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Identification of Nucleotide Binding Site of HIV-1 Reverse Transcriptase.

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We have utilized uv induced crosslinking of methy[^{3}H]dTTP to identify the nucleotide binding site on heterodimeric HIV-1 reverse transcriptase (RT). Crosslinking was carried out in a 2 ml reaction mixture containing 50 mM dTTP, 5 mM $MgCl_2$, and 0.9 mg of purified RT followed by irradiated with uv light for 10 min. Under these reaction conditions, only the 66 kDa subunit of the 66/51 RT heterodimer was labeled with dTTP. The [^{3}H]dTTP labeled RT was fragmented with trypsin and Asp-N, and fragments purified on reverse phase HPLC. The peptide covalently linked to [^{3}H]dTTP was subjected to amino acid sequence analysis. The sequencing data localized the nucleotide binding site of RT to the 6 amino acid peptide (KLVDFR) near the amino terminus of the protein.

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Sequence variations in the Reverse Transcriptase of HIV-1 from AZT-treated patients map to the binding site of a postulated HIV-1 RT model

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Objectives: 1.: To compare sequences of HIV-1 Reverse Transcriptase (RT) from 5 AZT treated patients, amplified from proviral DNA directly from the patients' lymphocytes, or from viral cDNA derived from plasma, or from primary virus derived from serial blood specimens and passaged virus. 2.: To relate the changes in the HIV RT of AZT treated patients to a three dimensional model of HIV-1 RT.
Methods: Lymphocytes from AIDS patients are FICOLL separated. Following phenol extraction and ethanol precipitation, cellular DNA is quantified and amplified using PCR. Either the entire 1.7 kbp RT gene or a 700 bp fragment is subjected to M13 cloning, or limiting dilution of DNA to single proviruses, followed by highly specific double PCR. cDNA generated by reverse transcription of plasma viral RNA is also sequenced after limiting dilution and double PCR. RT assays demonstrate activity of the amplified RT.
Results: RT sequences from our patients showed a number of aa changes. AA substitutions cluster between positions 60 to 70 and 180 to 220. In a highly resistant virus, derived from a patient who had received AZT for 2 years, 11 base mutations were found in the whole RT fragment leading to aa substitutions at 41, 138, 142, 210, 215, 248, 259, 467, 483 and 490. Plasma virus from another patient, who had received AZT for 10 months, demonstrated a single aa substitution at position 70 after generation of viral cDNA and amplification of the 700 bp fragment. Four viral sequences derived from the lymphocytes of a single patient isolate, treated with AZT for 9 months, show changes seen in sensitive and resistant viruses.
Discussion: Simultaneous mutations at aa's 67, 70, 215 and 219 have been implicated by others in the generation of high level viral resistance to AZT. We have observed more diverse mutations in drug resistant strains, as well as the ones established. The aa mutations concentrate in the proposed substrate binding site of a new RT model suggested by B. Lindborg. Also, both resistant and wild type RT sequences are present in cellular proviral DNA. We have seen only 'resistant' sequences in cDNA derived from plasma virus.