Association of Human Cytomegalovirus (HCMV) with Mink and Rabbit Lung Cells

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With 6 Figures

Accepted October 15, 1980

Summary

The association of human cytomegalovirus with mink and rabbit lung cells was studied. Strain AD-169 was used which was free of Mycoplasma and other contaminating agents. It was found to be incapable of productively infecting mink lung cells. Infection appeared to be initiated but aborted at an early stage. This was indicated by indirect immunofluorescence, assays of culture supernatants and cell lysates for infectious virus, electron microscopy of ultra-thin sections of infected cells, labelling of virus and viral DNA with \(^3\)H-thymidine and assay of virally-induced DNA polymerase at various times after infection. On the other hand, using these methods, AD-169 was found to infect rabbit lung cells, the virus being produced in low amounts over a period of up to one month after infection. At this time, focal areas of infection were still apparent and 15 per cent of the cells expressed nuclear viral antigens as shown by immunofluorescence. The viral genome was assumed to have become latent in some rabbit cells with a few being capable of producing infectious virus.

Introduction

Human cytomegalovirus (HCMV) normally replicates in the nuclei of human fibroblasts, although there have been reports of other cells being susceptible to infection. Two human systems have been described; firstly, lung epithelial cells being productively infected with slow release of virus over several weeks and accompanying cytopathic effect typical of cytomegalovirus (12); and secondly, amnion epithelial cells being productively infected, the virus yield being lower than from equivalent fibroblasts and the replication cycle being slower (4). In addition, human embryonic kidney epithelial cells which are normally non-permissive for cytomegalovirus replication can be converted into a permissive state by treatment with iododeoxyuridine before infection (15).
Various non-human systems have also been described in attempts to find a permissive cell for HCMV replication. Guinea-pig embryo cells were abortively infected, with no new virus particles being produced (5). Early virus antigens were formed and cellular DNA synthesis stimulated. However, the DNA polymerase activity in the infected cells did not have the properties of the enzyme induced by HCMV in permissive cells (8). Mouse fibroblasts can also be abortively infected, the block in replication being at the level of viral DNA synthesis (1).

In addition, Darai and Flugel (2) found that epithelial-like mink lung cells were highly susceptible for the replication of varicella-zoster and suggested that this might be a suitable cell line for the growth of other Herpes viruses, like Epstein-Barr virus and cytomegalovirus.

Finally Farber et al. (3) discussed the replication of HCMV in rabbit lung fibroblasts. They noted a cytopathic effect in this system after 5 to 7 days which increased for 2 weeks and then remained unchanged over a period of up to 28 days. CMV antigens could be detected in the nucleus in 40 per cent of the cells by immunofluorescence at this time. Although no infectious virus was detected in the culture supernatant 3 days after infection, virus could be recovered by co-cultivation of the rabbit lung cells with human embryo lung cells even 28 days post-infection.

We decided to investigate further the association of HCMV with rabbit lung cells and to ascertain whether mink lung cells could be infected. This included an examination of infected cells by electron microscopy, growth curves over periods of up to 20 days, labelling with 3H-thymidine and subsequent extraction of viral DNA, immunofluorescence over periods of 32 days and finally measurement of DNA polymerase activity inside infected nuclei.

**Materials and Methods**

**Cells**

Rabbit lung cells were prepared by trypsinization of the lungs of a 1 year old New Zealand White rabbit and grown in Earles based Eagles medium (MEM) containing 100 i.u./ml penicillin and 100 μg/ml streptomycin and supplemented with 2 to 5 per cent rabbit serum. After the cells had formed monolayers they could be split 1:3 every week and were fibroblastic in appearance. They were used between pass 8 and 20.

Mink lung cells (Mv-1-Lu) were obtained from the American Type Culture Collection, number CCL 64, and were cultured in MEM containing 2 to 5 per cent new born calf serum and antibiotics as above. They were epithelial-like in appearance and extensive microvilli on the cell surface could be seen on electron microscopy of ultra-thin sections.

Human embryo lung cells (HEL) were prepared as described for the rabbit lung cells except that the medium was supplemented with 5 to 10 per cent new born calf serum instead of rabbit serum.

Incubation of all cells was at 37°C in an atmosphere of air containing 5 per cent CO₂. All cells were free of contamination with Mycoplasma.

**Virus**

Human cytomegalovirus (HCMV), strain AD-169, which had been plaque purified by Dr. J. M. DeMarchi and was free of other contaminating viruses as tested by Dr. G. Darai, was obtained from Dr. J. Cameron, Institute of Virology, Glasgow. It was also free of contamination with Mycoplasma species.