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

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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.