O021
2-hour Oral Session
New insights in epidemiology, resistance and pathogenesis to improve HIV management

CHANGES IN PATTERNS OF EXPRESSION OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 SPLICED RNAS IN DIVERSE PERIPHERAL BLOOD MONONUCLEAR CELL TYPES ASSOCIATED WITH DISEASE PROGRESSION

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Objectives

Human immunodeficiency virus type 1 (HIV-1) RNAs undergo a complex splicing process generating a great diversity of transcripts, classified in three categories: unspliced, singly spliced (SS), and doubly spliced (DS) RNAs. Knowledge on in vivo HIV-1 splicing patterns and their association with disease progression is scarce. Here we aim at detecting and quantifying HIV-1 spliced RNAs in diverse peripheral blood mononuclear cell (PBMC) types of HIV-1-infected individuals, analyzing correlations with disease progression.

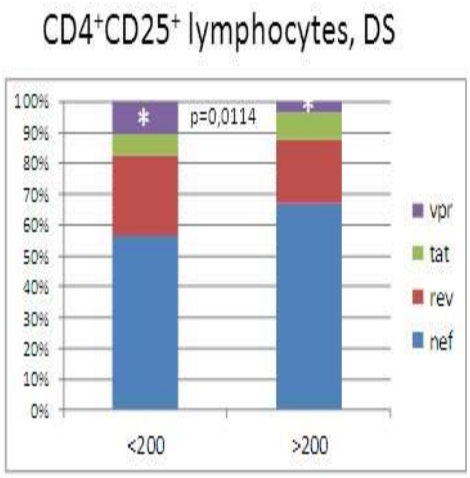
Methods

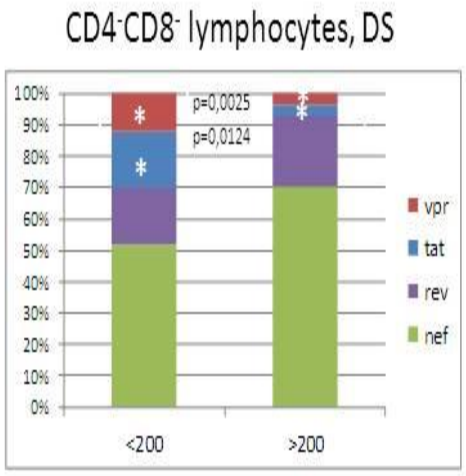
PBMCs from 37 HIV-1-infected antiretroviral drug-naïve individuals were immunomagnetically separated in different cell types: $CD4^+CD25^+$, $CD4^+CD25^-$, $CD8^+$, and $CD4^-CD8^-$ [double negative (DN)] lymphocytes, and monocytes ($CD14^+$). HIV-1 DS and SS RNAs were amplified from total cellular RNA by RT-PCR and nested PCR using primers recognizing 5' and 3' exons common to all RNAs of each class, yielding amplicons of different sizes, according to splice sites (ss) used. In nested PCR, a 5'-fluorescently-labeled primer was used, allowing precise size determination and quantification of amplified products by GeneMapper software after electrophoresis in an automated sequencer. Differences in relative expression of RNAs between groups with different $CD4^+$ cell counts and different cell types were analyzed using Mann-Whitney and Kruskal-Wallis tests, respectively. PCR products from HIV-1 DS RNAs from $CD4^+CD25^+$ cells from 17 individuals were analyzed by deep sequencing using GS FLX System.

Results

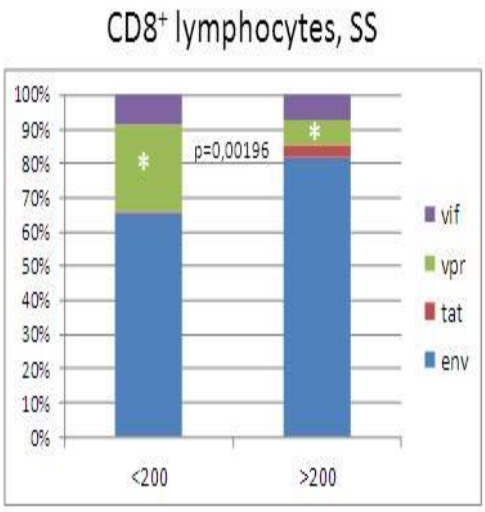
HIV-1 spliced RNAs were detected in all cell types, at frequencies of 78%, 70%, 59%, 59% and 65% individuals among $CD4^+CD25^+$, $CD4^+CD25^-$, $CD8^+$ and DN lymphocytes, and monocytes, respectively. Mean relative expression was, among DS RNAs, nef 65.5%, rev 22%, tat 8.8%, and vpr 4.4%; and among SS RNAs, env-vpu 79.3%, tat 4.5%, vpr 13.2% and vif 3%, without significant differences between cell types in the global analysis. Comparing patients classified according to $CD4^+$ cell counts ≥200 vs. <200/µl, significant differences ($p<0.05$) were found in expression of DS vpr RNAs in $CD4^+CD25^+$ lymphocytes (mean 3.6% vs. 10.3%), DS tat (3.7% vs. 18%) and vpr (3.2% vs. 12%) RNAs in DN lymphocytes, and SS vpr RNAs in

CD8⁺ lymphocytes (7.7% vs. 25.7%). Deep sequencing confirmed peak identifications made with GeneMapper and allowed the identification of three novel 3'ss used by HIV-1 RNAs in three samples, two used by rev RNAs and one by nef RNAs.





*p<0.05
(Mann-Whitney test)



Conclusions

HIV-1 individual spliced RNAs were identified and quantified in vivo in diverse PBMC types of most HIV-1-infected individuals, with relative expression within DS and SS classes being similar between cell types and generally consistent with reported in vitro results, except that vpr RNA expression was considerably higher than reported. Significant increases in vpr and tat RNA expression in some cell types associated with disease progression suggests a possible role of the regulation of HIV-1 RNA splicing in pathogenesis.