Preliminary Report on Virus-like Particles in a Transplantable Rat Mammary Carcinoma

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SUMMARY

Tumor, thymus, spleen, and plasma from rats inoculated with an X-irradiation-induced transplantable rat mammary carcinoma were examined with the electron microscope at various intervals following transplantation. Virus-like particles were found in all tumors at 15 or more days after transplantation but were not observed in the thymus, spleen and plasma, or in tumors examined earlier than 15 days. The particles were morphologically of the C-type, having a diameter of from 95 to 110 nm. The significance of these virus-like particles and their relationship to the transplantable rat carcinoma is unknown.

INTRODUCTION

A transplantable carcinoma induced in Northwest Sprague-Dawley (NWSD) rats (Northwest Rodent Company, Pullman, Washington) by X-irradiation was passaged through 63 generations over a period of 32 months (4). The original tumor was an adenocarcinoma. It consisted of groups of proliferating glandular epithelial cells which were pleomorphic and had large nuclei. There were also a few spindle-shaped cells, and small amounts of connective tissue were present. There was a gradual microscopic change in the morphology of the tumor until after the 19th transplant; the tumor was diagnosed as an undifferentiated carcinoma.

This report describes the examination of the transplanted tumor with the electron microscope and the finding of virus-like particles therein.

MATERIALS AND METHODS

The primary tumor was induced by irradiating a 1-month-old female NWSD rat with 720 R of X-ray. The focal skin distance was 50 cm. A 250 kv.p. X-ray machine, operating at 15 ma/second with 0.5 mm Cu and 1.0 mm Al filter, was used for the irradiation. The total dose was given in a single exposure. Five months after irradiation, the rat developed a mammary tumor. Tumor tissue (2 gm) was homogenized in a Ten Broeck grinder with 3 ml of physiologic saline solution, and the homogenate was inoculated subcutaneously with a 20-gauge needle into 8 newborn NWSD rats of both sexes. Each rat received 0.2 ml.

NWSD rats used to passage the tumor in this investigation were maintained as a small, randomly bred, closed colony. No evidence of clinical disease was manifested in any of the rats during the period of transplantation experiments.

Tumor, thymus, spleen, and plasma from 7 rats receiving transplants of tumor at 3 to 10 weeks of age were examined with an electron microscope at intervals of 8, 15, 22, and 30 days after transplantation.

The tissues were prefixed in 3% glutaraldehyde at 0°C for 2 hours, left in Millonig's buffered solution at 4°C overnight, and postfixed in 2% osmic acid for 2 hours at room temperature. The tumors were embedded in Epon 812 according to the method described by Luft (15).

The plasma was separated from heparanized veno-caval blood concentrated by ultracentrifugation in a #40 rotor in the Spinco L-2 centrifuge and processed as previously described for the tissue specimens.

Thin sections of Epon-embedded tissue and plasma were prepared with an MT-2 Porter Blum ultramicrotome and a diamond knife. Sections were stained with saturated uranyl acetate in alcohol and 0.04% lead citrate in 0.1 N NaOH.

The stained sections were examined with an RCA EMU 3 G electron microscope.

RESULTS

In the first transfer, 1-cm nodules were present in 5 of the 8 rats thirty days after inoculation. However, these tumors regressed in all rats except 1 (a male). The tumor in the male rat grew progressively and was used for the 2nd generation transfer.

The transplantable carcinoma (4) grew well in NWSD rats of either sex at various ages. It was invariably fatal, death of the host occurring within 15 to 35 days following the inoculation of tissue homogenates. Examination of the tumor with the light microscope at the sixty-first transfer showed a necrotic undifferentiated solid carcinoma with a slight fibrovascular stroma and numerous mitotic figures (Fig. 1).
When examined with the electron microscope, virus-like particles were found in all of the older transplanted tumors. In no case were particles detected earlier than 15 days after transplantation. On the other hand, no virus-like particles were observed in thymus, spleen, or plasma of either nontransplanted or tumor-bearing rats (regardless of the age of the tumor).

The virus-like particles were spherical and ranged from 95 to 110 m in diameter with a unit membrane and a densely staining nucleoid (Figs. 2, 3). The particles underwent replication by budding from the cell membrane (Figs. 4, 5) and were also localized in vesicles or extracellular spaces, singly or in groups (Figs. 3, 6, 7). The particles were tentatively identified as C-type according to Bernhard's classification (2) and a later-proposed classification scheme (3).

Extract prepared from the tumor did not react in the complement fixation test with a broadly reacting murine leukemia antiserum.

DISCUSSION

Spontaneous leukemia appears to be rare in rats. Kim et al. (14) reported six cases occurring in 66 old inbred (Wistar/Furth) rats and Schreiner and Will (23) described three cases of chloroleukemia in a group of adult Wistar rats. Gross (7) has stated that the incidence of spontaneous leukemia in Sprague-Dawley rats probably does not exceed 2%.

Leukemias have been induced in rats with chemicals (8, 9, 24), murine leukemia viruses (7, 16, 17, 21), injection of fractions of rat tumor tissues (25), and irradiation (18).

The electron microscopic examination of germ-free rats has thus far not revealed the presence of C-type particles (11, 12, 19, 20). However, leukemia and mammary adenocarcinomas have been induced by X-irradiation and the administration of dimethylbenzanthracene (DMBA) separately in germ-free rats (20). Electron microscopic examination of these X-irradiated and DMBA-induced leukemias and carcinomas have likewise not revealed the presence of C-type particles. However, Moloney (26) was successful in demonstrating C-type particles in a transplantable chloroleukemia of random-bred Wistar rats, and Bergs (1) has recently reported the development of leukemia in rats following their inoculation with filtered extracts of rat mammary tumor induced by DMBA.

In the present study, C-type particles were found in a transplantable rat carcinoma induced by X-irradiation. These virus-like structures are similar to those which cause murine, avian (6), and feline (10, 13, 22) leukemias.

While Hartley has detected the presence of antigen by complement fixation which reacts with a broadly reacting murine leukemia antiserum, in old rats of a variety of strains (J. Hartley, personal communication), no antigen was found in an extract of tumors in our study which contained C-type particles. Neither was antigen detectable following passage in rat embryo cell cultures. This would then suggest that if the particles seen with the electron microscope represent a leukemia virus, then the virus appears to be unrelated antigenically to known murine leukemia viruses.

Viruses may be found in tumor cells as passengers and be unrelated to the etiology of the tumor (5). Therefore, the relationship of the particles in the present experiment to the tumors in which they have been found and their possible oncogenic potential, particularly in relation to leukemia, has yet to be determined.

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REFERENCES


Fig. 1. Thin section through transplanted tumor (61st transfer) of a six-week-old female Northwest Sprague-Dawley rat. H & E, × 625.
Fig. 2. Immature C-type particle (arrow) having an outer membrane, an electron dense nucleoid, and an intermediate membrane between the nucleoid and the outer membrane. × 120,000.
Fig. 3. Mature C-type particles (arrow), which are irregular in shape and have a densely staining central nucleoid. × 120,000.
Fig. 4. Early budding C-type particle (arrow) protruding from the cell membrane into an extracellular space. × 82,000.
Fig. 5. Later budding C-type particle (arrow) in which the intermediate membrane between the nucleoid and the outer membrane of a particle is almost complete. × 78,000.
Figs. 6, 7. Virus-like particles (arrows) localized in the extracellular spaces. Fig. 6, × 36,000; Fig. 7, × 54,000.
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