70

Disruption of Epstein-Barr Virus Episomal DNA Maintenance By A Specific Oligodeoxyribonucleotide. Jung-Chung Lin, Nancy Raab-Traub, and Joseph S. Pagano. Lineberger Cancer Research Center, School of Medicine, University of North Carolina, Chapel Hill, NC 27514.

Although nucleoside analogs are potent inhibitors of Epstein-Barr virus (EBV) replication in productive infection, neither these nor other antiviral drugs affect latent EBV infection. Specifically, none of the drugs tested has had any effect on EBV episomes, the intracellular genomic form. Episomal replication and maintenance require not only cis-acting elements of the plasmid origin of replication (ori-P), but also a trans-acting function supplied by EBV nuclear antigen-1 (EBNA-1), brought about by binding of this protein to the ori-P elements. Synthetic oligodeoxynucleotides antisense to the coding strand for EBNA-1 specifically blocked the synthesis of EBNA-1 as analyzed by Western blot and anticomplement immunofluorescence. In contrast, similar treatment with sense oligomers had no apparent effect on EBNA-1 synthesis. With the reduction in EBNA-1 synthesis there was a striking concomitant reduction in episomal copy number. Thus, this experiment provides the first successful attempt to block both EBV latent gene expression and episomal replication and may provide a conceptual approach for treatment of latent herpetic infection.

71

Point Mutations in the Herpes Simplex Virus DNA Polymerase Selectively Affect Sensitivity of the Enzyme to Novel Nucleotide Antiviral Agents. M. L. Haffey, J. T. Stevens, R. D. Carroll, B. J. Terry, A. K. Field and J. T. Matthews. The Squibb Institute for Medical Research, P. O. Box 4000, Princeton, New Jersey 08543-4000 USA

The triphosphates of the synthetic antiviral compounds (±)-(1α, 2β, 3α)-9-(2,3-*bis*[hydroxymethyl]cyclobutyl)guanine (BHCG), (±)-(1α,2β,3α)-9-(2-hydroxy-3[hydroxymethyl]cyclobutyl) guanine (HHCG) and acyclovir (ACV) were prepared enzymatically with thymidine kinase, GMP kinase, nucleoside-5'-diphosphate kinase and purified by high performance liquid chromatography. These nucleoside triphosphates were then compared for their inhibitory activity against wild type and mutant herpes simplex virus type 1 (HSV-1) DNA polymerases. The HSV-1 polymerases were derived from extracts of HSV-1 infected HeLa cells or of yeast cells expressing the viral enzyme from an inducible vector containing either the wild type HSV-1 polymerase gene or the gene containing a point mutation introduced by site directed mutagenesis. Point mutations were analyzed in this study which altered the sensitivity of the HSV-1 enzyme to various polymerase inhibitors: TYR696 to HIS696 or ASN815 to SER815. The mutation at residue 815 conferred strong resistance to ACV triphosphate (TP), moderate resistance to HHCG-TP and no resistance to BHCG-TP. The mutation at residue 696 conferred resistance to aphidicolin but not to any of the nucleotide analogs tested. These results suggest that BHCG-TP interacts with HSV-1 polymerase in a manner distinct from either ACV-TP, HHCG-TP or aphidicolin.