Electron Microscopic Studies of Intracisternal Virus Particles in Soehner-Dmochowski Murine Sarcoma Virus-induced Bone Tumors of New Zealand Black Rats

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ABSTRACT

Soehner-Dmochowski murine sarcoma virus (Moloney)-induced bone tumors of New Zealand Black rats carry two morphologically different types of virus particles, namely, extracellular type C and intracisternal virus particles, which have thus far not been reported. These two types of virus particles have also been observed in the tissue culture cells derived from normal prostate tissues of A/Dm and BALB/c/Dm mice after inoculation of cell-free extracts of these bone tumors. The intracisternal virus particles, 90 to 120 nm in diameter, have always been found in the rough endoplasmic reticulum; they have two inner concentric layers with a relatively electron-lucent center, frequently showing cylindrical, chain-like, or multipolar budding forms. Type C virus particles produced by Soehner-Dmochowski murine sarcoma virus (Moloney)-infected prostate tissue culture cells from A/Dm and BALB/c/Dm mice belong to the murine sarcoma-murine leukemia virus group, as revealed by the fixed immunofluorescence test and by immunoelectron microscopy. The morphological and immunological relationship of intracisternal virus particles and other types of virus particles (such as type C, type H, and intracisternal type A virus particles) and intracisternal virus particles in guinea pig leukemia are defined by routine electron microscopy observations and by immunoelectron microscopy studies.

INTRODUCTION

Cell-free extracts of murine sarcoma virus (Moloney) (20)-induced bone tumors in NB rats are tumorigenic for rats, hamsters, and mice (11, 28). The virus from bone tumors of NB rats was designated SD-MSV(M). The SD-MSV(M)-induced bone tumors of rats, hamsters, and mice have been extensively studied by light and electron microscopy (10, 11, 14, 24, 29). Only type C virus particles have been commonly seen in these tumors, except that type H virus particles have been seen in the tumors induced in hamsters (14, 29).

The present studies revealed the presence of large numbers of ICVP not previously reported in the SD-MSV(M)-induced bone tumors of NB rats during serial passages. An attempt has been made to characterize these ICVP morphologically and immunologically.

MATERIALS AND METHODS

Tumors. Bone tumors in NB rats used here were serially transmitted by the inoculation of cell-free extracts of SD-MSV(M)-induced NB rat bone tumors, as reported previously (11, 28). Frozen sections of these tumors for FIF and IEM studies were cut in 6 to 10 μm slices by a cryostat without prefixation.

Cell Culture. Cultured cells used in these experiments were cells from fibroblastic lines derived from prostate tissues of 42-day-old normal A/Dm and BALB/c/Dm mice infected with SD-MSV(M) (11, 28). This virus originated from cell-free extracts of murine sarcoma virus (Moloney)-induced rhabdomyosarcoma of BALB/c mice (20). The virus-infected cells from the A/Dm mouse were named AP-MSV cells, and those from the BALB/c/Dm mouse were named BP-MSV cells.

Immune Sera. Antisera tested by FIF and IEM comprised goat antisera to Tween ether-disrupted MuLV(M), murine sarcoma virus (Kirsten), and RLV (obtained through the courtesy of Dr. Jack Gruber, Chief of Program Resources and Logistics, Viral Oncology Program, NCI) and rabbit antiserum to MuLV(F) produced in our laboratory and used after in vivo absorption. Normal goat and rabbit sera were used as control. These sera were used at 1:10 to 1:40 dilutions.

FIF Tests. The procedure of FIF tests on AP-MSV cells, BP-MSV cells, and frozen sections of SD-MSV(M)-induced NB rat bone tumors with immune sera was a slight modification of the technique previously described (16). Fluorescein-conjugated goat anti-rabbit IgG or rabbit anti-goat IgG sera were purchased from Hyland Laboratories, Los Angeles, Calif., and were used at 1:10 dilution.

IEM. The routine embedding method of tumors and tissue culture cells for electron microscopy examination was previously described (27). Ferritin- or peroxidase-conjugated IgG fraction of goat anti-rabbit IgG and of rabbit anti-goat IgG were purchased from Cappel Laboratories, Downingtown, Pa., and were used at 1:4 dilution. IEM studies of tissue culture cells and frozen sections were performed by a cell suspension method (19) or by an in situ embedding method of cells grown in BEEM capsules (21). One % acrolein or PLP containing 2% paraformaldehyde was used as prefixative (18, 23).
RESULTS

Electron microscopic examination of the SD-MSV(M)-induced rat bone tumor used in this experiment revealed large numbers of ICVP with peculiar morphology (Figs. 1 and 2) in some tumor cells in serial passages of 36 and 39 induced by the inoculation of cell-free extract. Few of these particles were found in the tumor cells in passage 4 embedded in 1968. These particles, 90 to 120 nm in diameter and budding, were always found in the rough endoplasmic reticulum. They were composed of a unit membrane and a core of about 70 nm, made of 2 inner concentric layers surrounding a relatively electron-lucent center (Fig. 1, inset). No ICVP with dense cores like those of mature type C virus particles were observed. Morphogenesis of the particles appeared to take place by budding of rounded or cylindrical forms, frequently united in chain-like formations from the wall of the rough endoplasmic reticulum (Fig. 2).

In several instances clusters of these particles were observed in the nuclei of tumor cells (Fig. 3). The clusters of particles were contained in cytoplasmic invaginations and sometimes apparently in plain nucleoplasm, as shown in Fig. 3. Besides these ICVP great numbers of typical immature and mature type C virus particles with tubular forms were observed by electron microscopic examination (Fig. 4), as was also previously reported (10, 11, 14).

Before inoculation of cell-free extract from SD-MSV(M)-induced NB rat bone tumors, prostatic tissue culture cells from mice of both A/Dm and BALB/c/Dm strains contained only a few intracisternal type A virus particles but not type C virus particles. Following virus inoculation the morphology of the cells remained unchanged, and the cells of both lines started to produce continuously type C virus particles with various stages of virus maturation. Furthermore, large numbers of ICVP with morphology identical with that of those observed in some tumor cells of SD-MSV(M)-induced bone tumors could be also detected in some AP-MSV and BP-MSV cells (Fig. 5), especially in AP-MSV cells. Following virus infection the AP-MSV cells have been maintained for 2 years and the BP-MSV cells have been maintained for 1 year.

In FIF of AP-MSV and BP-MSV cells, goat antiserum to MuLV(M) and rabbit antiserum to MuLV(F) gave strong positive cytoplasm fluorescence. Normal goat and rabbit sera were negative, as was the control stain, when used only with fluorescein-conjugated goat anti-rabbit or rabbit anti-goat IgG sera. Rabbit antiserum to MuLV(F) gave a positive cytoplasmic reaction by the immunoperoxidase method for light microscopy and was also positive in the FIF test on frozen sections of SD-MSV(M)-induced rat bone tumor.

In immunological tests by IEM with both immunoferritin and immunoperoxidase methods with the same antisera as those used in FIF tests and also with goat antiserum to murine sarcoma virus (Kirsten) and RLV, ferritin or peroxidase labeling of extracellular immature and mature type C virus particles with budding was observed in both AP-MSV and BP-MSV cells (Figs. 6 and 7). ICVP were not labeled with any of the antisera used in both AP-MSV and BP-MSV cells prefixed in 1% acrolein or even when they were treated in PLP solution, which was reported previously (18, 23) to allow the antisera and conjugates to penetrate cells in tissue culture and in frozen sections. Type C virus particles or ICVP in both AP-MSV and BP-MSV cells were not labeled with normal goat or rabbit serum by immunoferritin and immunoperoxidase. Ferritin labeling of extracellular type C virus particles, as well as of tubular forms, by anti-MuLV(M) serum was observed in cells of AP-MSV (Fig. 6a) and BP-MSV (Fig. 6b). Immature type C virus particles labeled with peroxidase by anti-MuLV(M) serum were found in AP-MSV cells (Fig. 7).

In frozen sections of rat bone tumor prefixed in PLP solution (18, 23), extracellular type C virus particles but not ICVP were labeled with peroxidase by rabbit antiserum to MuLV(F). No peroxidase labeling of virus particles was observed with normal rabbit serum.

Figs. 8 to 11 illustrate the general appearance of intracisternal type A particles, ICVP, and immature type C particles. The outer diameter of ICVP [105 nm in an average of 32 particles (Figs. 9 and 10)] was larger than that of intracisternal type A virus particles [77 nm in an average of 25 particles (Fig. 8)] but was similar to that of immature type C virus particles [101 nm in an average of 20 particles (Fig. 11)]. Although the diameters of the intermediate layer of both ICVP (73 nm average) and immature type C virus particles (75 nm average) were similar, the diameter of the inner dense layer of ICVP (54 nm average) was much smaller than was that of immature type C virus particles (61 nm average). ICVP had generally a translucent center; both ICVP from AP-MSV cells (Fig. 9) and from the cells of SD-MSV(M)-induced NB rat bone tumor (Fig. 10) gave similar measurements.

DISCUSSION

The reported ICVP appeared different from intracisternal type A virus particles in size and ultrastructure (8). They also appeared different from type H or R virus particles in fine structure of the viral core (1, 4, 22). ICVP similar to those observed in these studies could be also detected in some AP-MSV and BP-MSV cells (Fig. 5), especially in AP-MSV cells. Following virus infection the AP-MSV cells have been maintained for 2 years and the BP-MSV cells have been maintained for 1 year.

According to the description of type C virus particles (7-9), immature and mature type C virus particles should be present in the extracytoplasmic spaces. Exceptionally, intracisternal type C virus particles were reported in germ-free mice (5). As the ability to form dense nucleoids may be characteristic of noninfectious murine sarcoma virus (15), there may be some possibility that the observed ICVP, which do not show dense nucleoids, are immature and noninfectious forms of extracellular type C virus particles, in spite of their exceptional location for usual immature type C virus particles. However, ICVP observed in these studies differ from type C particles by their generally translucent core and by the negative results of ferritin or peroxidase labeling with antisera to murine sarcoma virus and murine leukemia virus by IEM. Furthermore, no morphological relationships between ICVP and extracellular type C virus particles could be detected.

Furthermore, the following results may support the possible individual transmission of these ICVP and extracellular...
type C virus particles by the inoculation of cell-free extracts of the original bone tumors in NB rats. (a) The inoculation of cell-free extracts of the original bone tumors caused the simultaneous appearance of ICVP as well as of extracellular type C virus particles in the bone tumor cells or in the cells of mouse prostatic tissue cultures. (b) The ability to produce ICVP and type C virus particles was maintained during 1 to 2 years after inoculation, and no transitional forms from ICVP to extracellular type C virus particles were observed in our electron microscopic observations, as previously reported in the case of guinea pig leukemic virus particles (1, 2). (c) Morphologically distinct immature forms of ICVP and extracellular type C virus particles were seen in the same tissue culture, as revealed by electron microscopic observations.

Other types of ICVP were also reported in the leukemic cells of guinea pigs and were described as simply ICVP (12, 25) or intracisternal type A virus particles (13). These particles, 80 to 90 nm in diameter, were smaller than were those observed in the present study. Further mature forms with dense nucleoids were present in the intercellular and rarely in the intracisternal spaces (12) and were not detected in the present study. As to the morphological relationship between ICVP and extracellular virus particles with dense nucleoids in guinea pig leukemia, it was suggested that ICVP could be released from the cytoplasm to the intercellular spaces by reverse pinocytosis (12). However, in the present studies no morphological or immunological relationship was observed between ICVP and extracellular type C virus particles. Further, ICVP in guinea pig leukemia had a thick rough outer coat, but ICVP in the present studies showed a clear unit membrane (Fig. 1, inset). Peculiar filamentous or multipolar budding patterns of ICVP observed in this study were not reported in guinea pig leukemia. Although ICVP in guinea pig were also observed in primordial germ cells of fetal guinea pigs (2, 3), ICVP reported here were not detected in the normal tissues of NB rats.

As far as our morphological and immunological studies by electron microscopy and IEM are concerned, it may be concluded that the observed ICVP were different from intracisternal type A, H, or R virus particles; extracellular type C virus particles; or ICVP in guinea pig leukemia (17, 26). Only ICVP closely related to those observed in the present studies were reported in the cells of human and simian tissue culture cells infected with RLV (6). Further investigations are obviously needed to clarify the biological and antigenic relationship of these ICVP and extracellular type C virus particles by using other approaches and methodology.

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REFERENCES

Fig. 1. Numerous ICVP including tubular forms in the rough endoplasmic reticulum of a tumor cell of SD-MSV (M)-induced NB rat leg bone tumor. × 40,000. Inset, higher magnification of a typical virus particle showing triple layers with a relatively translucent center. × 150,000.

Fig. 2. ICVP showing chain-like and multipolar budding forms in the tumor cell of SD-MSV(M)-induced NB rat jaw bone tumor. × 80,000.

Fig. 3. Virus particles in the nucleoplasm of the tumor cell of SD-MSV(M)-induced rat leg bone tumor. × 40,000.

Fig. 4. Extracellular type C virus particles with tubular forms in the SD-MSV(M)-induced NB rat jaw bone tumor. × 40,000.

Fig. 5. ICVP and extracellular type C virus particles in a prostate tissue culture cell from an A/Dm mouse infected with cell-free extract of SD-MSV(M)-induced NB rat bone tumor. × 80,000.

Fig. 6. Ferritin labeling of an immature type C virus particle with goat antiserum to MuLV(M) in AP-MSV cells (a) and of a tubular form of type C virus particle in BP-MSV cells (b). × 80,000.

Fig. 7. Peroxidase labeling of type C virus particles in an AP-MSV cell with the same antiserum as in Fig. 6. × 80,000.

Fig. 8. Intracisternal type A virus particles in normal prostate of an A/Dm mouse. × 150,000.

Fig. 9. ICVP in an AP-MSV cell. × 150,000.

Fig. 10. ICVP in the tumor cell of SD-MSV(M)-induced NB rat jaw bone tumor. Note the similarity to the virus particle of Fig. 9. × 150,000.

Fig. 11. Extracellular type C virus particles in BP-MSV cells. Note the differences in size of outer and inner layers to intracisternal type A particles (Fig. 8) and ICVP (Figs. 9 and 10). × 150,000.
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