 29) and murine (14) sarcomas.

Histologically, the subcutaneous neoplasms and the solid type metastatic tumors were typical fibrosarcomas (33). Their basic pattern was formed by interwoven bundles of parallel cells. The tumors were well vascularized and frequently showed invasion of surrounding soft tissues. As with Rous sarcomas, 3 basic cell types were observed by light and electron microscopy (13). Fibroblasts were the most numerous cells and were morphologically similar to normal fibroblasts (5, 24), except for the presence of replicating C-type virus particles. Additionally, tumor tissue contained a relative lack of collagen in contrast to normal fibrous connective tissue. The macrophage-like cells were also quite numerous. These cells, because of their tendency to form cytoplasmic pseudopodia, their paucity of organized endoplasmic reticulum, and their ability to phagocytize extravasated erythrocytes, were considered to be less differentiated than the fibroblastic cells. Other cells, morphologically intermediate between fibroblasts and macrophage-like cells, helped to substantiate further that the macrophage-like cells were undifferentiated neoplastic fibroblasts. Mast cells in the tumors were not thought to be neoplastic and were indistinguishable from normal mast cells. Although the role of the mast cells in the neoplastic process is not understood, it may be that they are involved in the formation of hyaluronic acid ground substance in a manner similar to that reported for Rous sarcomas (1).

Lymphocytes that were present in the few regressing tumors were often adjacent to tumor cells in various stages of degeneration and necrosis. The role of the lymphocytes in spontaneous tumor regression is also poorly understood and is being investigated.
The morphogenesis of the cystic blood-filled cavities seen in the liver, brain, and occasionally in other organs may be similar to that suggested by Duran-Reynals for avian sarcomas (10). He thought that the cavities may have resulted from the action of virus on endothelial cells. In our series, the tumors often closely resembled hemangiosarcomas and progression in size of the more discrete spherical cavities was obvious.

Measurements of the C-type virus particles seen in electron micrographs of sarcomas of the original source cat and of all experimental kittens show that these virions were morphologically indistinguishable from those associated with cat leukemia as reported by us (18, 34).

Additional pathogenesis studies involving continued serial transmission, different routes of inoculation, age effects, and species susceptibility are now being investigated in this and other laboratories. In addition, serological and tissue culture studies are in progress to test the hypothesis that a feline sarcomatous-leukemia system exists in a manner similar to the avian and murine systems.

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REFERENCES

Fig. 1. Experimental Kitten FS-31, 4 weeks postinoculation, showing a large multinodular tumor over the shoulders. X 0.4
Fig. 2. Brain of Experimental Kitten FS-32 showing a few cavernous metastases on the meningeal surface. X 1.8.
Fig. 3. Liver of Experimental Kitten FS-49 showing extensive replacement of hepatic parenchyma by dark, cystic structures. X 1.3.
Fig. 4. Subcutaneous tumor showing the basic fibrosarcomatous histological pattern. H & E, X 220.
Fig. 5. Subcutaneous tumor containing foci of hemorrhage and necrosis. H & E, X 220.
Fig. 6. Solid-type metastatic lesion in the iris and ciliary body of the eye. Lens is at left. H & E, X 80.
Fig. 7. Cavernous type of metastatic lesion in the liver. H & E, X 220.
Fig. 8. Impression smear of a tumor showing gradation from a foamy macrophage-like cell (left) to a fusiform fibroblastic cell (right). Giemsa, X 1500.
Fig. 9. Tumor containing several mast cells (arrows). Giemsa, X 550.
Fig. 10. Fibroblastic cell in a tumor. Adjacent tumor cells show extensive interdigitations of cell membranes. X 12,900.
Fig. 11. Macrophage-like cell in a tumor showing extensive cytoplasmic processes. X 12,900.
Fig. 12. Mast cell in a tumor. X 16,500.
Fig. 13. Tumor, showing relationship of a mast cell to the neoplastic cells. X 12,900.
Figs. 14 to 17. C-type viral particles, showing apparent mode of production and maturation in the sequence. X 80,500.
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