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Receptor targeted inhibition of HIV-I RNA production in cells by an IL-2 Diphtheria Toxin Fusion Protein. L. Zhang[1], C. Waters[2], J. Nichols[2], and C. Crumpacker[1]. [1]Beth Israel Hospital, Harvard Medical School; and [2]Seragen, Inc. Boston, MA. USA.

The recombinant fusion protein IL-2 Diphtheria toxin (DAB_{486}IL-2) was explored as an inhibitor of HIV replication in cultured human peripheral blood mononuclear cells (PBMC's). A sensitive and specific RNA-RNA target cycling hybridization assay which measures HIV reverse transcriptase mRNA in HIV infected cells was employed to show that a concentration of 10^{-8} M DAB_{486}IL-2 markedly decreases HIV RNA production in activated T lymphocytes and in quiescent cells after 7 days of infection with HIV ($H9/III_B$). Treatment of infected cells with DAB_{486}IL-2 was associated with a marked decrease in protein synthesis at a concentration of 10^{-8} to 10^{-6} M whereas in uninfected cells there was little decrease in protein synthesis at this concentration. An inactive control molecule which is identical to DAB_{486}IL-2 except for a single amino acid substitution which destroys its enzymatic activity was studied for comparison and shown to have no ability to inhibit HIV RNA production or protein synthesis in treated cells. HIV RNA production by a clinical isolate of HIV was also shown to be inhibited by DAB_{486}IL-2 and results in additional clinical isolates will be presented. These results indicate that receptor targeted toxins for HIV infected cells may provide a highly specific method to eliminate HIV infected cells in culture and in HIV infected patients.

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Crosslinking of Substrates Occurs Exclusively to the p66 Subunit of Heterodimeric HIV-1 Reverse Transcriptase. N. Cheng, G. Painter, P. Furman, Division of Virology, Burroughs Wellcome Co., Research Triangle Park, NC, USA

HIV RT catalyzes the synthesis of a duplex DNA copy of the viral RNA genome. The protein purified from virions consists of two polypeptides of molecular weights 66,000 and 51,000 (p66/p51). We have carried out photolabeling experiments using [α-^{32}P]dTTP as a representative substrate and [^{32}P]rA$_{12-18}$·dT$_{10}$ as a representative template-primer to determine on which subunit of the heterodimeric enzyme the polymerase activity resides. Both reactants crosslink to the heterodimeric and homodimeric (p66/p66, p51/p51) forms of the enzyme. Crosslinking is only observed to the p66 subunit of the heterodimer. Because the labelling efficiency was less than 100%, it was impossible to establish whether or not crosslinking occured to one or both of the subunits of the homodimers. Fluorescence binding assays indicate, however, that only one of the two binding sites available to either substrate is occupied. To determine the specificity of the reactions, the heterodimer was heated to 60° in the presence of 0.1% SDS, conditions which destroy all enzyme activity, before being irradiated in the presence of a substrate. No cross-linking of either reactant was observed to enzyme treated in this fashion. Competition experiments carried out with alternative nucleotide substrates, further demonstrated the specificity of [α-^{32}p]dTTP cross-linking. All results indicate that polymerase activity resides on the p66 subunit of the heterodimer.