OA3-4

Distinct HIV genetic populations in effector memory T-cells after prolonged therapy

Lee E.1,2, Hiener B.1,2, Bacchetti P.3, Shao W.4, Boritz E.5, Douek D.5, Somsouk M.6, Hunt P.6, Fromentin R.7, Deeks S.G.6, Hecht F.M.6, Chomont N.7, Palmer S.1,2

1Westmead Millennium Institute for Medical Research, Centre for Virus Research, Westmead, Australia, 2University of Sydney, Sydney Medical School, Sydney, Australia, 3University of California San Francisco, Department of Epidemiology and Biostatistics, San Francisco, United States, 4Leidos Biomedical Research, INC, Frederick National Laboratory for Cancer Research, Frederick, United States, 5National Institutes of Health, Immunology Laboratory, Vaccine Research Center, National Institute of Allergy and Infectious Diseases, Bethesda, United States, 6University of California San Francisco, Department of Medicine, San Francisco, United States, 7Vaccine and Gene Therapy Institute of Florida, Port Saint Lucie, United States

Background: The effect of prolonged antiretroviral therapy (ART) on the genetic composition of persistent HIV in cellular reservoirs is unknown. We examined the genetic makeup of HIV DNA sequences within T-cell subsets from peripheral blood and gut tissue of persons on ART for >15 years.

Methods: Using single-proviral sequencing, we isolated HIV DNA from naïve, stem cell memory (TSCM), central (TCM)-, transitional (TTM)- and effector (TEM)-memory and homing CD4+ T-cells (expressing CCR6, CXCR5 or both markers) sorted from peripheral blood and total CD4+ T-cells sorted from rectal biopsies. Samples were collected from 6 subjects on ART for >15 years: 3 who initiated therapy during early infection and 3 during chronic infection. Hypermutants, drug resistance mutations and identical sequences were identified by phylogenetic analysis. We used the tat/rev induced limiting dilution assay (TILDA) to measure the frequency of cells with inducible multiply spliced HIV RNAs (msRNAs).

Results: In subjects treated during chronic infection, TEM contained genetically distinct HIV populations, often clonal in nature, compared to other cells. In one subject all HIV sequences (n=62) from TEM were hypermutants and 82% clonal, whereas all other T-cell subsets had significantly fewer identical HIV DNA hypermutants (p=< 0.0001-0.001). Another subject, with a history of sequential ART regimens, had wildtype HIV sequences in TEM (92%) and more drug resistant HIV in other T-cell subsets (p=< 0.0001-0.0004). TEM from the third chronic subject contained 73% clonal drug resistant HIV sequences whereas the other cells had only 7-15% (p=< 0.0001-0.0002). In one subject treated during early infection, 44% of all HIV sequences were hypermutant: 56% in TEM and 86% in CD4+ T-cells from rectal biopsies. All subjects had inducible HIV msRNAs in memory T-cell subsets as measured by TILDA but msRNAs were lower in TEM from individuals containing hypermutant populations.

Conclusions: The distribution of HIV genetic material among memory subsets varied dramatically across the cohort after prolonged ART. TEM are marked by clonal expansions which may reflect random antigen-driven cellular proliferation and expansion. Enrichment of hypermutant HIV in TEM suggests new infection events during proliferative bursts are attenuated by cellular restriction factors and/or by death of cells expressing replication competent virus.