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**Expression And Functional Characterization Of Epstein-Barr Virus DNA Polymerase In Insect Cells Infected With A Recombinant Baculovirus**

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The DNA polymerase gene of Epstein-Barr virus (EBV) was cloned into baculovirus transfer vector (pBlueBac). The recombinant baculovirus (AcEBP-15) was obtained by co-transfection of Spodoptera frugiperda (SF9) cells with infectious DNA from Autographa californica multiple nuclear polyhedrin virus (AcMNPV) and pBlueBac plasmid carrying EBV polymerase gene. Infection of SF9 cells with the recombinant virus produced substantial quantities of the EBV DNA polymerase protein of the expected size (110 kDa). The identity of the EBV polymerase 110 kDa-polypeptide was determined by (i) immunoprecipitation and Western blot analyses with rabbit polyclonal antiserum specific for a synthetic peptide derived from the coding sequence of the polymerase gene; (ii) identification of a polypeptide of identical size (110 kDa) from EBV-infected cells; (iii) measurement of DNA polymerase activity similar to that of the enzyme induced in EBV-infected cells; and (iv) neutralization of the enzymatic activity by the rabbit antiserum and inhibition by phosphonoacetic acid. Our results indicate that the baculovirus expression system provides large quantities of functional polymerase suitable for biochemical and structural analyses, thereby furthering our understanding of the mechanism of viral DNA replication and its inhibition by antiviral drugs.

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**Comparative activity of penciclovir and acyclovir against Epstein-Barr Virus. T.H. Bacon, M.R. Boyd. SmithKline Beecham Pharmaceuticals, Great Burgh, Epsom, Surrey, KT18 5XQ, U.K.**

Epstein-Barr virus (EBV) is the cause of infectious mononucleosis (IM) which often persists for several months and may lead to serious complications in immunocompromised patients. Virus can be cultured from the throat during the acute phase of IM and intermittently thereafter, but as yet, a clinically effective chemotherapeutic agent has not been identified. In a comparative study of penciclovir (PCV) and acyclovir (ACV) against EBV in cell culture, the yield of infectious EBV released from B95-8 cells was reduced by 94% and 86% after treatment with 10μg/ml PCV or ACV respectively. Viral capsid antigen (VCA) expression by $P_3$HR-1 cells following treatment with PCV or ACV was assessed by an indirect immunofluorescence assay and also by an ELISA. Following treatment for 14 days, both compounds reduced the numbers of VCA-positive cells with $EC_{50}$s (50% effective concentration) of 4.4μg/ml and 3.3μg/ml for PCV and ACV respectively. Both compounds at 3μg/ml significantly reduced antigen expression in $P_3$HR-1 cells treated for 5 days ($p<0.05$) as measured by an ELISA. PCV and ACV were almost equally active at inhibiting EBV DNA synthesis in $P_3$HR-1 cells after 5 days treatment with $EC_{50}$s of 1.5μg/ml and 1.6μg/ml respectively. The selectivity of both compounds was confirmed in a 4-day $P_3$HR-1 cell proliferation assay, the $IC_{50}$ (50% inhibitory concentration) for PCV and ACV were >100μg/ml. PCV is thus a potent and selective inhibitor of EBV replication and has comparable activity in cell culture to ACV. ACV has no symptomatic benefit in patients with IM although limited virological responses have been observed. However, considering the greater bioavailability (1) of PCV (77%) after oral famciclovir (FCV) and the greater intracellular stability of penciclovir-triphosphate, the efficacy of FCV in IM should be tested. (1) Pue, M.A. and L.Z. Benet (1993) Antiviral Chem. Chemother. 4, Suppl. 1 47-55.