Intracisternal Type A Particles Occurring in Foreign Body-induced Sarcomas

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SUMMARY

Eight sarcomas induced in mouse strains CBA/H, CBA/H-T6, and C57BL/10ScSn (or their hybrids) by s.c. implantation of smooth or roughened plastic films or glass pieces were examined electron microscopically. Type A virus particles were detected within dilated ergastoplasmic cisternae of the sarcoma cells from five mice. The doughnut-shaped particles measured 90 to 100 nm and were seen to bud from membranes of the granular endoplasmic reticulum. All five virus-positive sarcomas developed in secondary hosts that had received segments of cell-laden implants and/or surrounding tissue capsules transferred from the original implant carriers during the period of nonneoplastic foreign body reaction. In all cases the tumors were derived from cells of the original host. No virus particles were found in three sarcomas that had developed directly in original implant carriers.

INTRODUCTION

The process of foreign body tumorigenesis in mice is being investigated in our laboratory (1, 2, 7, 8). Previous studies have revealed that few or single cells with neoplastic potential appear in foreign body-reactive tissue as early as 1 month following implantation of plastic films (2, 8). These cells were found to expand into clones which mainly adhered to the implant surfaces. At this early (nonneoplastic) stage of foreign body reaction the implants were excised and cut into smaller pieces. These pieces were then separated to histocompatible but karyologically distinguishable recipient mice, where they gave rise to tumors after many months of latency. In some cases the latency exceeded 2 years. On the basis of marker chromosomes (T6) it was proven that the tumors were derived from cells of the original implant carriers. Tumors that were derived from segments of the same implant were found to be closely related ("homologous") with regard to (a) duration of tumor latency, (b) histopathological features and criteria of malignancy, and (c) stem-line-specific aberrations of chromosome numbers and presence of abnormal marker chromosomes. These findings proved the clonal nature of transferable neoplastic cells.

Previous attempts by our group (unpublished data) and others (6, 10, 12) have failed to demonstrate an association of viruses with foreign body-induced sarcomas. Virus particles were not evident in our earlier electron microscopic studies of the preneoplastic tissue reactions in CBA/H and CBA/H-T6 mice (8). Tumors that developed directly in original implant carriers were also negative for murine sarcoma and leukemia viruses in molecular hybridization experiments previously carried out in collaboration with M. Green (unpublished data). The present communication reports the detection of intracisternal type A virus particles in some of these tumors.

MATERIALS AND METHODS

The basic experimental designs, procedures, and techniques are the same as those detailed in our earlier publications (1, 2).

The following inbred mouse strains and their hybrids were used: CBA/H and CBA/H-T6 (originally obtained from Harwell, Didcot, England), and C57BL/10ScSn (obtained from The Jackson Laboratories, Bar Harbor, Maine). The 8 tumors randomly selected for these electron microscopic studies had been induced by different materials and procedures (Table 1). Materials used as implants included (a) glass coverslips (15 x 22 mm in size, 0.2 mm thick), (b) plastic coverslips (unplasticized vinyl chloride acetate copolymer, 15 x 22 mm in size, 0.2 mm thick), and (c) plastic coverslips with surfaces finely roughened by sandpapering.

Five of the tumors developed in secondary implant carriers (i.e., segments of cell-laden films and/or surrounding tissue capsules were transferred from original implant carriers at specified times during the period of nonneoplastic foreign body reaction). Transfer to recipient animals was carried out either by direct s.c. implantation or by implantation into 2-month-old preformed tissue capsules. These capsules had been induced by previous implantation of plastic coverslips which were removed as the transfer material was slipped in. The remaining 3 tumors had developed directly in the original implant carriers.

The tumor specimens for electron microscopic study were fixed in 2% glutaraldehyde for 2 to 3 hr followed by 1% osmium tetroxide for 1 to 2 hr. Fixatives were buffered at pH 7.2 using 0.2 M phosphate buffer. The specimens were dehydrated in a graded series of 50 to 100% ethanol and passed through propylene oxide before being embedded in Epon-Araldite epoxy resin. Thin sections were cut with a diamond knife, picked up on uncoated copper grids, stained with uranyl acetate and lead citrate, and examined with a Zeiss EM 9S electron microscope.
RESULTS AND DISCUSSION

Numerous virus particles were identified in the cytoplasm of sarcoma cells from Mouse FF5453 (Table 1). Approximately 50% of the cells contained up to 12 particles in each cross-sectional cell profile. Significantly fewer particles of the same type were detected in tumors of 4 other mice (i.e., less than 1% of the cells contained 1 to 2 particles per cell profile).

In all 5 tumors the particles (Figs. 1 to 4) were confined to dilated cisternae of the granular endoplasmic reticulum. The particles were doughnut shaped and measured 90 to 100 nm in diameter. They consisted of 2 concentric electron-dense shells which enclosed an electron-lucent center. Budding occurred only from the membranes of the granular endoplasmic reticulum. Particles were not seen in extracellular spaces or intracellular areas other than the ergastoplasmic cisternae. These morphological features are consistent with intracisternal type A virus particles.

Virus particles were not detected in 3 of the 8 sarcomas studied despite the fact that several preparations from each tumor were extensively examined. As can be seen from Table 1, presence of virus particles did not depend on a specific type of implant material. Smooth or rough plastic as well as glass coverslips were capable of inducing virus-positive tumors. Also, it appears (Table 1) that mouse strain, tumor latency, or the timing of preneoplastic transfers was in no way correlated with the presence or absence of virus particles. However, a possible correlation is suggested by the observation that all 5 virus-positive tumors resulted from pretumor transfer experiments whereas the 3 virus-negative tumors had developed directly in original implant carrier animals. These findings may be purely coincidental and further studies are necessitated on larger numbers of selected tumors.

Intracisternal type A particles have been reported in a variety of transplantable murine tumors (17) and in rat (13), gerbil (15), guinea pig (11), and cat (5) tumors. In plasma cell tumors of mice, intracisternal type A particles have been observed in the primary as well as the transplanted tumors (4). These particles have also been demonstrated in normal adult tissues of certain strains of mice (17). Cell-free preparations from neoplastic tissue containing intracisternal type A particles have been reported to induce tumors (9), but the significance of the virus particles per se in neoplastic transformation has not been resolved. The presence of a DNA polymerase, different from that of known viral-associated DNA polymerases, was recently demonstrated in association with intracisternal type A particles isolated from several mouse tumors (16). It was proposed that this unique polymerase may be a defective tumor virus enzyme. Although there is increasing evidence that type A particles present in the cytoplasmic matrix (i.e., intracytoplasmic A particles) may become the nucleoid of budding type B particles (3, 14), there is no known direct relationship of intracisternal type A particles to type C particles.

Whether the type A particles demonstrated in this study are etiologically related to the foreign body-induced sarcomas or whether they are only passenger agents was not determined. Additional studies are needed to determine the significance of type A particles in foreign body-induced sarcomas. The unique possibilities of our experimental system, which permits analytical studies on preneoplastic cells during foreign body reaction, may provide a clue as to whether the gene activation

<table>
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<th>Experiment code no.</th>
<th>Strain</th>
<th>Sex</th>
<th>Implant material</th>
<th>To</th>
<th>Total tumor latency (mo.)</th>
<th>Tumor genotype</th>
<th>Virus particles</th>
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<td>FF5453</td>
<td>H × H1 F1</td>
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Abbreviations (see also "Materials and Methods"): H, CBA/H; H1, CBA/H-T6; C, C57BL/10ScSn; pf, preformed.

a A spontaneous i.p. tumor developed at Month 28 in the original implant carrier.

b Excision of half implant and half capsule at Month 10.
responsible for virus formation represents a primary etiological event or a secondary effect of foreign body tumorigenesis.

REFERENCES

Figs. 1 to 4. Type A particles (arrows) in foreign body-induced tumor cells. The particles are located within cisternae of the endoplasmic reticulum. A particle budding from the endoplasmic reticulum is evident in Fig. 4. M, mitochondria; N, nuclei. Fig. 1, X 21,600; Fig. 2, X 31,700; Fig. 3, X 50,700; Fig. 4, X 31,700.
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