121

CMV-Associated DNA Polymerase Activity: Kinetics, Isolation, Characterization and Its Potential Application in the Diagnosis of CMV Disease. M. A. Nokta, M. Hassan and R. B. Pollard from Department of Internal Medicine, University of Texas Medical Branch, Galveston, Texas, USA.

CMV has been reported to be associated with a DNA polymerase activity (DPA). In this communication the kinetics of its appearance, isolation and characterization were examined. CMV DPA was measured in cell free supernatants from CMV (AD 169) infected cultures on an oligo(dT)-poly(dA) template primer. The peak of DPA activity preceded the peak of CMV replication observed at 96 hours. The use of metabolic inhibitors, actinomycin D, cycloheximide and phosphonoacetic acid (PAA) inhibited this activity. Isolation and purification of the enzyme was accomplished by PEG precipitation and gradient centrifugation of CMV followed by column chromatography of the lysed virus. SDS-PAGE and western blot analysis of the purified polymerase, under reducing conditions using an anti-CMV early antibody showed an 80 KDa protein band. However, CMV isolates and CMV from urines from CMV retinitis patients immunoblotted by the same Ab revealed 140 KDa and 80 KDa bands under non-reducing and reducing condition respectively. The diagnostic value of the CMV associated DPA was tested using CMV positive urines. The latter demonstrated high PAA-sensitive DPA activity, compared to normal and HSV positive urines and urines from HBSAg positive patients. Taken collectively, these findings indicate the potential usefulness of CMV-associated DNA polymerase activity in the diagnosis and follow-up of patients with CMV-related illnesses.

122

Combination Effects of a Human Monoclonal Anti Cytomegalovirus (CMV) Antibody (MSL 109) and Foscarnet or Ganciclovir on CMV Replication In Vitro. **M. Nokta[1], M. Tolpin[2], P. Nadler[2], and R. Pollard[1]. [1]University of Texas Medical Branch, Galveston, TX, and [2]Sandoz Research Institute, East Hanover, N.J., USA.**

Ganciclovir (GCV) and Foscarnet (FOS) are the drugs of choice for management of CMV disease. Both are not without hazards and have a narrow margin of safety. In this report the effects of human neutralizing monoclonal antibody MSL-109 (MSL, Sandoz Pharmaceutical) on CMV replication was examined alone or in combination with GCV or FOS. Human fibroblasts were infected with CMV that had been either previously neutralized with MSL (0.1-3 μg/ml) or not. Then GCV (0.3-30 μM) or FOS (50-400 μM) were added. CMV replication was determined by DNA/DNA probe hybridization on day 5 of infection. MSL in combination with GCV had an additive effect that was observed at doses of GCV of 3-10 μM and MSL of 1-10 μg. The combination index (CI) of various drug ratios at ED_{70} and ED_{95} were $\simeq$1. Meanwhile, MSL (3-10 μg) together with FOS (100 -400 μM) showed a synergistic effect on CMV replication with a CI at ED_{70} and ED_{95} of less than 0.5. Drug interactions were evaluated by the isobologram technique assuming mutually non exclusive conditions for GCV/MSL and FOS/MSL mixtures. Peak and trough MSL-109 serum levels of 15 and 4 μg/ml respectively have been attained in AIDS patients receiving 0.5 mg/kg of MSL-109 every 2 weeks without noticeable side effects. The data suggest that MSL at doses achievable in humans, enhanced GCV and FOS induced anti CMV effect and that they may be clinically useful in the treatment of CMV disease.