A New Virus in a Spontaneous Mammary Tumor of a Rhesus Monkey

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SUMMARY

The present studies were undertaken to investigate the ultrastructure and development of virus particles detected in a spontaneous mammary tumor of a rhesus monkey. Thin-section electron microscopy of the tumor tissue has revealed 2 types of particles, viz., an intracytoplasmic, electron-dense, ring-shaped particle measuring about 70 μm in diameter, and an extracellular particle with an outer unit membrane and a central dense nucleoid measuring about 110 μm in diameter. From the electron micrographs, the intracytoplasmic development and virus maturation by a process of budding at the level of the cell membrane are reconstructed. The observations are discussed in view of the known oncogenic RNA-type virus particles.

INTRODUCTION

The search for virus particles associated with cancer in primates has been of special interest in view of the fact that murine (33), avian (21), and feline (24) leukemias, as well as mouse mammary tumors (30), are caused by the viral agents. During the last 10 years, many reports have described virus-like particles in the blood and tissues of cancer patients (14, 19, 32); however, none of these particles has been established to be the causative factor. Particles resembling oncogenic RNA types have been reported in the milk (7), blood (27), and tumor biopsies (31) of cancer patients. Also, herpes-like virus(es) has been detected in cultures from humans and several chimpanzees (16, 23, 26).

Following the discovery of virus particles, by one of us (H. C. C.), morphologically resembling the known oncogenic RNA viruses in a spontaneous breast carcinoma of a rhesus monkey, the present investigations were undertaken to study the ultrastructure and development of these virus particles.

MATERIALS AND METHODS

Tumor

At Mason Research Institute, Worcester, Mass., an 8-year-old female rhesus monkey (Macaca mulatta) developed a spontaneous breast carcinoma near the left nipple. During a 3-month period of tumor growth, several biopsies were taken for investigations. At the time of autopsy, tumor specimens and small pieces of spleen, thymus, liver, bone marrow, and lymph nodes were also procured. The samples were immediately fixed in cold 2% glutaraldehyde in phosphate-buffered saline fixative for electron microscopy.

Electron Microscopy

Thin-Section Study. Small pieces of the glutaraldehyde-fixed biopsy samp1 were rinsed several times in buffer and postfixed in Dalton's chrome-osmium (9). The samples were dehydrated in successive changes of 50% ethyl alcohol (containing 2% uranyl acetate) and 70, 95, and 100% ethyl alcohol. The dehydrated samples were placed in various proportions of propylene oxide and the embedding medium. The material was embedded in Araldite (Fluka AG Chemische Fabrik, Buchs, Switzerland) or Epon and allowed to harden in an oven at temperatures of 37° and 60°. Thin sections were cut with a diamond knife on an LKB ultramicrotome, and cut sections were mounted on carbonized Formvar membranes on 200 mesh copper grids. The sections were stained with lead citrate (34) and examined in a Siemens Elmiskop Model I-A electron microscope.

Negative Stain Study. The virus pellets were concentrated by ultracentrifugation from the tumor homogenate, and the supernatant tissue culture medium of cell cultures of the tumor was grown in 1640 medium with or
without calf serum. The concentrated virus suspension was diluted 1:4 with 2% phosphotungstic acid at pH 5.0 (5). Carbon-coated grids (200 mesh) were applied to the surface of the virus-phosphotungstic acid suspension and dried at room temperature before examination in the electron microscope.

RESULTS

Electron microscopy of many thin sections of the monkey mammary carcinoma revealed the presence of a large number of virus particles in the intercellular spaces of the epithelial cells (Fig. 1). These virus particles were continuously observed in all the biopsies and in autopsy material procured from this monkey tumor. A few extracellular virus particles were also observed in the stroma of the mammary tumor.

At higher magnification, 2 types of virus particles were found to be associated with the tumor cells; one was always observed in the cytoplasm, and the other was found extracellularly. The latter were found in the intercellular spaces, and the pictorial evidence suggested that they were formed by the budding process at the cell membrane. While both forms of the particles were observed in the tumor, only a few extracellular particles were detected in the thymus and lymph nodes, and none of the particles were found in the spleen, bone marrow, and liver examined during the present investigations.

The intracytoplasmic particles were smaller, dense, ring-shaped structures measuring 60 to 95 μm in diameter. The density of the viral material is usually higher in the peripheral portion than in the central portion of the virus, thus appearing as a doughnut-shaped particle. These particles may occur singly or in clusters in the cytoplasm (Fig. 2). These particles were seen enveloped in the plasma membrane at the cell surface, where the particles belonging to the 2nd category are formed. The budding process of the virus particles at the cell surface, by the incorporation of intracytoplasmic particles into the plasma membrane, seemed to be the transitional phase of the virus cycle between the proviral stage and the formation of extracellular particles (Fig. 3). These budding forms were often observed connected to the cell by the cytoplasmic peduncle or stalk. The rupture or break of the narrow cytoplasmic peduncle or stalk probably liberates the virus into the extracellular or intercellular spaces. The extracellular forms of the budded virus particles can also be detected in the cytoplasmic vacuoles of the tumor cells.

The extracellular virus particles are well defined, with oval or spherical configuration (Fig. 4). The measurements made on several particles gave a range in size from 100 to 120 μm; most particles measured 110 μm in diameter. Estimates on particle size of the larger diameter of spherical profiles were made on the assumption that these were changed least by sectioning compression.

The ultramorphology of these extracellular virus particles demonstrated an outer unit membrane and a central nucleoid (Figs. 4 and 5). The virus particles with partially dense nucleoids were either observed still connected to, or lying very near, the plasma membranes, whereas the forms consisting of a central electron-dense nucleoid were found free in the intercellular spaces. The central condensed nucleoid of the virus particles is observed as either a dense sphere or a rod-shaped structure and measures 30 to 50 μm in diameter (Fig. 6). The inner or intermediate membrane binding the nucleoid is distinctly recognizable only in a few of the particles, and it is not yet clear whether it should be considered a definite component of the virus or whether it corresponds to the condensation of the material between the outer envelope and inner nucleoid. The unfixed and PTA\(^2\)-negative-stained preparation of extracellular virus particles recovered from the tissue culture medium show various sized or tadpole shapes (Fig. 7). The virus particles consistently showed head and tail morphology, formed by salt concentration and surface tension phenomenon.

DISCUSSION

While the Bittner agent has been found to be the causative agent in mouse mammary cancer (4, 30), little is known about the viral etiology of breast cancer of other species. C-type particles have been reported in several strains of rats to be associated with mammary tumors which originated spontaneously or by chemical and X-irradiation carcinogenesis (6), but their etiology has not been established so far. Similarly, recent reports have described virus-like particles in the milk and biopsy samples of human breast cancer patients, although no causative role to these particles has been ascribed (7, 17).

So far, no oncogenic RNA-type virus particles of subhuman primates have been reported. The findings relating to tumor induction in newborn hamsters by SV40 virus (15, 18, 22) represented a breakthrough in studies of neoplasia in being the first demonstration of a malignant oncogenic quality for a virus of primate (monkey) origin. Herpes saimiri, a latent DNA viral agent from the squirrel monkey (Saimiri sciureus), has been found to induce acute malignant lymphoma in owl and marmoset monkeys (28). Oncogenicity of poxviruses in monkeys has also been demonstrated (1).

With electron microscope techniques, it is now possible to differentiate between known oncogenic viruses and to devise a classification on the basis of morphological data (10). Within such a framework, it would appear that the virus particles detected in the monkey breast adenocarcinoma herein reported bear general resemblance to the C-type viruses of murine, avian (20), and feline (25) leukemias, inasmuch as the mature particles consist of an outer unit membrane and a central nucleoid. The virus particles also resemble C-type structures in PTA-negative-stained preparations (Fig. 7). However, the development process of these particles appears to be similar to the oncogenic virus particles described as B type observed in the mouse mammary tumors (3, 29).

The monkey mammary carcinoma has also revealed the presence of intracytoplasmic particles which bear re-

\(^2\)The abbreviation used is: PTA, phosphotungstic acid.
semblance to the intracytoplasmic A-type particles of Bernhard (2, 13), described in mouse mammary tumors. They consist of a dense ring- or doughnut-shaped morphology. Similar particles have also been described in a spontaneous leukemia by de Harven and Friend (12), in L1210 leukemia by Chopra et al. (8), and in 2 spontaneous plasma cell tumors by Dalton et al. (11). In mouse mammary cancer, the incorporation of intracytoplasmic A particles into buds of B-type particle has been described (3, 29). From our electron micrographs, the occurrence of a similar phenomenon in the monkey breast tumor cells, which results in the formation of an extracellular particle by a budding process can be reconstructed (Fig. 3). Although the origin and budding process of these particles is similar to B-type particles, the particles differ from B forms in consisting of a centric nucleoid, instead of one in the eccentric position. They can further be differentiated from B-type particles by the absence of characteristic surface spikes in PTA-negative-stained preparations.

The electron microscopic identification of virus particles in the monkey mammary carcinoma does not in any way provide a proof that the particles are etiologically related to the tumor. A proper evaluation of virus-like particles in tumor tissue can be obtained only if combined with biological tests; this is now in progress in monkeys, hamsters, and mice. The distinct resemblance of the virus reported herein to the known oncogenic RNA-type viruses is of great significance and warrants further investigation to determine its role in breast cancer.

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REFERENCES


Fig. 1. Thin-section electron micrograph through spontaneous mammary tumor of the monkey showing virus particles in the intercellular spaces of the epithelial cells (arrows). X 5000.

Fig. 2. Electron micrograph showing the intracytoplasmic particles. X 35,400. Inset, X 71,000.

Fig. 3. Electron micrograph showing the intracytoplasmic particles and the extracellular virus particles during the budding process. X 71,000.

Fig. 4. High-magnification electron micrograph showing ultrastructure of the virus particles (arrows) with electron-lucent nucleoids. X 110,800.

Fig. 5. Electron micrograph showing virus particles containing dense nucleoids in the intercellular space. X 35,400.

Fig. 6. High-power magnification of the virus particles showing an outer unit membrane and central spherical or rod-shaped condensed nucleoids. X 78,000.

Fig. 7. Negative-stained electron micrograph of virus particles (in PTA) showing head and tail morphology. X 124,300.
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