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RNA-Dependent DNA Polymerases (Reverse Transcriptases) of Normal Cells: A Possible Basis for Nucleotide Analogue Toxicities? D.J. Hart*, S.R. Gogu†, K.C. Agrawal†, and R.F. Garry*, *Departments of Microbiology and Immunology, and †Pharmacology, Tulane University School of Medicine, New Orleans, Louisiana USA 70112

Retroviruses contain a virion-associated RNA-dependent DNA polymerase (reverse transcriptase, RT) that plays an essential role in the replication cycle. Reverse transcriptase activities were associated with an intracisternal A-type retroviral particle (HIAP) recovered from T-lymphoblastoid cells (RH9) exposed to salivary gland homogenates from patients with Sjögren's Syndrome (SS), an autoimmune disease. This retrovirus, named human intracisternal A-type particle (HIAP) was shown to be antigenically related to HIV-1, but differed in morphology and intracellular location. Unlike the RT of HIV which prefers Mg^{++} in the RT reaction using poly rA:oligo dT, the HIAP-associated RT had significantly greater activity with Mn^{++}. The fact that the HIAP RT had considerably lower activity when poly dA:oligo dT or oligo dT alone were included in the reaction mixtures distinguished it from cellular DNA polymerases or terminal nucleotidyl transferase. We also detected a Mn^{++}-dependent RNA-dependent DNA polymerase activity in uninfected RH9 cells. This polymerase differed from the HIAP-associated RT in that it utilized Mg^{++} to a significantly lesser extent. The hydrodynamic mobilities of the RT isolated from uninfected RH9 cells and the HIAP-associated RT also differed. In addition to the RT from uninfected lymphoid cells, we also detected significant levels of RNA-dependent DNA polymerases in normal mouse bone marrow cells, spleen cells, and liver cells. The role of these RT activities in normal cells is not known. It is possible that the RT activities of normal cells might incorporate activated 2'-3' dideoxynucleosides into DNA resulting in the toxicities associated with the use of these drugs in treatment of HIV diseases.

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Reduction of Herpes Simplex Virus Infection using Phosphorothioate Oligonucleotides Complementary to Viral mRNA. K.G. Draper and V. Brown-Driver. ISIS Pharmaceuticals, Carlsbad, CA. 92008 USA

Oligodeoxyribonucleotides (oligos) which are complementary to selected herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2) mRNA sequences were synthesized and tested for their ability to reduce the infectious yield of HSV-1 and -2 in vitro. A number of oligos with phosphodiester backbones were screened for antiviral activity. Treatment of infected cells with ISIS 1049, which is complementary to a secondary translation initiation region within the open reading frame of the UL13 mRNA, gave the best reduction of virus yield. Parallel assays showed that oligos with phosphorothioate (P=S) backbones gave greater inhibition of virus yield than oligos of the same sequence which contained either phosphodiester or methylphosphonate backbones. In an in vitro assay, the addition of either ISIS 1049 or the P=S analog, ISIS 1082, resulted in RNase H-mediated target-specific cleavage of an in vitro synthesized UL13 transcript but did not affect the integrity of a heterologous RNA. In vitro translation of the same transcripts further demonstrated that ISIS 1049 and 1082 could inhibit synthesis of the UL13 protein but had little effect upon the synthesis of protein from the heterologous RNA. Dose response data with HSV-2 strain HG52 and HSV-1 strains, KOS, DM2.1 (a TK deletion mutant) and $PAAr^5$ (a viral DNA polymerase mutant) show that ISIS 1082 is inhibitory to a broad spectrum of HSV strains and does not require activation by the viral thymidine kinase.