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Potent Anti-Viral Activity of an Antisense Oligonucleotide Complementary to the Intron-Exon Boundary of Human Cytomegalovirus Genes UL36/37.

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We have identified an antisense phosphorothioate (PS) oligonucleotide complementary to the intron-exon boundary of human cytomegalovirus (HCMV) genes UL36 and UL37 that exhibits potent antiviral activity. This oligonucleotide, UL36ANTI, was shown by Southern analysis to inhibit HCMV DNA replication at concentrations as low as 0.08μM. This concentration also reduced the yield of infectious virus by 99% when compared to infected untreated controls. Studies of inhibition of viral DNA replication demonstrated that UL36ANTI was 1200-fold more potent than ganciclovir and 6000-fold more potent than PFA. Northern analysis showed that treatment with UL36ANTI decreases steady state UL36 mRNA to undetectable levels while RNA levels of a second immediate-early gene, HCMV IE2 were unaffected. This indicates that UL36ANTI was selective for UL36 mRNA and strongly suggests an antisense mechanism of action. In addition, base substitutions which result in base-pair mismatches showed lesser degrees of activity, indicating a sequence specific antisense mechanism. Anti-viral activity was not observed with oligonucleotides complementary to the UL36/37 sense strand or with other control PS oligonucleotides. UL36ANTI was also shown to inhibit DNA replication of ganciclovir and PFA-resistant HCMV strains and recent clinical isolates.

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Inhibition of HCMV DNA Processing by a New Class of Anti-HCMV Compounds (Benzimidazole Ribosides) is Mediated Through the UL89 Gene Product

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The benzimidazole ribosides, 2- bromo- 5,6- dichloro- 1- β- D- ribofuranosyl benzimidazole (BDCRB) and 2,5,6- trichloro- 1- β- D- ribofuranosyl benzimidazole (TCRB), inhibit HCMV *in vitro* with IC50s of 0.5 and 2 μM respectively[1]. These antiviral compounds act by an entirely novel and selective mechanism of action. In the presence of BDCRB, or TCRB, HCMV DNA is synthesized at rates equivalent to untreated controls[1]. However, both BDCRB and TCRB prevent processing of precursor high molecular weight HCMV DNA to unit genome length[2]. (Herpesvirus DNA replication proceeds through the formation of high molecular weight concatemers which must be precisely cleaved for packaging. This is a process unique to the DNA replication of herpes viruses and not of mammalian cells). In the presence of TCRB, immature capsids are synthesized[1] which are similar in appearance to capsids produced by a temperature sensitive mutant of HSV UL15 at the non-permissive temperature[3]. Finally, a BDCRB-resistant HCMV is cross-resistant to TCRB[2]. To map the viral gene responsible for benzimidazole resistance, discrete DNA fragments from an *in vitro* selected BDCRB-resistant HCMV genome were used for recombination into the wildtype AD169 genome. The BDCRB-resistance phenotype was mapped to within a 394 basepair region which encodes 122 amino acids of exon 2 in UL89. UL89 (the HCMV homologue of HSV UL15) encodes the most highly conserved of the herpes virus proteins and has significant amino acid similarities to known terminases from the dsDNA bacteriophage T3, T4 and T7. Sequencing of UL89 exon 2 identified 2 amino acid changes in BDCRB-resistant HCMV relative to the BDCRB-sensitive wildtype AD169.

1) Drach, J.C. and Townsend, L.B., *et. al.*, 1992 International Conference on Antiviral Research.
2) In preparation. 3) Poon, A.P. and Roizman, B. (1993) J Virol. 67, 4497-503.