O023
2-hour Oral Session
New insights in epidemiology, resistance and pathogenesis to improve HIV management

HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 TRANSCRIPTS USING SPLICE SITES NEAR THE 3' END OF THE VIRAL GENOME ARE FREQUENTLY EXPRESSED IN VIVO AND ENHANCE VIRAL REPLICATION

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Objetives:

HIV-1 RNAs have been usually classified in three major categories: unspliced (~9 kb), singly spliced (~4 kb), and doubly spliced (~2 kb). We recentlydescribed a fourth class of spliced RNAs of ~1 kb using 3' splice sites (3'ss) near the 3' end of the viral genome in the Nef coding sequence, most of them potentially coding for a 33-34 amino acid peptide corresponding tothe carboxy-terminus of Nef (Carrera et al. 2010; AIDS Res Human Retroviruses; 26:815-820). The objectives of this study are to examine the expression of these RNAs in diverse cell populations *in vivo* and to analyze their effect on HIV-1 replication.

Methods:

Peripheral blood mononuclear cells (PBMCs) from HIV-1-infected individuals were separated from whole blood by centrifugation on Ficoll and subjected to immunomagnetic separation to obtain the following cell types: $CD4^+CD25^+$, $CD4^+CD25^-$, $CD8^+$, and $CD4^-CD8^-$ lymphocytes, and monocytes ($CD14^+$). HIV-1 1 kb RNAs were amplified from total RNA by RT-nested PCR using primers recognizing sequences near both ends of the HIV-1 genome. In 21 individuals, PCR-amplified DNA bands with sizes consistent with 1 kb RNAs were extracted from agarose gels and sequenced. In 22 individuals, clones from RT-PCR products obtained from $CD4^+CD25^+$ lymphocytes were sequenced. Four clones from 1 kb RNAs were subcloned into an expression vector under cytomegalovirus transcriptional promoter control, and their effect on HIV-1 replication in PBMCs was examined by nucleofection with HIV-1 infectious molecular clone p 89.6, with determination of p24 antigen in supernatant at 48 hours.

Results:

Through direct sequencing from gel-extracted bands, HIV-1 1 kb RNAs were detected in 9 (43%) of 21 individuals, with a higher frequency in $CD4^+CD25^+$ lymphocytes (33%) and sporadically in other cell types. In clones from $CD4^+CD25^+$ lymphocytes, HIV-1 1kb RNAs were detected in 8 (36%) of 22 individuals (a frequency which was similar to that of *rev* RNAs, 41%). Notably, in 6 (27%) individuals, 1 kb RNA clones were more abundant than clones derived from HIV-1 doubly spliced RNAs. In nucleofection assays in activated PBMCs, all four cloned cDNAs derived from 1 kb RNAs induced an enhancement of HIV-1 production by p89.6 isolate by factors of 1.4 to 2. In nonactivated PBMCs from a second donor, the two clones that were assayed induced increases in p24 production by factors of 2.5 and 3.2, respectively.

Conclusions:

HIV-1 1 kb RNAs using 3'ss near the 3' end of the viral genome are detected *in vivo* in different PBMC types from HIV-1-infected individuals, more frequently in $CD4^+CD25^+$ lymphocytes, where they were more abundant than HIV-1 doubly spliced RNAs in 27% individuals. In nucleofection assays in PBMCs, plasmids expressing 1 kb RNAs enhanced HIV-1 replication, both in activated and nonactivated cells.