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Poster presentation

Detection of A Shared HIV Protease-RT Deletion in Patient Plasma & Cells: A Role For ARV-mediated Selection and Viral Complementation

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Background

A deletion in HIV pol spanning amino acid residues 59–99 of protease and 1–204 of reverse transcriptase (RT) was detected in 12/22 plasma specimens from patients receiving antiretovirals (ARVs). It was subsequently detected in cerebrospinal fluid, blood monocytes and T-cells, brain and lymphoid tissues. It was always accompanied by an I54V mutation in protease, and L210W, R211K and L214F mutations in RT. Sequencing and other experiments confirmed that it is not a PCR artifact.

Materials and methods

To study the functional impact of this defect, the 738 bp deletion was inserted into an infectious macrophage-tropic molecular clone of $HIVSF_{162}$ carrying green fluorescence protein (GFP).

DNA from the GFP-containing defective virus clone was transfected into 293T cells alone, or along with DNA from the wildtype (wt) SF162 clone lacking GFP. Supernatants were collected, assessed for HIV by RT-PCR, and used to infected primary macrophages and T-cells, and CEM cells expressing CCR5 (CEM-R5). Transfected and infected cells were monitored for GFP expression by microscopy, and for the presence of defective and wt genomes by PCR. To determine if ARVs can influence the selection or persistence of this mutant, long-term cultures of CEM-R5 harboring both wt and mutant viruses were cultured with these drugs.

Results

GFP-expression was detected in cotransfected 293T cells, and in all cells infected with virus from cotransfected 293T. Cotransfection sups contained mutated and wt viruses suggesting that complementation, in addition to recombination within 293T, could have occurred. Mutated genomes persisted in CEM-R5 for 51 days, but decreased with time. Increased levels of mutants were detected in persistently-infected CEM-R5 at 3 and 9 days after exposure to AZT and saquinavir, respectively.

Conclusion

Exposure to ARVs may lead to creation of particles with large deletions in pol. These defective genomes could recombine with, or be complemented by, ones with functional protease and RT genes. Their persistence may represent a form of viral latency or mode of viral escape.