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Analysis of Reverse Transcriptase (RT) Nucleotide Sequences from AZT Resistant Strains of HIV-1 Suggest further Possible Sites of Mutation May be Involved in the Development of Drug Resistance

Levantis, P., Stein, C. A., *Ekstrand, H., *Gronowitz, S. and Oxford, J. S.; London Hospital Medical College, Academic Virology, Turner Street, London E1 2AD, UK; *Unit of Replication Enzymology, University of Uppsala, Uppsala, Sweden.

Objectives: Molecular analysis of the RT sequence of the HIV-1 *pol* gene from a total of 8 patients receiving AZT, to elucidate further potential sites of mutation associated with development of drug resistance, to assign those sites to three dimensional models of the RT polymerase domain and to investigate the role of these sites in drug resistance.

Methods: Phenol extracted genomic DNA of PBMCs from AZT treated HIV-1 infected patients served as template for PCR amplification of entire RT genes. M13 cloning and limiting dilution of DNA and nested PCR with direct sequencing were used for sequence analysis. Cloned RT genes from patients IPBMCs, lacking mutations are being used as substrate for mutagenesis.

Results: In addition to previously described mutations at position 41, 67, 70, 215 and 219, multiple mutations have been observed, varying both between individuals and within a single individual at any one time, as well as during the course of therapy. More than thirty different mutations were noted compared to samples derived prior AZT treatment, clustering in the region of amino acids between positions 60 to 70 and 180 to 220. Mutations could occur in both areas simultaneously or independently, within a particular isolate. When plotted onto a three dimensional model of the HIV-1 RT polymerase domain the two mutational domains appeared to be associated with the proposed nucleotide binding site. Multiple residues at positions 60, 62, 93, 180, 186 and 259 appear to be associated with the substrate binding site of the RT protein.

Conclusions: Demonstration of functional significance of mutations at these residues, both individually and collectively, is necessary to establish their relevance to development of drug resistance. Site directed mutagenesis of cloned RT genes and their expression in *E. coli* for enzymological studies , using a 'template destruction' assay is being used as a forerunner to whole virus reconstruction. *In vitro* enzymatic characterisation is required to examine putative resistance engendering mutations and their activity *per se* relating to catalysis and RT function.

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Development of AZT and ddI Resistance in Individuals Infected with HIV.

C.Birch, S.Stamatakos, D.Tyssen, D.Magliano, C.McGavin, S.Land, G.Tachedjian, D.Hooker, A.Gurusinghe and N.Deacon. Virology Department and Macfarlane Burnet Centre, Fairfield Hospital, Fairfield Victoria, 3078, Australia.

We compared the development of AZT and ddI resistance in patients undergoing monotherapy with either drug. Patients undergoing ddI therapy had already undergone AZT treatment and 6 of 7 were resistant to AZT when ddI therapy commenced. In individuals commencing AZT therapy, virus resistant to AZT could be isolated from 48% after 9 months AZT therapy. In contrast, resistance to ddI developed slowly, with only 1 of 7 patients being ddI resistant after at least 8 months therapy, and the difference in IC50 between ddI-sensitive and -resistant isolates was narrow compared to that seen with AZT. Resistance to AZT was more likely to occur in individuals with CD4 counts less than 200/μl at the start of therapy, but the effect of immunosuppression on the development of ddI resistance appeared less pronounced. The proportion of individuals reverting to AZT sensitivity on cessation of the drug was the same whether antiretroviral therapy was discontinued or whether the patient commenced ddI therapy. Biological data and the results of molecular studies on individuals treated with ddI will be presented.