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Rapid Assay for Determining the Mode of Action of Compounds Effective Against Human Cytomegalovirus (HCMV). J. McSharry and C. Chutkowski Albany Medical College, Albany, NY 12208

A flow cytometric assay was developed to monitor the expression of immediate early (IE) and late (L) HCMV antigens by infecting MRC-5 cell cultures with HCMV at an MOI of 10. After incubation at 37°C for various times in the absence or presence of various concentrations of different anti-HCMV compounds, the virus-infected cells were removed by trypsinization, permeabilized with 90% methanol, treated with monoclonal antibodies to the IE or L HCMV antigens followed by FITC-conjugated goat antimouse antibody to tag the viral antigens, and RNAse and propidium iodide to specifically stain the DNA. Two color fluorescence was assayed by quantitative flow cytometry. In the absence of antiviral agents, the expression of the IE antigen commenced at 2.5 hrs and peaked at 14.5 hr post infection (PI), whereas the expression of the L antigen commenced at 48 hr and peaked at 72 hr PI. In the presence of inhibiting concentrations of ganciclovir, foscarnet or DHMPG, the expression of the IE antigens was not inhibited, but that of the late antigens was inhibited by 95%. These results confirm that these compounds block HCMV DNA synthesis which is required for the expression of the late antigens and suggest that this rapid procedure can be used to screen antiviral compounds for their effects on the various early or late stages of HCMV replication. This assay can be used to rapidly detect antiviral resistant HCMV isolates.

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Rapid determination of antiviral sensitivity of human cytomegalovirus

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The appearance of strains of human cytomegalo virus (CMV) which, in the clinic, apparently do not respond to treatment with antiviral drugs motivates the development of methods to rapidly determine the sensitivity of clinical isolates to various antiviral drugs. Previous methods to measure antiviral sensitivity have been too slow to be of help in treatment decisions. We here present a new method which utilises the expression of the strongly immunogenic CMV-antigen pp65 as an indicator of CMV replication for studies of CMV sensitivity. The monoclonal antibody CF5 was found to selectively and sensitively bind to pp65 from CMV. Isolates of CMV in five different dilutions were used to infect human lung fibroblast cells in 96 well microplates. In order to assess sensitivity to ganciclovir and foscarnet various concentrations of the drugs were added to the tissue culture medium after the virus had been removed. After 72 hours the cells were fixed with acetone/water and dried. The monoclonal antibody CF5 was added and an enzyme-linked immunosorbent assay was performed. The degree of inhibition of virus replication was calculated as IC_{50} values using a computer programme. Using this method 15 CMV isolates have been investigated for sensitivity to ganciclovir and foscarnet. This method is approximately one week more rapid than previously used techniques and will therefore be useful as a tool to select antiviral therapy, and possibly to monitor the development of resistant strains during therapy.