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Efficient Propagation of Measles Virus in Suspension Cultures

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Suspension cultures of a human prostate cell line (MA160) supported abundant growth of the Edmonston strain of measles virus. The virus yields obtained with these suspension cultures (150 to 800 PFU/cell) were at least 20- to 100-fold higher than those frequently reported in the literature. Monolayer cultures of MA160 cells did not support a virus replication nearly as efficiently (progeny yield, 25 PFU/cell).

A continuous cell line derived from human prostate, MA160, (Microbiological Associates) grows well in monolayer and suspension cultures and easily adapts from one culture form to the other with only a 1-day lag in growth. In Fig. 1 we compare the growth of the Edmonston strain of measles virus in Vero cell monolayers with MA160 cell monolayers and suspension cultures. To facilitate direct comparison, the yields are reported as PFU/cell obtained after the infected cells had been frozen and thawed one time to liberate the cell-associated virus. Under these conditions, the maximum yield of virus obtained with Vero monolayers is 9 PFU/cell. Monolayers of MA160 cells yielded approximately three times as much measles as Vero cells cultured in the same way. However, the yield from suspension cultures of MA160 cells was about 20 to 100 times more than that from Vero cell monolayers and corresponded to 100 to 800 PFU/cell or 2 $\times 10^7$ to 1.6 $\times 10^8$ PFU/ml. These yields were at least 20- to 100-fold higher than those frequently reported in the literature (1, 5, 6). The yields of released virus (obtained without freezing and

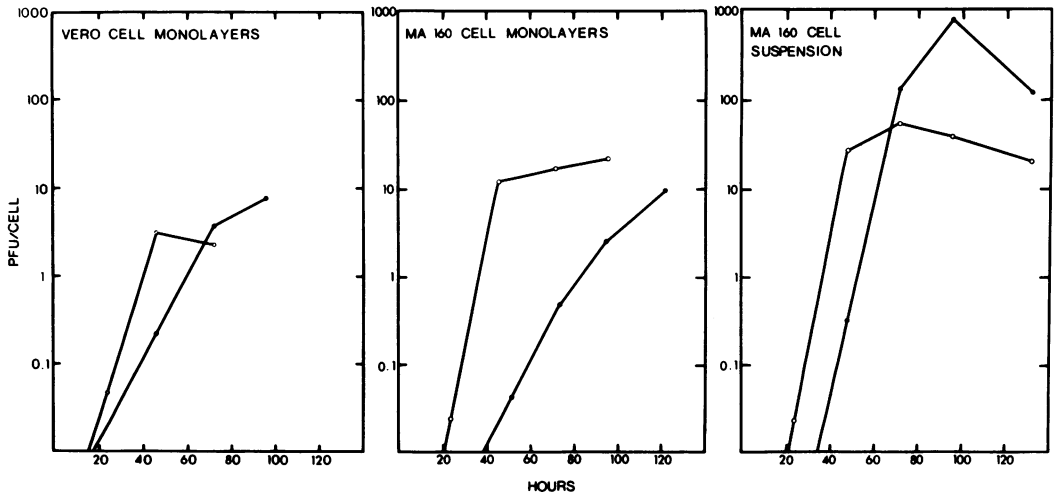

FIG. 1. *Growth of measles virus in Vero and MA160 cells. Monolayer cultures (75 cm^2) of Vero or MA160 cells were infected with measles virus at a multiplicity of infection of 0.1 (●) or 10 (○). Vero cells were cultured in Eagle minimum essential medium containing nonessential amino acids and 2% fetal calf serum. MA160 cells were cultured in the same medium but containing 10% fetal calf serum. The culture medium of the MA160 cells was changed every 48 h. Suspension cultures were infected at a multiplicity of infection of 0.1 (●) 10 and (○) at a density of 10^6 cells per ml. After a 1-h adsorption period, the infected cells were diluted to 2 $\times 10^5$ cells per ml with Eagle minimum essential medium modified for suspension culture but containing nonessential amino acids, twice the normal concentration of vitamins, and 5% fetal calf serum. The medium was replaced every 48 h. Viral titers were determined on frozen and thawed cultures by plaque assay on Vero cell monolayers.*

thawing of the cells) were in each case about 1/10 of the total virus progeny.

For the above experiments, monolayer cultures were grown in Eagle minimum essential medium fortified with nonessential amino acids and 10% fetal calf serum; suspension cultures were grown in Eagle minimum essential medium modified for suspension culture containing nonessential amino acids, twice the normal concentration of vitamins, and 5% fetal calf serum. To maintain continuous growth, suspension culture cells were collected by centrifugation and resuspended at a density of 1.5×10^5 to 2.0×10^5 cells per ml in fresh prewarmed medium every 48 h. Under these conditions, the doubling time of MA160 cells is 1.2 days.

The released and cell-associated virus was shown to be measles by two methods. First, in all three cases shown in Fig. 1, the progeny virus could be neutralized with dilutions of serum from subacute sclerosing panencephalitis patients who had high anti-measles titers but not by control human serum. Second, the progeny virus was purified as described by Bellini et al. (W. J. Bellini, A. Tardgett, and D. E. McFarland, J. Gen. Virol., in press), and the viral proteins were shown to have the same molecular weights by sodium dodecyl sulfate-gel electrophoresis (2) as those reported for measles virus proteins (3, 4). Coomassie brilliant blue staining of the gel (data not shown) revealed that the level of host proteins contaminating the virus is quite low. We suspect that this is a direct result of the high yield of virus per cell obtained in suspension cultures of MA160 cells.

In summary, we have shown that measles virus can be grown to high titers with suspension cultures of MA160 cells. This procedure appears to be admirably suited to the large-scale preparation of the virus.

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