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Abstract withdrawn

PP 3.24

HIV-1 mediated insertional activation of STAT5B promotes the formation of a viral reservoir in T regulatory cells

D. Cesana[1], F.R. Santoni de Sio[1], L. Rudilosso[1], P. Gallina[1], A. Calabria[1], L. Passerini[1], S. Nozza[2], E.Vicenzi[3], G. Poli[3], G. Tambussi[2], E. Montini[1]

[1]San Raffaele Telethon Institute for Gene Therapy (HSR-TIGET), San Raffaele Scientific Institute, Milan, Italy, [2]Department of Infectious Diseases, San Raffaele Scientific Institute, Milan, Italy, [3]AIDS Immunopathogenesis Unit, San Raffaele Scientific Institute, Milan, Italy

It has been suggested that HIV-1 by integrating near cancer-associated genes could promote the expansion and persistence of infected cells in patients under antiretroviral therapy (ART). However, the molecular mechanism/s of insertional mutagenesis used and the physiological impact on the cells harboring these integrations are completely unknown.

Here, we found that in peripheral blood mononuclear cells (PBMC) from 54 HIV-1 infected patients under ART, *BACH2* and *STAT5B* were targeted by a significantly higher number of integrations ($P<0.0001$) and with the same orientation of gene transcription compared to other lentiviral integration datasets. Furthermore, aberrant chimeric transcripts containing viral sequences fused to the first protein coding exon of *BACH2* or *STAT5B* and predicted to encode for unaltered full-length proteins were found in PBMC of 34% of HIV-1 patients under ART (30/87). Tracking the expression HIV-1/*STAT5B* transcripts in purified T cell subpopulations and monocytes ($n=6$) we found a specific enrichment of chimeric HIV/STAT5B mRNAs in T-regulatory (reg) and T-central memory (cm) cells in all patients tested ($n=6$). *In vitro* experiment on CD4+ T-cells isolated from healthy donor showed that forced expression of these transcription factors significantly increased their proliferation rate and do not alter the immune-suppressive function of T-reg cells.

Hence, our findings provide novel evidence that HIV-1 takes advantage of insertional mutagenesis to favor its persistence in the host by activating *STAT5B* and *BACH2*. Indeed, the selective advantage conferred by these integrations should favor the survival and proliferation of T-reg and T-cm cells which are long lived, potentially able to diminish the immune surveillance against infected cells thus favoring long-term viral persistence.

PP 3.25

Diverse proviral structure of HIV integrants in clonally expanded cells

F.R. Simonetti[1,2], J. Spindler[1], X. Wu[3], F. Maldarelli[1], J.M. Coffin[4]

[1]HIV Drug Resistance Program, National Cancer Institute, National Institutes of Health, Frederick, MD, USA, [2]Department of Biomedical and Clinical Sciences Luigi Sacco, University of Milan, Italy, [3]Leidos Biomedical Research, Frederick, MD, USA, [4]Tufts University, Department of Molecular Biology and Microbiology, Boston, MA, USA

Background: Clonally expanded populations of HIV infected cells persist during prolonged cART. Recently, we identified several highly expanded lineages, including a clone with an integrant in the HORMAD2 gene that accounted for c. 50% of all of the infected cells. Integration into specific introns of BACH2 or MKL2 increased the expansion and/or survival of infected cells. In one instance a clonally expanded provirus was infectious. The proviruses in these clonally expanded cells have not been adequately characterized. We describe characterization of other proviruses in highly expanded clones.

Methods: Longitudinal PBMC samples were obtained from volunteers with chronic HIV infection. Integration sites were determined as previously described. Proviruses integrated in HORMAD2, MKL2, and an intergenic region of the X chromosome were selectively amplified using specific primers for HIV and the flanking host DNA.

Results: The HORMAD2 integrant was present both pre- and on-cART and accounted for 50–80% of all of the infected cells after 7–8 years on cART, implying that expansion of the clone started early in infection. Proviral sequence analysis revealed a 675 nt single LTR with intact promoter elements. Analysis of a highly expanded provirus in an intergenic region of the X chromosome also revealed evidence for a solo LTR. Analysis of seven integrants in MKL2 were also characterized. One of the proviruses had a large pol-U3 internal deletion; of the remaining, four proviruses showed evidence GtoA mutation leading to multiple stop codons and three were found to lack intact tat or rev. All seven, however, had intact LTR promoter elements and retained the major splice donor sequence.

Conclusions: We found proviruses in expanded clones that were intact, hypermutated, partially deleted or consisting of a solo LTR. MKL2 proviruses, have intact LTR regulatory elements but some lack tat, which is normally required for HIV transcription.

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No selection of CXCR4-using variants in cell reservoirs of dual-mixed HIV infected patients receiving suppressive maraviroc therapy

J. Izopet[1–3], S. Raymond[1–3], F. Nicot[1,3], N. Jeanne[3], P. Delobel[1,2,4] and the ANRS 145 MARIMUNO Study group

[1]INSERM, U1043, Toulouse, France, [2]Université Toulouse III Paul-Sabatier, Faculté de Médecine Toulouse-Purpan, Toulouse, France, [3]CHU de Toulouse, Hôpital Purpan, Laboratoire de Virologie, Toulouse, France, [4]CHU de Toulouse, Hôpital Purpan, Service des Maladies Infectieuses et Tropicales, Toulouse, France