A Transmissible Feline Fibrosarcoma of Viral Origin


SUMMARY

Histological examination of an invasive neoplasm from an 18-month-old female domestic shorthair cat revealed it to be a pleomorphic fibrosarcoma that ranged in microscopic appearance from well-differentiated collagenous connective tissue to anaplastic sarcomatous tissue that infiltrated surrounding musculature and skin.

The fibrosarcoma was transmitted by whole cells or cell-free preparations to 8 to 10 neonatal kittens. The original neoplasm and the induced fibrosarcomas that were examined electron microscopically contained mature and budding C-type particles. Immunodiffusion studies with serum prepared in rabbits against purified ether-disrupted feline leukemia virus revealed identity between the feline leukemia virus group-specific antigen and extracts of the original fibrosarcoma.

INTRODUCTION

Previous reports indicated the cell-free transmission to kittens and puppies (1, 9) of feline fibrosarcomas containing C-type virus particles, which induced cell-free transformation in feline embryo fibroblasts.

The feline fibrosarcoma reported here, SM-FS, differed from those previously reported in that it exhibited a great deal of pleomorphism. The purpose of this study was to describe the histopathology of this tumor and to determine its transmissibility by cell-free preparations and its possible serological relationship to feline leukemia.

MATERIALS AND METHODS

A spontaneous tumor occurred s.c. in the right thigh of an 18-month-old, spayed, female domestic shorthair cat and was surgically excised. It recurred at the same site 6 weeks later, and by 12 weeks multiple, rapidly growing, s.c. tumors were present dorsal to the left shoulder and in the right flank. At postmortem examination 5 months after the initial excision, several tumor masses were harvested for study.

For electron microscopy, tissue was prepared by fixing in glutaraldehyde followed by osmic acid. After rapid dehydration in ethanol, it was stained en bloc in 5% uranyl acetate and embedded in Epon or Maraglas. Sections were stained with uranyl acetate and lead citrate and examined with a Siemens Elmiskop 1A electron microscope.

Several transmission experiments were conducted.

Experiment A. Two 3-day-old kittens (Kittens 1 and 2) received whole cell suspensions of fresh tissue prepared by mincing 1 g of SM-FS in 10 ml of warm 0.25% trypsin in PBS, mixing at room temperature for 1 hr, and allowing the large pieces of tissue to settle out. Each kitten was inoculated s.c. with 2 ml of this preparation/site.

Experiment B. A 3rd littermate (Kitten 3) received a cell-free suspension prepared by a modification of the Moloney technique (7). Fresh SM-FS was finely minced in 0.25% trypsin in PBS and sonicated twice for 20 sec each time (Setting 7, Crest Ultrasonic). The volume was doubled with distilled water, and the preparation was mixed at room temperature for 1 hr. After two 5-min centrifugations at 3,000 rpm (RC2B Sorvall centrifuge with SS34 rotor) and a 1-min centrifugation at 10,000 rpm, the decanted supernatant was spun at 35,000 rpm for 1 hr (Beckman 60 with A-170 rotor). The final pellet was resuspended in 2 ml of PBS (equivalent of 0.5 g/ml) and inoculated s.c. into a single kitten.

Experiment C. Each of three 1-day-old littermate kittens (Kittens 4, 5, and 6) was inoculated s.c. with 1 g equivalents of trypsin digests of SM-FS prepared from tumor tissue that had been stored at —70° for 118 days.

Experiment D. Four 1-day-old littermate kittens (Kittens 7, 8, 9, and 10) received filtrates of SM-FS prepared from tumors stored at —87° for 305 days. Tumor tissue (5 g) in 50 ml of BME with 10% fetal calf serum was homogenized in an Omnimix apparatus and centrifuged at 2000 rpm for 15 min, and the supernatant was filtered through a 0.25-μ filter (Nalge Co., Rochester, N. Y.). Three ml were injected s.c./site into the left flank and right axilla of each kitten. The 5th kitten in the litter (Kitten 11) was not inoculated and was housed as a contact control.

An extract from SM-FS was tested in the immunodiffusion (Ouchterlony) system with rabbit standard antiserum prepared against group-specific antigen of ether-disrupted FeLV (3). Purified ether-disrupted FeLV was used as standard antigen in adjacent wells. Plasma or serum from the experimental kittens was tested for antibody and antigen activity by diffusing against the standard antigen and antiserum, respectively.
RESULTS

Pathology and Histopathology of SM-FS. The cat was euthanatized 5 months after the original excision because of the invasive nature of the neoplasm.

At necropsy, firm grey-white nodules, 0.4 to 4.0 cm in diameter, occurred singly and in aggregations in the skin, the subcutis and adjacent musculature of the thorax, the lumbosacral region, and both thighs. The total mass of neoplastic tissue was approximately 300 g. Microscopically, the tumors were pleomorphic fibroblastic growths ranging from rather well-differentiated, quiescent-looking collagenous connective tissue, as shown in Fig. 1, to very cellular, anaplastic sarcomatous tissue containing numerous mitoses and infiltrating into surrounding structures (Fig. 2). Some areas contained large, bizarre, multinucleated giant cells, and central necrosis was frequent in the more rapidly growing areas. Microscopically, the invasive nature of the neoplasm.

Electron Microscopy of SM-FS. Numerous immature and mature C-type virus particles were observed extracellularly and within cytoplasmic vacuoles (Fig. 3). Budding virus was observed infrequently. The complete particle had an average external diameter of 90 to 100 μm. The nucleoid measured 60 to 70 μm in diameter. Many of the particles had a fringe visible on the outer envelope (Fig. 4). This may be an artifact, but similar C-type morphology has been reported by other investigators (6).

Transmission Experiments. Table 1 summarizes the results of the transmission experiments. All the inoculated kittens, except Kittens 7 and 10, developed fibrosarcomas.

Both the kittens receiving fresh tumor homogenates (Kittens 1 and 2) developed fibrosarcomas. By Day 11 after inoculation, Kitten 1 had s.c. tumors at both sites of inoculation, in the left flank and right axilla. On Day 12, 1 of the nodules was biopsied; by Day 22, both had completely regressed. Nodules reappeared at the same sites on Day 68. Kitten 2 developed a tumor in the right pinna on Day 30 and another dorsal to the right scapula on Day 56. By Day 60, other nodules had appeared in both axillae. Kitten 3 developed a tumor in the right axillary region 6 days after inoculation of a Moloney concentrate of the homogenate at that site. This kitten died of bronchopneumonia 3 days later.

Kittens 4, 5, 6, 8, and 9 developed tumors at the sites of inoculation after latent periods of 41, 51, 53, 51, and 52 days, respectively. Kitten 7 and 10 died 50 and 58 days after inoculation; neither these nor the control contact, Kitten 11, developed tumors.

Pathology and Electron Microscopy of Experimental Tumors. The tumors in the 8 kittens ranged from approximately 2 to 10 mm in greatest dimension. Histologically, they were less pleomorphic than the original tumor. They presented an appearance, shown in Figs. 5 and 6, that was intermediate between the extremes seen in SM-FS. No metastases were detected.

The results of electron microscopic examination of tumors from Kittens 1, 2, 8, and 9 are summarized in Table 1. A few mature and budding C-type virus particles were seen in the fibrosarcoma from Kitten 1. Fig. 7 shows immature and budding particles in the tumor from Kitten 9. No virus particles were observed in approximately 150 cell sections from the tumor in Kitten 2. Tumors in the other kittens were not examined electron microscopically.

Immunodiffusion Results. A precipitin line was formed between rabbit antiserum against the group-specific antigen of FeLV and the soluble tumor extracts from SM-FS. Lines of identity were formed between this soluble feline fibrosarcoma extract and purified ether-disrupted FeLV. After regression and after the reappearance of neoplasms, unconcentrated plasma from Kittens 7, 8, 9, 10, and 11, as well as serum from Kitten 1, was negative for FeLV group-specific antigen.

DISCUSSION

C-type particles occur in spontaneous feline mammary adenocarcinoma (2), spontaneous and transmitted feline lymphosarcoma (4, 6), fibrosarcoma (1, 9), and liposarcomas that developed in kittens inoculated with FeLV (8). Virus particles observed in the fibrosarcoma we report are morphologically similar to those seen in feline lymphosarcoma (5).

Table 1
Summary of in vivo experiments with homogenates and cell-free preparations of SM-FS

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Kitten</th>
<th>Inoculum</th>
<th>Tumor</th>
<th>Latent period (days)</th>
<th>EM a</th>
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<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>Fresh whole cells</td>
<td>+</td>
<td>11</td>
<td>+</td>
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<td></td>
<td>2</td>
<td></td>
<td>+</td>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>3</td>
<td>Moloney preparation</td>
<td>+</td>
<td>7</td>
<td>ND</td>
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<tr>
<td></td>
<td></td>
<td>of fresh SM-FS</td>
<td></td>
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<tr>
<td>C</td>
<td>4</td>
<td>Trypsin digest of frozen</td>
<td>+</td>
<td>41</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>SM-FS</td>
<td>+</td>
<td>51</td>
<td>ND</td>
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<td></td>
<td>6</td>
<td></td>
<td>+</td>
<td>53</td>
<td>ND</td>
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<td>D</td>
<td>7</td>
<td>Filtrate of frozen SM-FS</td>
<td>–</td>
<td>NA</td>
<td>ND</td>
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<td>8</td>
<td></td>
<td>+</td>
<td>51</td>
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<td></td>
<td>9</td>
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<td>52</td>
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<td>10</td>
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<td></td>
<td>11</td>
<td>None</td>
<td>–</td>
<td>NA</td>
<td>ND</td>
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</tbody>
</table>

a EM, electron microscopy; +, positive; –, negative; ND, not done; NA, not applicable.
Immunodiffusion experiments indicate that SM-FS contains the group-specific antigen of the FeLV. Immunodiffusion assays done on plasma or serum from the experimental kittens revealed no antibody to the group-specific antigen of FeLV. This most likely is due to tolerance of this antigen by the cat (3). One experimentally induced tumor (Kitten 9) was established in tissue culture, C-type virus was isolated, and FeLV group-specific antigen was detected in immunodiffusion tests of virus pellets.

Sarcoma viruses of the mouse and chicken require excess helper leukemia virus to be oncogenic. The feline sarcoma virus isolates are similar, in that the finding of FeLV group-specific antigen in tumor extracts probably reflects excess FeLV. One cannot be sure if the feline sarcoma virus contains the group-specific antigen of the FeLV until pure sarcoma virus is obtained. Also, one cannot be certain whether any of the observed C-type virus is actually a sarcoma virus particle or merely excess FeLV.

ACKNOWLEDGMENTS

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REFERENCES


Fig. 1. Portion of SM-FS showing rather well-differentiated collagenous connective tissue. H & E, X 145.
Fig. 2. Another area of SM-FS illustrating sarcomatous appearance with infiltration of skeletal musculature. H & E, X 145.
Fig. 3. Higher magnification of sarcomatous portion of SM-FS showing anaplastic features of cells. H & E, X 475.
Fig. 4. Mature and immature C-type virus particles within cytoplasmic vacuoles (from SM-FS). X 48,000. Inset, 2 particles with fringed appearance of outer envelope. X 96,000.
Fig. 5. Histological appearance of s.c. neoplasm induced with cell-free filtrate from SM-FS, Kitten 9. H & E, X 145.
Fig. 6. Electron micrograph of immature C-type particle from the tumor illustrated in Fig. 5. X 90,000.
Fig. 7. C-type particle budding from endothelial cell into lumen of small vessel (from the tumor illustrated in Fig. 5). X 90,000.
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