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Growth Of Epstein-Barr Virus-Immortalized Cell Lines Is Inhibited By Specific Antisense Oligodeoxyribonucleotides.

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The Epstein-Barr virus (EBV) immortalizes latently infected B-lymphocytes, which will grow indefinitely when explanted into tissue culture. Such cell lines are initially polyclonal and resemble B-lymphoma cells that arise in patients with AIDS and in recipients of organ transplants. During latency, which persists for life, the virus genome exists as episomes within the cell nucleus that replicate synchronously with host DNA. The only gene product needed for episomal replication is the virally encoded Epstein-Barr nuclear antigen 1 (EBNA-1), which activates the episomal origin of replication (*ori*-P). Using antisense oligonucleotides, we have attempted to block the expression of EBNA-1 in order to disrupt episomal maintenance, and hence latent infection and the immortalized state. Recently EBV-immortalized lymphocyte lines (derived from human cord blood) were treated 4 times between days 0 and 7 with P=S modified oligonucleotides that were antisense to a region near the start codon of the EBNA-1 mRNA. Cell proliferation was monitored over a period of seven days. The growth of antisense-treated cells was significantly inhibited in a dose-dependent manner by day 4 (3 of 6 cell lines), followed by cessation of cell growth and cell death in some cases. Cells treated with "sense" or "scrambled" oligonucleotides were also inhibited, but to a lesser extent. Kinetics of growth of cells recovered from the treated cultures also differed. We are presently correlating effects on cell growth with levels of EBNA-1 protein and viral episome copy number. Finally since the EBNA-2 protein is essential for immortalization, is made before EBNA-1 and activates the promoter for EBNA-1 (Cp), we are testing a combination of antisense oligomers to mRNAs for both proteins. Use of EBNA-1 antisense oligomers is the first treatment shown to produce reversal of the EBV-immortalized cell state.

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Inhibitors of *Herpes Simplex* Virus 1 Uracil-DNA Glycosylase. F. Focher,[1] A. Verri,[1] J. Gambino,[2] S. Spadari,[1] R. Manservigi[3] and G. Wright.[2] [1]Istituto Genetica ed Biochimica Evoluzionistica, Consiglio Nazionale delle Ricerche, Pavia, Italia. [2]Department of Pharmacology, University of Massachusetts Medical School, Worcester, MA, USA. [3]Dipartimento di Microbiologia, Universitá di Ferrara, Ferrara, Italia.

The post-replicative DNA repair enzyme uracil-DNA glycosylase (UDG) has been purified from nuclei of HSV1-infected HeLa cells harvested 8 hours post-infection, when induction of the enzyme is maximum. The enzyme (MW 37 kDa) has been shown to be distinct from the host UDG isolated from uninfected HeLa cells. We have identified several 6-anilinouracils that inhibit the viral enzyme with high selectivity. In an assay measuring uracil release from [^{3}H]dUMP-containing DNA, several uracil analogs inhibited both the human and viral UDGs, but the HSV1 enzyme was more sensitive to a series of 6-(*p*-alkylanilino)-uracils. Strongest inhibition of the viral enzyme was observed in the order: *p*-n-butyl < *p*-n-pentyl = *p*-n-hexyl < *p*-n-heptyl < *p*-n-octyl. The most potent inhibitor, 6-(*p*-n-octylanilino)-uracil (OctAU), with IC_{50} = 8 μM, was highly selective for the viral enzyme, and inhibition appeared to be competitive with the DNA substrate. [^{3}H]Thymidine incorporation into DNA of cultured HeLa cells was partially inhibited by OctAU, but was unaffected by the n-hexyl analog, HexAU. These compounds are the first potent and selective inhibitors of HSV1 uracil-DNA glycosylase, and studies of their use to evaluate the role of the enzyme in virus infections and reactivation will be presented.