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## PRELIMINARY REPORT

## 70 NM PARTICLES DETECTED IN LYMPHOBLASTOID CELL LINES DERIVED FROM MAREK'S DISEASE TUMORS

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The first lymphoblastoid cell lines from Marek's disease (MD) lymphomas were established by Akiyama et al. (1973) and by Akiyama and Kato (1974), and the biological characters of these cell lines, named MOB-1 and MSB-1, have been extensively investigated (Akiyama and Kato, 1974; Nazerian and Witter, 1975). In several respects the cell lines are quite analogous to those established from Burkitt's lymphoma or normal human lymphoid cells transformed by Epstein-Barr (EB) virus. Namely, a few percent of the cells always produced viral specific antigens, detected by the fluorescent antibody technique, and intranuclear viral particles, observed by electron microscopy, and the number of MDV genomes in the MSB-1 cell line was found to be between 60 and 130, which was comparable with values established for EBV DNA (Lee et al., 1975).

The ultrastructures of the various viral forms detected in the MOB-1 and MSB-1 cell lines were essentially the same as those of the viral forms observed in duck embryo fibroblasts infected with MDV. However, the two lymphoblastoid cell lines contained fewer enveloped particles and these particles were rarely found in extracellular spaces (Nazerian and Witter, 1975; Nii, unpublished observations). Moreover they contained a form of particle of about 70 nm diameter, which we have never seen before during electron microscopic observations of duck embryo fibroblasts infected with MDV, and which has never been mentioned in previous reports on the morphology of this virus. This paper is a preliminary report of the ultrastructure of this 70 nm particle.

MSB-1 and MOB-1 cell lines were both used at low passage levels of in vitro cultivation. The cells were cultured as described previously (Akiyama and Kato, 1974). Two different cell suspensions of the MSB-1 cell line, prepared from 2 and 5 day old cultures and one cell suspension of MOB-1 cells, obtained from a 5 day old culture, were used to make samples for electron microscopy. Samples were prepared essentially as described in a previous report (Nii and Yasuda, 1976).

Intranuclear viral capsids of about 100 nm diameter were detected in a few percent of the cells in all three samples. About a quarter of these capsid bearing cells also contained smaller spherical particles of about 70 nm diameter.

The nucleus in Fig. 1 contains several capsids with or without a less dense core (long arrow) and a similar number of smaller parti-



FIGURE 1 Parts of the nucleus and cytoplasm of an MSB-1 cell. The nucleus contains several viral capsids with or without cores (long arrows) and smaller spherical particles of about 70 nm diameter (short arrows).

cles of about 70 nm (short arrow). Similarly, in Fig. 2 three particles with capsids and cores and two 70 nm particles (arrow) are seen in the nucleus near the nuclear surface. Of the latter, the upper one has a clear contour of moderate density, while the lower one looks rather opaque. This difference may be due to a difference in the levels of section relative to the centers of the two particles.

Most 70 nm particles appear to consist of an inner spherical structure of high electron density and outer radially arranged projections of moderate density. This structure is



FIGURE 2 Parts of the nucleus and cytoplasm of an MSB-1 cell. In the nucleus three capsids with cores and two 70 nm particles (arrows) are seen.

especially clearly seen in the particle shown in Fig. 3. The arrow in Fig. 4 indicates a peculiar structure, looking like a distorted 70 nm particle. Possibly this peculiar form may be an early stage of these particles. At present, however, it is uncertain whether the 70 nm



FIGURE 3 A 70 nm particle in part of the nucleus of an MSB-1 cell. This spherical particle seemingly consists of an inner ring-like structure and radially arranged projections.



FIGURE 4 A particle, indicated by an arrow, consisting of an inner, distorted ring-like or polygonal structure of high electron density with several rodshaped structures of low density attached to it.

particle actually has a substructure with the appearance of rod-shaped projections or whether these projects merely represent cross sections of some strand-like structures which twin around the inner spherical structure.

The 70 nm particles are easily distinguished from the following three particles or granules: 1) 35 nm nuclear particles, which were observed in nuclei infected with MDV and HVT (herpesvirus of turkeys) (Nazerian et al., 1971), 2) adenovirus-associated viruses (AAV), which have an average diameter of 23 nm (Henry et al., 1972), and 3) perichromatin granules, which are occasionally observed in cells infected with herpes simplex and which have a characteristic appearance (Nii, 1971).

At present the most plausible explanation of the 70 nm particle is that it is an aberrant form of MDV. This particle was observed in about a quarter of the capsid-bearing nuclei

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of MSB-1 cells, but not in non-capsid bearing cells, and in general, numerous 70 nm particles were only detected in nuclei containing numerous viral capsids. The 70 nm particles were detected in both the MSB-1 and MOB-1 cell lines, but not in duck fibroblasts infected with MDV, although another aberrant form of MDV with a tubular appearance could be found in both infected duck embryo fibroblasts and lymphoblastoid cell lines (in preparation). Therefore, the question arises of whether the 70 nm particle is specific to MDVassociated lymphoblastoid cell lines and studies are required on this problem.

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