Naturally Occurring Bone Tumors in C57BL/Icrf Mice

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SUMMARY—Seven bone tumors were found in a colony of C57BL/Icrf mice. All occurred in females. Histologically these were osteosarcomas. Lymph node metastases were present in 1 mouse. Transplants from 2 primary tumors were bone forming. The ultrastructure of 1 of these is described.—J Natl Cancer Inst 50: 431–438, 1973.

SPONTANEOUS BONE tumors in mice are rare. Pybus and Miller (1, 2) described a subline of Simpson mice which had a high incidence of bone tumors, but descendants of these mice no longer develop bone tumors. Apart from this series, most reports have been of single tumors usually in C3H mice (3) or their hybrids (4). Dunn (personal communication) observed an osteogenic sarcoma with metastases in a 21-month-old female C3H mouse. Transplantation was carried through 125 generations and metastases to the lungs were frequent. One osteogenic sarcoma has been reported in a feral mouse (4) and one in an AKR mouse (5).

In the present paper, we report on the occurrence and histology of bone tumors in our colony of aging C57BL mice.

MATERIALS AND METHODS

The establishment of the C57BL/Icrf subline and its husbandry were described in (6, 7).

After formol fixation and decalcification in Versene, paraffin sections were prepared from bone tumors and stained with hematoxylin and eosin.

Tissues for electron microscopy were sliced and fixed for 4 hours in 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer at 4°C, rinsed overnight in 0.1M sodium cacodylate buffer at 4°C, and postfixed in Palade's fluid for 1 hour over ice. Tissue blocks were dehydrated in graded ethanol, stained with 5% uranyl acetate in absolute alcohol, and embedded in Araldite with epoxypropane as transitional solvent. Ultrathin sections were cut on a Sorvall MT2 ultramicrotome, stained with lead citrate (8), and viewed in a Hitachi HS7S or Siemens Elmiskop 1 electron microscope.

Radiographs were made by 60-second exposure at 40 kV and 3 mA at a distance of 33 cm.

Two tumors were transplanted subcutaneously into syngeneic mice, with the use of a modified Bashford needle. Chopped fragments of some tumors were suspended in 10% dimethyl sulfoxide and stored in liquid nitrogen.

RESULTS

All tumors were classified as osteogenic sarcomas but varied in the amount and type of bony and cellular elements. These are described in more detail for each tumor which is then classified on this basis. They are summarized in table 1. In 4,381 autopsies on C57BL/Icrf mice from birth to 38 months of age, 7 bone tumors were found. Only 3 of these were diagnosed during life. The age distribution at autopsy is given in table 2.

Tumor #1 (St. 1219/66)

This tumor was found in a mouse killed at 27 months because of a swelling above the right eye. On dissection there was a bony projection 0.4 cm in diameter above the orbit. It extended into the cranial cavity, compressing the right cerebral hemisphere. Sections showed an osteoblastic osteosarcoma with osteoid and bone formation (figs. 1, 2).

Tumor #2 (St. 402/67)

The mouse was killed at 31 months because of a mass in the left inguinal region. The animal was

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2 We are grateful to Dr. C. H. G. Price and Dr. P. Kossey for their comments, to Mr. G. D. Leach for the photographs, to Mr. E. B. Brain for the X-rays, and to Mr. M. U. Sheriff for excellent technical assistance.
TABLE 1.—Bone tumors in female C57BL/1crf mice

<table>
<thead>
<tr>
<th>Tumor ref. No.</th>
<th>Age (months)</th>
<th>Site</th>
<th>Histology</th>
<th>Transplantation*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. St 1219/66</td>
<td>27</td>
<td>Orbit</td>
<td>Osteoblastic osteosarcoma</td>
<td>0</td>
</tr>
<tr>
<td>2. St 402/67</td>
<td>31</td>
<td>Left femur</td>
<td>Osteogenic sarcoma; lymph node metastases.</td>
<td>0</td>
</tr>
<tr>
<td>3. AE 165</td>
<td>12</td>
<td>Right femur</td>
<td>Osteogenic sarcoma with bone and cartilage.</td>
<td>+</td>
</tr>
<tr>
<td>4. AE 413</td>
<td>20</td>
<td>Left femur</td>
<td>Osteogenic osteosarcoma</td>
<td>+</td>
</tr>
<tr>
<td>5. St 20/69</td>
<td>25</td>
<td>Sacrum</td>
<td>Pleomorphic osteosarcoma</td>
<td>0</td>
</tr>
<tr>
<td>6. St 754/64</td>
<td>29</td>
<td>Sacrum</td>
<td>Osteoblastic osteosarcoma</td>
<td>0</td>
</tr>
<tr>
<td>7. St 13/72</td>
<td>30</td>
<td>Left orbit</td>
<td>Osteoblastic osteosarcoma</td>
<td>0</td>
</tr>
</tbody>
</table>

*0 = Not attempted; + = successful transplant.

Table 2.—Age distribution of C57BL/1crf mice at autopsy

<table>
<thead>
<tr>
<th>Age range (months)</th>
<th>Percent of males</th>
<th>Percent of females</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-5</td>
<td>2.6</td>
<td>1.6</td>
</tr>
<tr>
<td>6-11</td>
<td>27.7</td>
<td>15.4</td>
</tr>
<tr>
<td>12-17</td>
<td>6.1</td>
<td>7.8</td>
</tr>
<tr>
<td>18-23</td>
<td>10.9</td>
<td>10.8</td>
</tr>
<tr>
<td>Over 24</td>
<td>52.7</td>
<td>64.4</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Total No</td>
<td>2674</td>
<td>1707</td>
</tr>
</tbody>
</table>

weak and emaciated, with paralyzed hind quarters and no pedal reflex. On dissection the mass was attached to the left femur and spread directly into the lower abdominal cavity. There were metastases above the uterus but not directly involving it. Sections showed an osteogenic sarcoma, with much osteoid in some areas, and some uninucleate and multinucleate giant cells. There were similar giant cell areas in regional lymph node deposits.

Tumor #3 (AE 165)

The mouse was killed at 12 months of age. The tumor, 0.5 cm in diameter, in the right thigh, was hard and knobby with gritty, hard, or cartilaginous areas. An X-ray taken after death of the animal showed a bone-forming tumor with radiating bony spicules (fig. 3). Sections indicated an osteosarcoma (fig. 4) with newly formed bone, osteoid, and cartilage (fig. 5), some of which had areas of calcification. There were some cysts containing amorphous material. This tumor was carried through 14 transplant generations. In some areas of these transplants, the histology was similar to the primary tumor, but usually the tumors contained spindle cells predominantly. The ultrastructure is described below.

Tumor #4 (AE 413)

This tumor was in a mouse killed at 20 months because of a swelling approximately 1.5 cm in diameter on the left foreleg. Histology showed a well-differentiated osteogenic osteosarcoma with numerous mitoses. Transplants had a similar histology with extensive bone formation (figs. 6, 7). This tumor is now in its tenth generation transplant and has massive bone formation.

Tumor #5 (St. 20/69)

This mouse was killed at 25 months of age because of a 2.4×1.8 cm swelling on the back, which extended into the lower abdominal cavity. Histology showed a pleomorphic osteosarcoma (fig. 8) with some collagen, osteoid, and bone formation.

Tumor #6 (St. 754/64)

This tumor was found in a mouse which died at 29 months of age. Autopsy showed that the descending colon was obstructed by a hard mass in the left sacral region. Histology indicated an osteoblastic osteosarcoma with much bone formation.

Tumor #7 (St. 13/72)

Tumor #7 was in a mouse killed when 30 months old. The animal had a swelling about 0.4 cm in diameter above the left orbit (fig. 9). Histology showed an osteogenic sarcoma with many spindle cells.
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Electron Microscopy

An electron microscopic study was made of the first transplant generation of tumor #3. The tumor consisted of single cells and small cell masses surrounded by randomly arranged collagen fibers lying in a fine fibrillar matrix. In some areas the collagen was arranged in more definite parallel bundles. There were small electron-dense areas on and between some cells (figs. 10, 13, 15) probably representing early calcification.

There were 2 main cell types. The predominant cell was spindle shaped and electron dense and resembled the normal osteoblast (fig. 10). The nucleus was convoluted with a thick peripheral chromatin zone and a single prominent nucleolus. The cytoplasm was packed with many fine filaments (fig. 11) often arranged in parallel bands, both around the nucleus and at the periphery of the cell. Rough endoplasmic reticulum (RER) and ribosome clusters were present. The Golgi zone was not prominent. These cells had many long, thin processes projecting into the surrounding collagen for a considerable distance, sometimes making contact with similar processes of neighboring cells.

The other cell type (fig. 12) was similar in basic structure but much paler and contained fewer cytoplasmic filaments. The RER was arranged in parallel stacks. These cells had only a few short blunt processes and resembled tissue fibroblasts.

Occasional uninucleate and multinucleate giant cells lay in the collagenous matrix (fig. 13). These cells resembled osteoclasts but did not have ruffled membranes. Some contained large and small electron-dense granules and laminated pleomorphic electron-dense inclusions (fig. 14). The tumor pattern was similar to that described by Ghadially and Mehta (9) and Kay (10) in human osteosarcomas.

Discussion

Bone tumors are rare because they usually occur in elderly mice. Only one of the tumors described here was found in a young adult (#3, table 1). This was the only one with cartilage formation. In another study of a series of 113 C57BL/ICRF mice between the ages of 6 and 30 months, no bone tumors were found in whole-body radiographs (Rowlatt and Chesterman, unpublished observations). The age distribution differs from that seen in man where there are 2 peaks, one in adolescence and the other in old age usually associated with Paget’s disease of bone which was not seen in our mice. Unlike human osteosarcomas which are more common in males, all our mouse tumors occurred in females, even though the proportion of males to females in the colony was about 2:1. This agrees with other reports (11).

Unlike bone tumors in man, blood-borne metastases were not seen in our mice, though in one case, metastases were in the regional lymph nodes. The histology and ultrastructure of the tumors were similar to those in man. Tumors detected in our mice did not resemble histologically those induced by FBJ virus (12, 13), or most of those induced by polyoma virus after perinatal injection (14, 15), or in adult immunosuppressed mice (16, 17) or those induced by X-rays and bone-seeking isotopes (18).

REFERENCES

(2) ———: The gross pathology of spontaneous bone tumors in mice. Am J Cancer 40:47-53, 1940
(4) Dunn TB, Andervont HB: Histology of some neoplasms and non-neoplastic lesions found in wild mice maintained under laboratory conditions. J Natl Cancer Inst 31:873-885, 1963


All sections stained with hematoxylin and eosin (figs. 1–9). T refers to the tumor Ref. No. as given in table 1. Figures 10–15 are from the first transplant of T3.

**Figure 1.**—T1. Orbital tumor. Bone appears black in section. X 10

**Figure 2.**—T1. Higher power showing bone formation. X 300

**Figure 3.**—T3. Radiograph. Bone forming tumor, right hind leg.

**Figure 4.**—T3. Section through tumor showing cysts. X 10

**Figure 5.**—T3. Cartilaginous areas from tumor. X 300

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Figure 6.—6th generation transplant from T4 showing bone formation (black). X 10. Figure 7.—Higher power of tumor in figure 6 showing anaplastic cells, giant cells, and osteoid, partly calcified (bottom left). X 300. Figure 8.—T5. Pleomorphic area of tumor. X 300. Figure 9.—T7. Orbital tumor invading muscle. X 10.
FIGURE 10.—Electron-dense osteoblast-like dark tumor cells with many processes. Dense area in stroma (arrow). X 5000

FIGURE 11.—Edge of dark cell showing cytoplasmic filaments. X 25,000

FIGURE 12.—Fibroblast-like tumor cells. X 25,000
FIGURE 13.—Giant cell with electron-dense inclusions (above) and dark cell processes, in collagen. Small dense areas in stroma (arrows). X 5000. FIGURE 14.—Laminated inclusion from giant cell in figure 13. X 25,000. FIGURE 15.—Electron-dense area in stroma at edge of osteoblast-like dark cell, probably early calcification. X 20,000