THE GENETIC TRAITS OF FULL-LENGTH HIV SEQUENCED FROM MEMORY T CELL SUBSETS

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Background
A thorough understanding of the distribution and genetic traits of replication-competent HIV will be needed to design eradication therapies. We used the Full-Length Individual Proviral Sequencing (FLIPS) assay to examine the traits of proviruses within memory CD4+ T cell subsets, and their contribution to the latent reservoir during ART.

Methods
Naïve, central (CM), transitional (TM) and effector (EM) memory CD4+ T cells, and CD45RA-HLA-DR+ and CD45RA-HLA-DR- CD4+ T cells, were sorted from the peripheral blood of six participants who initiated ART during either acute or chronic infection (n=3 each). FLIPS was used to obtain near-full-length HIV DNA sequences, using LTR-specific primers to amplify proviruses at limiting dilution followed by next-generation sequencing. Proviruses were characterized as defective or genetically-intact. Expansions of identical sequences (EIS) were determined as ≥2 identical proviral sequences.

Results
Of the 728 sequences isolated, 5% were considered intact. Intact provirus was found in all cell subsets except CM (0/125 sequences intact). The proportion of intact provirus was different across the cell subsets (EM>TM>CM and HLA-DR+> HLA-DR-; p=0.001). The frequency of cells infected with intact provirus was highest in HLA-DR+ memory T cells (48 vs <10 infected cells/million cells in HLA-DR+ vs all other subsets). Eighty-three percent of intact proviruses were CCR5-tropic. The percentage of intact and defective sequences contributing to an EIS was 34% (12/35) and 46% (319/693) respectively. In one participant, 56 identical sequences contained a defective packaging signal but were intact in the coding region. Despite this, corresponding intracellular RNA was detected.

Conclusions
Genetically intact and therefore likely replication-competent, CCR5-tropic HIV is enriched in cells expressing HLA-DR and EM cells. This indicates the latent HIV reservoir is established early and maintained by cell proliferation and differentiation. Defective proviruses can also produce viral transcripts, demonstrating that RNA quantification is not specific for cells containing intact HIV.