Virus-like Particles in Chemically Induced Sarcomas in High- and Low-Leukemia Strains of Mice

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SUMMARY

Well-differentiated fibrosarcomas and anaplastic sarcomas developed in the s.c. tissues of four low-leukemia strains of mice (BALB/cf/Ki, Af/Ki, C3H/Ki and C3Hf/Ki) as well as two high-leukemia strains (AKR/Ki and C58/Ki) following a single injection of benzpyrene (0.004 mg/g body weight) as young adults. The incidence of sarcomas was significantly less in both high-leukemia strains than that in all four low-leukemia strains. Abundant type C murine leukemia virus particles were found in primary and transplanted sarcomas of AKR and C58 origin as well as in normal tissue of either tumor-bearing or non-tumor-bearing mice. Type C virus particles were seen less frequently in primary and transplanted sarcomas arising in BALB/cf, C3Hf, and C3H mice. No virus-like particles were seen in either primary or transplanted sarcomas or normal tissues obtained from Af mice. Intracisternal type A virus particles were also seen in both primary and transplanted tumor tissue as well as normal tissue of AKR and C58 mice but not in the tumor or normal tissue of the other four strains. Type B mammary tumor virus particles were not seen in any sarcomas developing in any strain of mouse.

Cell-free extracts of Af transplanted sarcomas in which no virus-like particles could be seen by electron microscopy proved to have biological activity when injected into newborn mice of the BALB/cf/Ki, C3Hf/Ki, C57BL/Ki, and Af/Ki strains. This activity was characterized by an increased incidence of the tumors, which occur in low to moderate incidence in these strains. No sarcomas were seen in any of the injection-treated mice of any strain.

Cell-free extracts of transplanted AKR sarcomas with abundant type C virus particles had no oncogenic effects when injected into newborn BALB/cf mice. Whereas an extract of the 1st transplant generation of C58 sarcomas with abundant virus particles was negative when injected into newborn BALB/cf mice, an extract from the 8th transplant generation of sarcomas was associated with an increase in lung tumors in the same strain of mice.

These results indicate the complexity of the interactions between host genetic factors and the different murine oncopogenic viruses found in chemically induced sarcomas. A relationship between the virus-like particles found in these sarcomas and their etiology was not established.

INTRODUCTION

The role of viruses in the spontaneous development of reticular tissue neoplasms and mammary cancer in mice has been well documented in the literature. A viral agent has also been implicated in the development of chemically induced leukemia (18, 32) and X-ray-induced leukemia (13, 17, 22) in mice.

Recently, the isolation of a sarcomagenic virus was reported which rapidly induced s.c. sarcomas following injection into newborn mice (16, 25). Subcutaneous sarcomas are readily induced in adult mice with various chemical carcinogens. Chemically induced sarcomas (35) and certain transplanted mouse tumors (11, 24) have been shown to be the sources of biologically active leukemogenic viruses. Electron microscopic studies have revealed the presence of Type A virus particles in transplanted and chemically induced tumors in mice (4, 5, 20, 34) and transplanted avian sarcomas (14). However, MC3-induced pulmonary tumors in mice (30) did not contain virus-like structures. The major question focuses on the relationship between the presence of virus-like particles in chemically induced sarcomas and the etiology of these tumors. Recently, it has been suggested from electron microscopic studies of MC-induced sarcomas in germ-free mice and rats that the presence of type A and C virus particles in these tumors arising in mice but not rats represents "viral contamination in vivo" (19).

It was the purpose of the present experiments to examine by electron microscopy chemically induced s.c. sarcomas arising in 2 high-leukemia strains of mice (AKR and C58) and 4 low-leukemia strains (Af, BALB/cf, C3Hf, and C3H) to determine the influence of genetic factors on tumor morphology and the presence or absence of virus particles. In turn, cell-free extracts were prepared from selected tumors that were later classified by electron microscopy as positive or negative for the presence of virus-like particles to determine whether oncogenic activity could be demonstrated following injection into newborn mice.

MATERIALS AND METHODS

Mice. Approximately equal numbers of male and female mice of the low-leukemia strains Af, BALB/cf, and C3Hf, high-leukemia strains AKR and C58, and high-mammary-cancer strain C3H mice were used in this study. All mice were...
obtained from the Kirschbaum Memorial Laboratory, Baylor College of Medicine (29). Males and females were separated at weaning and fed Purine laboratory chow (St. Louis, Mo.) while maintained in plastic cages.

**Induction of Sarcomas.** Six- to 8-week-old mice of all 6 strains were given s.c. injections in the right axillary region with 0.1 ml of 0.07% BP in tricaprylin (0.004 mg/g body weight). This dose was originally selected to produce an incidence of sarcomas of approximately 33% in Af mice for the purpose of another study concerned with the 2-stage mechanisms of sarcoma induction. Twenty newborn AKR mice were given injections s.c. in the dorsal region with 0.05 ml of 0.5% MC in olive oil (23, 33). All mice were observed until either a s.c. tumor developed at the injection site or the mice became moribund because of the development of a tumor elsewhere or other reasons. Mice with s.c. tumors at the injection site were killed when the tumor reached a diameter of approximately 1 to 2 cm. Some primary tumors were transplanted into 6- to 8-week-old recipients of the same strain by the trocar method and carried in serial transplantation.

**Light and Electron Microscopy.** Tumor tissue arising at the injection site as well as other tissue from the tumor-bearing mice including spleen, lymph node, and thymus were used for light and electron microscopic examination. Subcutaneous- reactive tissue which appeared as a small nodule at the site of injection 4 to 5 weeks after BP injection was also taken from selected mice of all strains for electron microscopic study. Tissues for electron microscopy were fixed in 10% neutral, buffered formalin and selected mice of all strains for electron microscopic study. Tissues were fixed in 10% neutral, buffered formalin and stained with routine hematoxylin and eosin techniques for light microscopy. Tissues for electron microscopy were fixed in 1% phosphate-buffered osmic acid or in 4% glutaraldehyde in cacodylate buffer (28) followed by osmic acid fixation. Tissues were dehydrated in graded ethanol and embedded in Epon. Thin sections were mounted on uncoated grids and stained with saturated uranyl acetate followed by lead citrate (27). Electron micrographs were taken on an RCA-EM-3G microscope.

A total of 28 primary tumors and 22 transplanted tumors were examined by electron microscopy. Eight primary and 5 transplanted tumors (1st through 10th TG) were obtained from Af mice. Tissues were obtained from 5 primary and 3 transplanted tumors (1st through 3rd TG) of AKR mice. BALB/cf mice were the source of 5 primary and 4 transplanted tumors of the 1st TG. C3H mice managed to identify 5 primary tumors as compared to 4 primary tumors from C3H/f mice. Four transplanted tumors (1st and 4th TG) were obtained from C3H mice and 3 transplanted tumors (1st TG) from C3H/f mice. One primary and 3 transplanted tumors (1st, 2nd, and 6th TG) were obtained from C58 mice.

Approximately 10 small pieces of tissue were dissected from the peripheral nonnecrotic portion of each tumor. Eight to 10 blocks from each tumor were selected at random for thin sectioning. At least 5 grids mounted with 3 to 4 sections from each block (80 to 100 sections from each tumor) were examined under the electron microscope for the presence of virus particles.

**Bioassays of Cell-free Extracts of Primary and Transplanted Sarcomas.** Immediately after the animals were killed and portions taken for electron microscopy, the remainder of the selected tumors were dissected free and minced with scissors. Five volumes of chilled 0.9% NaCl solution were added to the minced tissues and homogenized with a glass homogenizer resulting in a 20% w/v suspension of homogenized tissue. The homogenate was then centrifuged in a Sorvall Model RC12 centrifuge, at 3000 rpm (1085 × g) for 15 min at 4°. The final supernatant was used for injection into test animals either immediately after preparation or after storage at −20° for a period no longer than 2 weeks. All recipient animals were newborn mice of either the Af, BALB/cf, C3H/f, or C58BL/t mice and were given injections within 24 hr after birth.

**RESULTS**

**Occurrence of Tumors.** The incidence of tumors arising at the site of BP injection varied in the different strains of mice. Tumors developed with comparable frequency in the Af, C3H, C3H/f, and BALB/cf mice, but the incidence was much lower in mice of the 2 high-leukemia strains AKR and C58 (Table 1). The time of appearance of the sarcomas was similar in all strains when they did occur.

All tumors developing at the injection site were found in the s.c. tissue and were well encapsulated. Several were found to be invasive into the thoracic wall or thigh muscles. No metastases were identified macroscopically or microscopically.

**Light and Electron Microscopy.** The majority of tumors at the injection site were diagnosed as fibrosarcoma. Some tumors were diagnosed as anaplastic type sarcomas with multinucleated giant cells and occurred in all strains of mice. One rhabdomyosarcoma was found in a C3H female mouse (Figs. 1 to 3). No other tumors were found in the mice bearing sarcomas except for lung adenomas which developed in a varying percentage in the different strains and will be reported elsewhere.

Four females out of 20 (10 females and 10 males) AKR mice that received a neonatal injection of MC developed s.c. sarcomas after latency periods of 84, 91, 100, and 110 days.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Sex</th>
<th>Incidence of sarcomas</th>
<th>Mean latency period (days and range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Af</td>
<td>d</td>
<td>33/91 (36%)</td>
<td>168 (152–186)</td>
</tr>
<tr>
<td></td>
<td>f</td>
<td>31/94 (33%)</td>
<td>166 (157–180)</td>
</tr>
<tr>
<td>BALB/cf</td>
<td>d</td>
<td>10/43 (23%)</td>
<td>176 (140–192)</td>
</tr>
<tr>
<td></td>
<td>f</td>
<td>10/46 (21%)</td>
<td>188 (153–199)</td>
</tr>
<tr>
<td>C3H</td>
<td>d</td>
<td>10/20 (50%)</td>
<td>127 (110–136)</td>
</tr>
<tr>
<td></td>
<td>f</td>
<td>10/30 (33%)</td>
<td>145 (121–156)</td>
</tr>
<tr>
<td>C3H/f</td>
<td>d</td>
<td>10/24 (41%)</td>
<td>142 (120–161)</td>
</tr>
<tr>
<td></td>
<td>f</td>
<td>8/22 (36%)</td>
<td>137 (125–150)</td>
</tr>
<tr>
<td>AKR</td>
<td>d</td>
<td>1/28 (3.6%)</td>
<td>141 (130–162)</td>
</tr>
<tr>
<td></td>
<td>f</td>
<td>0/29</td>
<td></td>
</tr>
<tr>
<td>C58</td>
<td>d</td>
<td>1/12 (8%)</td>
<td>141 (130–162)</td>
</tr>
<tr>
<td></td>
<td>f</td>
<td>0/10</td>
<td></td>
</tr>
<tr>
<td>AKR</td>
<td>f</td>
<td>0/10</td>
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</tr>
</tbody>
</table>

Table 1

Occurrence of chemically induced sarcomas in high- and low-leukemia strains of mice

All mice received 0.004 mg/g body weight of BP at 6 to 8 weeks of age except as otherwise noted.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Incidence of sarcomas</th>
<th>Mean latency period (days and range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Af</td>
<td>33/91 (36%)</td>
<td>168 (152–186)</td>
</tr>
<tr>
<td>BALB/cf</td>
<td>10/43 (23%)</td>
<td>176 (140–192)</td>
</tr>
<tr>
<td>C3H</td>
<td>10/20 (50%)</td>
<td>127 (110–136)</td>
</tr>
<tr>
<td>C3H/f</td>
<td>10/24 (41%)</td>
<td>142 (120–161)</td>
</tr>
<tr>
<td>AKR</td>
<td>1/28 (3.6%)</td>
<td>141 (130–162)</td>
</tr>
<tr>
<td>C58</td>
<td>1/12 (8%)</td>
<td>141 (130–162)</td>
</tr>
<tr>
<td>AKR</td>
<td>0/10</td>
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</tr>
</tbody>
</table>

*AKR mice received a single injection of MC at newborn age.*
respectively. One had a mediastinal lymphosarcoma involving the thymus in addition to the s.c. sarcoma. The remaining 16 mice died of lymphoma by 220 days of age.

The morphological characteristics of the tumor cells seen in electron microscopy were basically similar in all of the well-differentiated fibrosarcomas, irrespective of strain of origin. The sarcoma cells were usually fusiform with centrally located, enlarged nuclei.

The shape and size of tumor cells varied greatly in the anaplastic cell sarcomas. In these tumors, cells often showed a rounded form with many cytoplasmic processes (Fig. 4). Binucleated cells were occasionally observed. The production of collagen was a constant feature of all the sarcomas examined and either appeared as a bundle of fibrils or as irregular flocculent aggregates loosely arranged in the intercellular spaces. Regular, thick bundles of fibers with periodic bands as seen in normal connective tissue were never found.

The nuclei of the tumor cells were large and located in the center of elongated cells. They appeared oval with smooth contours in the well-differentiated fibrosarcoma, but in anaplastic sarcoma the nuclei became irregular and often showed deep infolds of the nuclear envelope (Fig. 4). Cytoplasmic organelles were found in both poles of the elongated cells or irregularly distributed in the cytoplasm of the rounded cells. Mitochondria were generally round or oval, with cristae being scarce and with a moderately dense matrix. The number of mitochondria seemed increased in most of the sarcoma cells compared to normal fibroblasts. The well-organized ergastoplasm was a characteristic feature of the well-differentiated fibrosarcoma. The cisternae of the endoplasmic reticulum was filled with fine flocculent material of moderate electron density which seemed to be identical with that filling the distended cisternae of normal fibroblasts actively synthesizing collagen (15).

The Golgi apparatus consisted mostly of flattened sacs. Two or more Golgi zones were occasionally seen in 1 section of a cell in an anaplastic sarcoma. In general, however, the Golgi development was not prominent.

The cytoplasmic matrix was rich in free ribosomes and arranged in rosettes of irregular clusters. The presence of numerous fine filaments scattered throughout the cytoplasm was another characteristic feature of the fibrosarcoma cell. They often formed loose bundles beneath the cell membrane or around the nucleus. Other cytoplasmic organelles and inclusions, such as lysosomes, fat droplets, and cytoplasmic vacuoles, were occasionally observed, but none of them were considered characteristic or unique to the tumors.

The striking feature of the sarcomas induced in the high-leukemia-strain mice was the presence of many virus-like particles and the results are summarized in Table 2. Numerous particles appeared in the primary and transplanted sarcomas in adult AKR and C58 mice (Figs. 4, 5, and 7). Virus-like particles were located either extra- or intracellularly. They were round and consisted of an outer membrane and central nucleoid. Outer diameter of the particle was approximately 100 to 200 mμ, and a nucleoid was 60 to 70 mμ in diameter. These virus-like particles were morphologically consistent with the murine leukemia virus classified as type C (4). Immature C particles appeared less frequently in the sarcomas than in the normal tissues. They were round and consisted of an outer membrane and central nucleoid. Outer diameter of the particle was approximately 100 to 200 mμ, and a nucleoid was 60 to 70 mμ in diameter. These virus-like particles were morphologically consistent with the murine leukemia virus classified as type C (4). Immature C particles with electron-lucent centers and budding particles from the cell membrane of sarcoma cells were often observed. Mature C particles appeared less frequently in the sarcomas which developed in young AKR mice that received a neonatal injection of MC, but many doughnut-shaped particles were observed in the distended cisternae of endoplasmic reticulum.
Table 4

Tumorigenic activity of cell-free extracts of Af chemically induced sarcomas injected into newborn mice of 4 different strains

<table>
<thead>
<tr>
<th>Newborn recipients</th>
<th>TG</th>
<th>No. of mice and sex</th>
<th>Reticular tissue</th>
<th>Type of neoplasm (%)</th>
<th>Mean survival time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>I&lt;sup&gt;b&lt;/sup&gt;</td>
<td>C&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Lung I</td>
</tr>
<tr>
<td>BALB/cf</td>
<td>T1</td>
<td>15&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0</td>
<td>7</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0</td>
<td>28</td>
<td>60&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>12&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0</td>
<td>31</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>T8</td>
<td>10&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C3H/f</td>
<td>T1</td>
<td>13&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>T8</td>
<td>17&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>14&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C57BL</td>
<td>T1</td>
<td>10&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td>60&lt;sup&gt;d&lt;/sup&gt;</td>
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<td></td>
<td></td>
<td>10&lt;sup&gt;d&lt;/sup&gt;</td>
<td>30&lt;sup&gt;f&lt;/sup&gt;</td>
<td>6</td>
<td>30&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>T3</td>
<td>11&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>16</td>
<td>25&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0</td>
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<td>Af</td>
<td>T2</td>
<td>13&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0</td>
<td>2</td>
<td>15&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>20</td>
<td>0</td>
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<tr>
<td></td>
<td></td>
<td>10&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0</td>
<td>40&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Tumor-bearing animals.
<sup>b</sup> Injection-treated mice.
<sup>c</sup> Incidence of neoplasms in 100 male and female colony controls.
<sup>d</sup> p < 0.01.
<sup>e</sup> Ovarian tumor.
<sup>f</sup> p < 0.05.

of these sarcoma cells (Fig. 6). Their structure and size were consistent with intracisternal type A particles (4). Occasionally, 2 or 3 areas containing A particles were seen in a section of a single sarcoma cell (Fig. 4). Type A virus particles were also found in both AKR and C58 primary and transplanted sarcoma as well as control tissue (Figs. 4 and 5) but were not found in tumor or normal tissues of the other 4 strains of mice.

Small numbers of extracellular virus-like particles were also present in the primary sarcomas in strains BALB/cf, C3H, and C3H/f mice, but no virus-like particles have thus far been seen in sarcomas of Af mice. Type C particles were observed in cytoplasmic vacuoles of rhabdomyosarcoma cells in a C3H mouse (Figs. 2 and 3).

Examinations of the lymph node, spleen, and thymus of tumor-bearing mice in high-leukemia strains consistently revealed the presence of extracellular mature and immature C particles, but the frequency of appearance and the number of particles were far less than those in sarcomas.

Small s.c. nodular lesions developed at the site of BP injection in some of the mice 3 to 4 weeks after the treatment. Some of these nodules disappeared gradually in the following few weeks, while others remained unchanged for a longer period of time. The possibility that these represented precancerous growths seemed unlikely, since some tumors developed independent of the lesion, which remained unchanged adjacent to the growing tumor. These lesions were cystic nodules containing a small amount of turbid fluid surrounded by a thick connective tissue wall.

Electron microscope examination of these reactive tissues in the high-leukemia-strain mice revealed apparently normal fibroblasts and muscle fibers. No virus-like particles could be observed in the connective tissue wall of the cystic nodules. A few immature C particles were found in the sarcoplasmic reticulum of the muscle fibers involved.

An estimation of frequency of virus-like particles in primary and transplanted tumors as well as the s.c.-reactive tissue nodules and normal tissue (spleen, lymph node, and thymus) was attempted. A scale ranging from 0 to 4+ was used and based on the approximate number of virus-like particles seen per grid (200 mesh) holding 3 to 4 sections. The results are summarized in Table 3. Sarcomas obtained from either of the high-leukemia strains AKR and C58 had an abundance of virus-like particles (3+ and 4+, respectively). The other strains ranged from 0 (Af) to 2+ (C3H) with both BALB/cf and C3H/f classified at 1+. A few virus-like particles were seen in the normal tissues of only the AKR and C58 strains, and in the s.c.-reactive tissue of only AKR mice.

Bioassays. Three primary Af fibrosarcomas were serially transplanted as separate tumors into at least five 6- to 8-week-old male and female recipients for each of 10 TG's. Three tumors from each of the 3 tumor lines were prepared for cell-free extracts at T1, T2, T3, and T8. The extracts from the 3 tumors of a given TG of each transplant line were pooled.
Table 5
Incidence of neoplasms after injection of cell-free extracts of C58 and AKR chemically induced sarcomas into BALB/c newborn mice

<table>
<thead>
<tr>
<th>Newborn recipient (tumor source)</th>
<th>T G</th>
<th>No. of mice and sex</th>
<th>Reticular tissue</th>
<th>Lung</th>
<th>Liver</th>
<th>Mean survival time&lt;sup&gt;c&lt;/sup&gt; (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>C&lt;sup&gt;b&lt;/sup&gt;</td>
<td>I</td>
<td>C</td>
<td>I</td>
</tr>
<tr>
<td>BALB/c (C58 sarcoma)</td>
<td>T1</td>
<td>12&lt;sup&gt;d&lt;/sup&gt;                       0</td>
<td>7</td>
<td>0</td>
<td>17</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>T6</td>
<td>15&lt;sup&gt;d&lt;/sup&gt;                       0</td>
<td>28</td>
<td>0</td>
<td>19</td>
<td>0</td>
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<tr>
<td></td>
<td></td>
<td>12&lt;sup&gt;d&lt;/sup&gt;                       0</td>
<td>58&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>BALB/c (AKR sarcoma)</td>
<td>T2</td>
<td>13&lt;sup&gt;d&lt;/sup&gt;                       0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>12&lt;sup&gt;d&lt;/sup&gt;                       0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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</tbody>
</table>

<sup>a</sup> Tumor-bearing animals.
<sup>b</sup> Injection-treated mice.
<sup>c</sup> Incidence of neoplasms in 100 male and female colony controls.
<sup>d</sup> p < 0.01.

and then injected into BALB/cf, C3H/f, C57BL, and Af newborn mice. The incidences of various types of tumors in the injection-treated mice of each strain were compared to colony controls for that strain. The results are shown in Table 4. None of the mice given injections of any of the cell-free extracts developed sarcomas.

BALB/cf newborns given injections of cell-free extracts of Af tumor tissue obtained from T1, T3, or T8 did not develop any s.c. sarcomas or reticular neoplasms. The incidence of lung tumors in female BALB/cf mice was significantly increased over that of controls when the mice were given injections of cell-free material from T1 of the Af tumors; however, extracts from T3 and T8 did not cause a comparable increase. The incidences of lung tumors in male mice given injections of all extracts were comparable to male controls.

C3H/f newborns given injections of the same Af cell-free material of T1 and T8 showed a significant increase in the incidence of hepatomas in males, but none appeared in the females. No reticular tissue neoplasms or lung tumors were found in the injection-treated males or females.

Injection of the T1 and T3 Af tumor extracts into C57BL newborns resulted in a significant increased incidence of lung tumors in both sexes. A significant increase in the incidence of reticular cell neoplasms developed in females given injections of extracts from T1 but not T3.

Af newborn mice when given injections of material from T2 and T3 of the Af tumor showed no change in reticular tissue tumor incidence as compared to controls, but there was a significant increase in lung tumors in only the females. The mean survival time of some of the tumor-bearing animals in the group was always less to that of colony controls dying with tumors.

Cell-free extracts of T1 and T6 of a C58 sarcoma (3 tumors pooled for each TG) were injected into newborn BALB/cf mice and caused a significant increase in lung tumors after injection of the T6 preparation only (Table 5). The incidence of reticular tissue neoplasms was decreased in both sexes as compared to that of controls for both T1 and T6. No sarcomas appeared in the injection-treated mice.

A transplanted AKR sarcoma was used for preparation of cell-free extracts at T2 and T3 (3 tumors pooled for each TG) and also injected into newborn BALB/cf mice (Table 5). No increase in tumor incidence of any type was found in injection-treated animals as compared to controls. Actually, no lung tumors or reticular tissue neoplasms developed in the injection-treated BALB/c mice, in contrast to a lung tumor incidence of 17 and 19% in male and female controls, respectively, and a 7 and 28% incidence of leukemia in male and female controls, respectively.

Four of the hepatomas that developed in the C3H/f mice given injections of extract from the transplanted Af sarcomas (T1) were examined by electron microscopy, and no virus particles were observed. Five of the lung tumors that arose in BALB/cf mice given injections of the T6-transplanted C58 sarcoma were also negative for virus particles by electron microscopy.

DISCUSSION

The morphological and ultrastructural characteristics of the tumor cells found in well-differentiated fibrosarcomas arising in all 6 strains of mice studied were similar to those of normal fibroblasts (2, 15, 26). The tumors diagnosed as anaplastic sarcomas arose in both the high- and low-leukemia strains of mice, and the round cells of these tumors were almost identical to Rous sarcoma cells or cells infected with the Rous virus (9, 14).

Electron microscopic studies have revealed the presence of virus particles in some types of chemically induced tumors (3, 12, 31), but similar virus particles have not been found in others (30). The role of these virus particles in the etiology of the tumors in which they are seen remains uncertain. Type C virus particles have been described in MC-induced sarcomas in
Virus Particles in Induced Sarcomas

germ-free AKR and C3H mice, but these were absent in similar tumors induced in germ-free rats (19). It was proposed that the presence or absence of virus particles in sarcomas arising in germ-free animals corresponded to the presence or absence of the same viruses in the experimental animals. The results of the present study with conventionally bred mice are essentially the same as those reported for the germ-free mice and rats. Type C and type A virus particles were found in both BP- and MC-induced sarcomas in the 2 high-leukemia strains of mice (AKR and C58), and both of these types of virus particles were seen in the normal tissue of mice of these same strains (10). Only the type C particle was found in sarcomas arising in the 3 low-leukemia strains (BALB/cf, C3H/f, and C3H), but neither this type of virus particle nor type A virus particles were seen in control tissues of these 4 strains. The findings for the primary tumors were the same for the transplanted tumors. However, the type C particles were more abundant in the tumor tissue of the high-leukemia strains than in the low-leukemia strains. No virus particles were seen in either primary or transplanted sarcomas obtained from Af mice. Finally, no type B virus particles were seen in any sarcomas of any of the strains studied including C3H mice, although this type of virus particle is abundant in mammary tumors arising in our C3H mice (1).

In spite of the failure to see virus particles in Af primary or transplanted sarcomas, cell-free extracts of these tumors injected into newborn mice of 4 different strains resulted in an increase in the incidence of lung tumors in BALB/cf mice, whereas C3H/f male recipients showed an increase in hepatomas. C57BL mice, on the other hand, showed an increase in incidence in reticular tissue neoplasms in females as well as an increase in lung tumors in both sexes. No sarcomas developed in any of the mice given injections of the Af sarcoma extract. These findings indicate that the inability to detect virus particles in the Af sarcomas by electron microscopy need not preclude that they were not present in these tumors. It would appear that the detection of viruses in Af chemically induced sarcomas can best be ascertained by bioassay techniques or perhaps more extensive searching by electron microscopy. The different incidences as well as kinds of tumors arising in the injection-treated newborn recipients of different genetic backgrounds in spite of receiving the same cell-free extract is of importance. It raises the question as to the significance of negative bioassays of a particular cell-free extract when tested in only 1 strain of mouse or for that matter only 1 sex of a particular strain of mouse. It also emphasizes the importance of the influence of host genetic factors on the expression of a potentially oncogenic virus.

The bioassay studies of C58 and AKR sarcomas are of interest because abundant type C particles were found by electron microscopy in all of the tumors assayed. However, the cell-free extracts of AKR sarcomas injected into newborn BALB/cf recipients did not cause any increase in any type of tumor development. Indeed, there appeared to be a suppression of the normally occurring neoplasms in this strain. No reticular tissue neoplasms appeared in over 50 injection-treated animals as compared to an incidence of 7 and 28% in male and female controls, respectively. The reason for this decrease is unknown. There was also a decreased incidence of reticular tissue neoplasms in the BALB/cf mice that received an injection of a cell-free extract of C58 sarcomas with numerous type C particles. Yet the extracts of the 6th TG were associated with a striking increase in lung tumors in these recipients. Cell-free extracts of neither AKR nor C58 sarcomas injected into BALB/cf newborn mice resulted in the development of sarcomas. Thus, the bioassay studies failed to ascertain whether the virus particles found in the primary sarcomas and subsequently transplanted tumors played any etiological role in the induction of the sarcomas or merely represented a passenger type of virus. With respect to the latter consideration, the number of type C particles in sarcomas developing in the 2 high-leukemia strains of mice was greater as compared to those arising in the low-leukemia strains of mice.

The significance of the presence of intracisternal type A virus particles in the sarcoma cells arising only in mice of the 2 high-leukemia strains in this study is unknown. This type of particle was found in both C3H and AKR germ-free mice (19). Intracisternal type A virus particles have been described in a number of neoplasms in conventionally bred mice (5, 6, 20, 34). It has been suggested that the intracytoplasmic type A particles which are not obviously associated with any organelle in cells of spontaneous mammary tumors become free as immature type B particles in cytoplasmic vacuoles, ducts, or intercellular spaces and then presumably develop into mature type B particles (7). Although neither virgin nor force-bred AKR and C58 mice of our colony develop mammary tumors spontaneously, these same 2 strains of mice showed a significant increased incidence of mammary tumors following the feeding of MC (21). Whether there is any causal relationship between MC-induced mammary tumors and type A particles is not known, and a similar conclusion can be held for significance of type A particles in BP-induced sarcomas.

Although no virus particles were observed in the Af sarcomas, electron microscopic examination of milk from Af mice of our colony revealed "a few" type C virus particles (8). On the other hand, no virus particles were found in the milk of BALB/cf mice from our colony (8), yet sarcomas induced in mice of this strain in the present study showed occasional type C particles. Finally, mammary tumors arising in C3H mice in our colony revealed an abundance of type B virus particles by electron microscopy (1), but only type C particles were found in the sarcomas induced with BP in mice of this strain.

These studies reemphasize the caution that must be exercised in describing a tumor as being free of virus particles when based solely upon electron microscopic examination. The data also indicated the need for a better understanding of the biological differences and interrelationships between the type A as well as the types B and C murine viruses and what role, if any, they perform in the development of chemically induced sarcomas.

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REFERENCES


Fig. 1. A rhabdomyosarcoma produced in a C3H mouse given an injection s.c. of BP. H & E, × 450.

Fig. 2. Partial magnification of a myosarcoma cell from the same tumor shown in Fig. 1. Arrow, mature C particle in a cytoplasmic vacuole; mf, remnant of myofilament. × 30,000.

Fig. 3. A portion of a rhabdomyosarcoma cell induced in a C3H mouse given an injection of BP. Note the remnant of myofilament (mf) in the periphery of the cell. Arrows, virus-like particles in cytoplasmic vesicles. × 10,000.

Fig. 4. A rounded cell from an anaplastic cell sarcoma produced in an AKR mouse given an injection of MC at newborn age. Note numerous type A particles (A) in the distended cisternae of endoplasmic reticulum. B, virus particle budding from tip of a small cytoplasmic process; C, extracellular C particle; cf, cytoplasmic filaments surrounding the nucleus. × 22,000.

Fig. 5. A portion of a cell from a transplanted fibrosarcoma induced in an AKR mouse given an injection of BP. Note extracellular type C particles (C) and intracisternal type A particles (A). N, nucleus. × 58,000.

Fig. 6. A portion of a sarcoma cell from the same tumor shown in Fig. 4 showing structural detail of intracisternal A particles. Two particles sharing a common outer shell are seen. × 70,000.

Fig. 7. Numerous type C particles appeared in a transplanted sarcoma in a C58 mouse. × 38,000.
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