Morphological and Biological Characteristics of the M-P Strain of Lymphocytic Choriomeningitis Virus

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Based on present data, the M-P virus appears antigenically and morphologically to be a strain of the lymphocytic choriomeningitis virus, with some different biological properties.

The Molomut-Padnos virus (MPV), now determined to be a strain of the lymphocytic choriomeningitis (LCM) virus, was originally isolated from Ehrlich carcinoma and a cell culture derived from this tumor. It produces lymphocytopenia in mice as well as lymphocyte depletion in thymus and spleen and lymph nodes. MPV has been studied for its tumor inhibitory properties in murine spontaneous and transplantable leukemia and carcinoma as well as in Rauscher virus-induced leukemia and FBJ virus-induced osteogenic sarcoma (9, 10, 11; Padnos, Molomut, and Satory, Bacteriol. Proc., p. 153, 1967; Reilly and Finkel, Bacteriol. Proc., p. 217, 1971). The effect of MPV on terminal cancer in man has also been investigated in clinical trials (5, 16).

MPV is heat sensitive and other sensitive, unstable at pH 3.0 and below, and is not inactivated by 5-fluoro-2-deoxyuridine (14; Padnos, Molomut, and Schirone, unpublished data). MPV was propagated on HeLa S3 cell monolayers grown in Eagle basal medium with Hanks salts, supplemented with 10% fetal calf serum and antibiotics. After MPV absorption, cells were maintained on the same medium containing 5% serum. HeLa cell cultures were free from contamination by pleuropneumonia-like organisms as determined by broth culture agar plate assay and by the orcein method (2, 3). Peak infectivity occurred 5 days postinfection and yielded $10^7$ to $10^8$ mean infectious doses/ml when assayed in Swiss mice.

For electron microscopy, HeLa cell monolayers were grown on cover slips in Leighton tubes and in 8-oz (240 ml) rectangular bottles.

After virus inoculation cultures were incubated at 35°C without change of medium. Separate tubes and bottles were removed for study at daily intervals for 5 days. Each infected culture had a parallel uninfected control.

MPV-infected and control HeLa cell cultures were scraped from cover slips or bottles with a rubber spatula, suspended in 1% phosphate-buffered osmium tetroxide (pH 7.2-7.5), and centrifuged at 2120 x g for 10 min. The packed cells remained undisturbed for an additional 50 min in the buffered osmium tetroxide and then were dehydrated in graded ethanol and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate.

Coincident with the recovery of high titers of virus in the tissue culture fluids (days 3 to 5), large numbers of virus particles were found on the surface of the cells as a consequence of budding from the plasma membrane and from the villus-like projections of the membrane (Fig. 1–3). Occasionally, particles were seen in membrane-bound spaces near the surface of the cell. It could not be determined whether these spaces were cytoplasmic vacuoles or surface indentations cut tangentially (Fig. 2). Virus particles were usually round, rarely oval, not noted to be pleomorphic, and measured up to 160 nm in greatest diameter. They consisted of a thickened envelope, the unit membrane structure of which was indistinct in the majority of particles. Projections or spikes appeared to be attached to the surface of the viral envelope (Fig. 2). In contrast to the Fo-2 strain of LCM virus, no cup forms were noted (1).

Internally, particles contained randomly distributed electron-dense granules varying in size and shape. These granules usually had a circular outline and varied in diameter from 20 to more than 300 nm. Most of the granules were associated with the viral genome, and they appeared to be packed into subunits of the particle (Fig. 3).

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in number from 1 to 12. These granules measured up to 17 nm in diameter. Similar granules, but measuring up to 26 nm, were observed in the cytoplasm adjacent to an indistinct plasma membrane covered with projections, the apparent site of a budding virion, or as clumped aggregates randomly distributed in the cytoplasm of some cells (Fig. 4). These aggregates may represent an early stage in virus maturation prior to their distribution along the plasma membrane.

Phase microscopy revealed no evidence of cytopathic effects in HeLa cells associated with the virus infection.

Evidence for the incorporation of intact HeLa cell membrane antigens into the viral envelope is suggested by earlier studies where the specific neutralization of HeLa-grown MPV by rabbit anti-HeLa cell serum, or the neutralization by rabbit anti-mouse thymus serum of mouse-grown MPV, was demonstrated. Each antiserum was mutually exclusive for the unrelated viral envelope (Padnos, Ferris, and Molomut, Bacteriol. Proc., p. 153, 1969).

MPV is serologically related to LCM virus by cross-reactive complement-fixing antigen. LCM virus is not infective by intracerebral inoculation in mice previously immunized with MPV. Biological differences between MPV and LCM are reflected in the time required for the development of neutralizing antibody in the mouse host. Neutralizing antibody to MPV is present in titratable amounts in 28-day convalescent mice and is developed in increasing titers during the acute viremia (11, 14; Padnos and Molomut, unpublished data). LCM virus develops neutralizing antibody slowly and in titratable amounts 6 to 10 months postinfection (7). MPV does not exhibit the high-dose immune paralysis described for most strains of LCM (6) and in this respect is similar to strain E350 of Armstrong (8).

MPV-infected cell cultures possess antigens reactive with fluorescent antibody (FAB) from LCM-infected mice obtained from John Hotchin. Mouse serum samples positive for neutralizing antibody to MPV, when tested at

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**Fig. 1.** Extracellular M-P virus accumulated in large numbers in HeLa cell culture. ×25,000.
FIG. 2. M-P virus in apparent membrane-bound space of HeLa cell. Projections appear to be attached to the surface of the viral envelope. ×85,000.

FIG. 3. M-P virus in HeLa cell. Virions may be seen budding from the plasma membrane. ×37,000.

Adult mice made neonatally tolerant to MPV induce normal titers of serum interferon when superinfected with NDV, whereas LCM-tolerant mice exhibit reduced titers of serum interferon under similar conditions (4, 15).

MPV appears antigenically to be a strain of LCM and morphologically identical to LCM and other members of the arenaviruses (12, 13). The serological relationship between MPV and members of the arenavirus group has not yet been studied. MPV possesses, in addition, other immunobiological properties not exhibited by the LCM virus which are reflected by differences in the immunological responsiveness of the host.

LITERATURE CITED
Fig. 4. Moderately packed aggregates of electron-dense granules located in the cytoplasm of infected HeLa cell. The edges of the cell nucleus may be seen in the upper left portion of the figure. x31,000.


