Mammary Tumor Virus (MTV) Virions in a Transplantable Ependymoblastoam[1] (34163)

D. H. Moore, J. Charney, E. Y. Lasfargues, N. H. Sarkar, R. C. Rubin, and R. P. Ames

Institute for Medical Research, Camden, N.J. 08103; Montefiore Hospital and Medical Center, Bronx, N.Y. 10467; and Roosevelt Hospital, N.Y. 20019

The mouse mammary tumor virus (MTV) has been shown to replicate in the mammary gland (1), in seminal vesicles (2), and possibly in the thymus (3). An examination of thymuses from high and low mammary tumor mouse strains was reported by Karl Hollmann (3) who found B particles in only the New Zealand black strain. No other tissues have thus far been shown to replicate this virus. Although MTV production in completely mammectomized females (4) has indicated other sources, the present report is the first to give evidence of MTV virion (B particle) replication in tissues other than the hormone dependent organs of the reproductive system. Many attempts have been made to grow the virus in a variety of cells and tissues.

Materials and Methods. Tissue. The MTV virions were found in a transplantable ependymoblastoma induced with methylcholanthrene in a C57BL/6/J mouse by H. M. Zimmerman. The tumor has been passaged intracranially in the same mouse strain since 1948 and the identity of the tissue has been well established (5). After finding MTV virions in the tumor (6), explants from it were introduced into culture (see Fig. 4) where virion production has continued since May, 1968.

Tests for MTV virions and MTV antigen. Electron microscopy. Thin section electron microscopy of the tumors and of packed cells from the culture showed the presence of intracytoplasmic A particles, (Fig. 1a) and budding B particles (Fig. 1b), and mature B particles with eccentric nucleoids and spiked outer membranes. The budding always contained complete A particles and never a crescent-shaped inner component characteristic of C particle budding. In negative contrast (phosphotungstate) electron microscopy, particles concentrated from tumor extract by centrifugation on a preformed density gradient of Ficoll had outer membranes covered with 90 Å spikes with thin stalks and knobby distal budends characteristic of B particles (Fig. 2). The particle in Fig. 2b shows a characteristic core (7). The overall size and tail formation are uniquely characteristic for B particles.

Immunodiffusion. Two g of tumor were homogenized with 20 ml of distilled water in a Virtis blender; and the homogenate was freeze–dried, then reconstituted with 1 ml of distilled water. This concentrate was tested in a micro-Ouchterloney diffusion plate (8) against rabbit anti-MTV serum, prepared according to Nowinski *et al.* (9), absorbed with 2 vol of C57BL/Haag MTV-free milk. The tumor extract shows a line of identity with the antigen–antibody precipitate formed with virus-rich RIII milk (Fig. 3).

Fluorescent antibody. The same anti-MTV serum absorbed with MTV-free milk used for the immunodiffusion test was employed to detect surface antigens in freshly trypsinized cell suspensions obtained from cultures. By indirect fluorescence microscopy, the membrane-fluorescence test of Klein (10) showed the presence of a specific surface antigen in about 20% of the cells in suspension (Figs. 5a and 5b).

[1] Supported in part by USPHS Grants CA-08740, 08515 and CA-08008 from the National Cancer Institute.

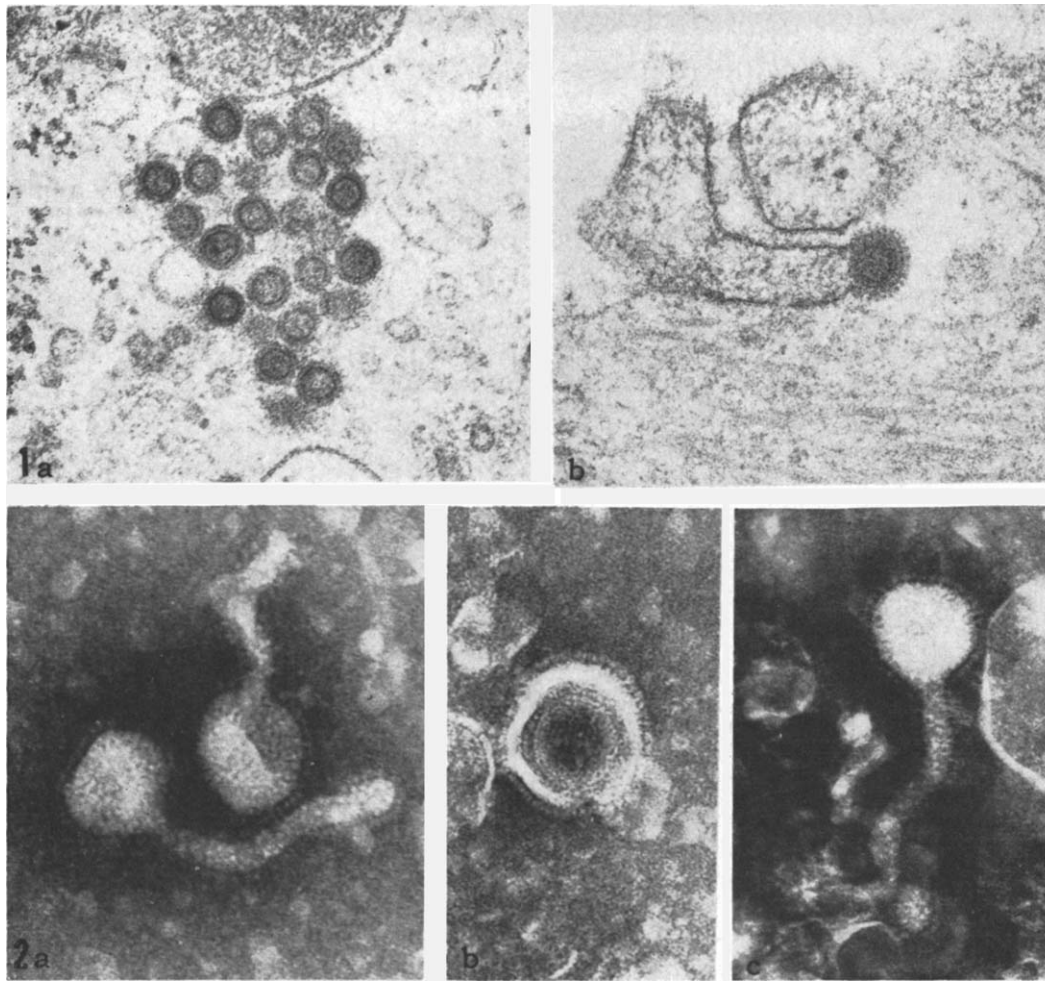

FIG. 1. Thin section of ependymoblastoma showing (a) cytoplasmic A particles and (b) budding B particle; (a) ×75,000, (b) ×90,000.

FIG. 2a–c. Negatively stained B particles separated from tumor extract by density gradient centrifugation in Ficoll. Center particle (Fig. 2b) shows internal core containing fine structures.

Infectivity. Nine of 12 C57BL/Haag mice inoculated with the tumor extract had strong MTV antigen in their milks at third lactation.

Discussion. It is surprising that ependymoblastoma cells are able to proliferate MTV virions, because it has not been possible to infect any cells *in vitro* with this virus; only explants of mammary tumors have been shown to proliferate MTV in culture. The C57BL/6/J mice are generally thought to be resistant to MTV. It is not known whether the ependymoblastoma has been proliferating B particles since its initiation or whether it has acquired the virus during the many intracranial passages. Consequently, any casual relationship between the tumor and the virus cannot yet be determined.

Summary. A methylcholanthrene-induced transplanted ependymoblastoma was found to be proliferating MTV virions (B particles). Identity of the particles was affirmed by thin section and negative contrast electron microscopy, by immunodiffusion precipitation against absorbed anti-MTV rabbit serum by infectivity and by fluorescent antibody microscopy of cell cultures. Cell cultures have continued to proliferate virions for a period of 10 months.

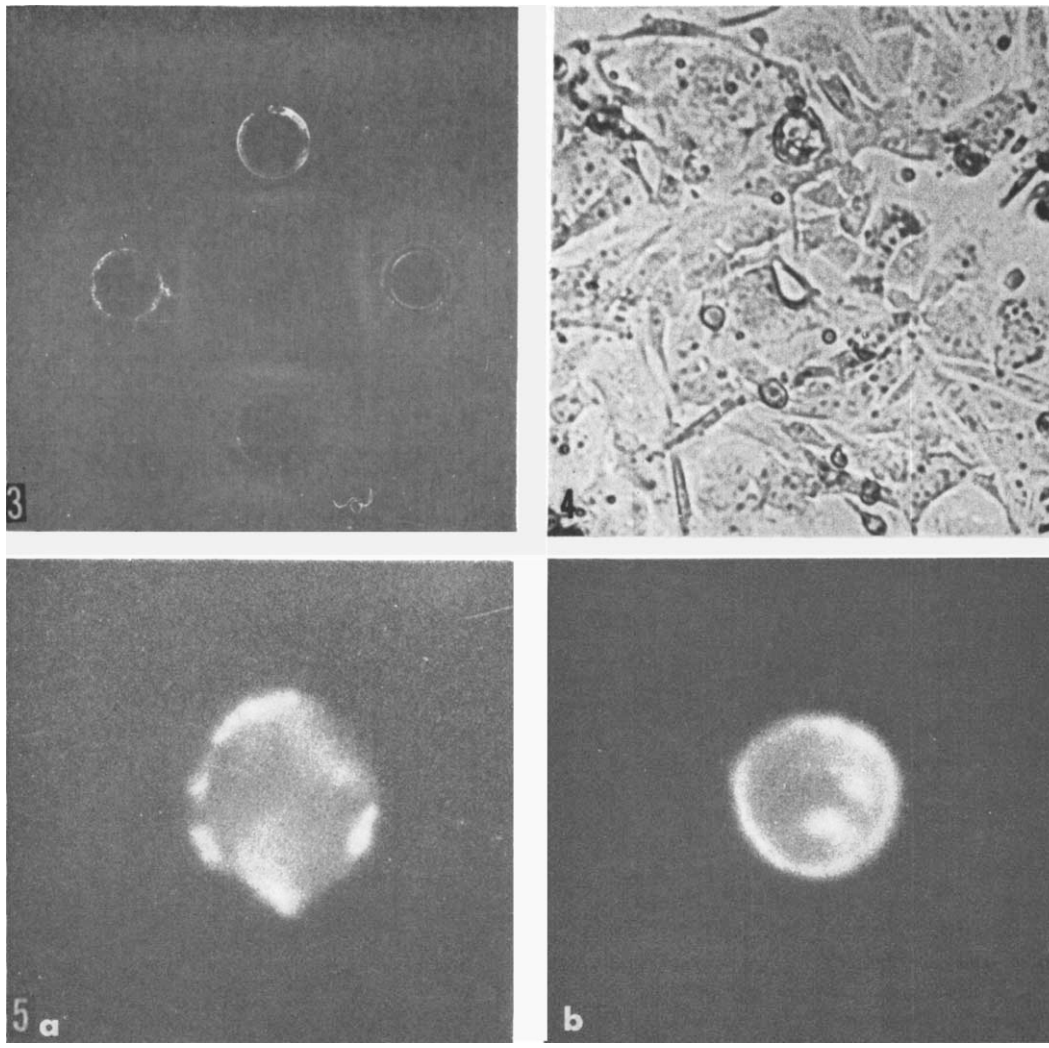

FIG. 3. Immunodiffusion plate: absorbed rabbit anti-MTV serum, central well; tumor extract, left and upper wells; milk from high mammary tumor strain RIII mice, right and lower wells.

FIG. 4. Culture cells after 2 months in culture.

FIGS. 5a and 5b. Indirect fluorescence microscopy showing reaction of 2 cells from culture with absorbed rabbit anti-MTV serum.

1. Lasfargues, E. Y. and Feldman, D. G., Cancer Res. **23**, 191 (1963).

2. Smith, G. H., J. Nat. Cancer Inst. **36**, 685 (1966).

3. Moore, D. H., Science **163**, 1230 (1969).

4. Dux, A. and Mühlbock, O., Proc. Soc. Exptl. Biol. Med. **115**, 433 (1964).

5. Rubin, R., Sutton, C. H., and Zimmerman, H. M., J. Neuropathol. Exptl. Neurol. **27**, 421 (1968).

6. Rubin, R., Ames, R., Sutton, C. H., and Zimmerman, H. M., J. Neuropathol. Exptl. Neurol. **28**, 371 (1969).

7. Sarkar, N. H. and Moore, D. H., J. Microscopie **7**, 539 (1968).

8. Charney, J. and Coriell, L. L., J. Natl. Cancer Inst. **33**, 285 (1964).

9. Nowinski, R., Old, L. J., Moore, D. H. Geering, G., and Boyse, E., Virology **31**, 1(1967).

10. Klein, G., Clifford, P., Klein, E., Smith, R., Minowada, J., Kourilsky, F., and Bruchenal, J., J. Natl. Cancer Inst. **39**, 1027 (1967).

Received May 7, 1969. P.S.E.B.M., 1969, Vol 132.