Oral Session III — Retrovirus Infections II

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DISULFIDE-CONTAINING MACROLIDES THAT INHIBIT A LATE STAGE OF THE REPLICATIVE CYCLE OF HUMAN IMMUNODEFICIENCY VIRUS

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Macrocyclic diamides possessing a disulfide linkage (SRR-SB3, SRR-SB26 and SRR-SB28) were found to inhibit human immunodeficiency virus type 1 [HIV-1(III_B)] replication at a concentration of 1.8 to 6.5 μg/ml in MT-4, CEM and peripheral blood mononuclear cells (PBMC). SRR-SB3 was toxic to MT-4 cells at a concentration of 15.9 μg/ml resulting in a selectivity index of about 10. However, newborn mice did not suffer from any visible side effects of the drug when SRR-SB3 was administered at 75 mg/kg/day for 10 subsequent days. SRR-SB3 was also effective against various other HIV-1 strains, including clinical isolates and HIV-1 strains resistant to protease inhibitors, nucleoside and non-nucleoside reverse transcriptase (RT) inhibitors. It was also active against various HIV-2 strains, simian immunodeficiency virus (SIV/MAC251) and murine (Moloney) sarcoma virus (MSV), but not against viruses other than retroviruses. In addition, the compounds were found to inhibit chronic HIV-1 infection *in vitro*. SRR-SB3 showed a subsynergistic effect on HIV replication when combined with other antiviral agents, such as AZT (zidovudine), ddC (zalcitabine) and d4T (stavudine). Time-of-addition experiments indicated that SRR-SB3 acts at a late stage of the HIV-1 replicative cycle. Additional results to elucidate the exact mechanism of action of these compounds will be presented.

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Novel isoforms of HIV-1 *nef* are expressed by frameshifting and selenium-dependent suppression of UGA termination codons.

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Retroviruses use either ribosomal frameshifting or termination suppression to synthesize their *pol* gene products. By genomic analysis, we recently identified additional potential frameshift and suppression sites in HIV-1, e.g. in the *nef* coding region, which contains an "ideal" heptameric -1 frameshift sequence, UUUAAAA, followed by a potential RNA pseudoknot. In addition, the *nef* ORF terminates in a highly conserved UGA codon (which can potentially encode selenocysteine), followed by a conserved "readthrough" ORF of 32 amino acids. All of these *nef*-associated features are as well conserved in HIV-1 isolates as the *nef* ORF itself. Using standard *in vitro* assays, we have found that the putative -1 frameshift sequence in *nef* is functional, and that translational readthrough suppression of the UGA terminator is selenium dependent. To see if these *nef* variants are formed *in vivo*, we examined post-mortem brain tissues from pediatric AIDS cases, in which "restricted" HIV-1 infection characterized by *nef* overexpression has been shown to occur in astrocytes. Antisera directed against a synthetic peptide representing the putative readthrough sequence immediately following the terminal UGA codon of *nef* gave positive immunocytochemical reactions in astrocytes, in HIV+ brain sections. In addition to being the first experimental demonstration of new coding potential in HIV since 1988, these findings are clearly significant in the light of recent data showing that serum selenium predicts outcome in HIV infection (e.g. Constans et al., *J. AIDS Human Retrovirol.* **10**: 392, 1995).