AN HIV-INFECTED INDIVIDUAL ON SUPPRESSIVE ART WITH A MASSIVE EXPANSION OF EFFECTOR MEMORY T-CELLS CONTAINING A DEFECTIVE PROVIRUS

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Background:
Cellular proliferation can contribute to the maintenance of the persistent HIV reservoir within individuals on antiretroviral therapy (ART). Here we report the unique case of an ART-treated individual with a massive expansion of a defective provirus.

Methods:
Memory CD4+ T-cell subsets were sorted based on the expression of CD45RA, CD27 and CCR7. The frequency of memory cells harbouring integrated HIV DNA was quantified using Alu-PCR, and integrated proviruses were genetically analysed using single-genome sequencing targeting the HIV envelope (ENV) region. T-cell receptor clonality was analyzed in memory CD4+ T-cell subsets by high-throughput sequencing of the T-cell receptor (TCR) β-chain gene using CDR3 sequences. HIV integration sites were found by extracting and sonicating genomic DNA followed by ligation-mediated PCR and next-generation sequencing (NGS). To genetically characterise the provirus in effector memory T-cells, we used a modified full-length individual proviral sequencing (FLIPS) assay, which amplifies HIV provirus at limiting dilution followed by NGS.

Results:
A high percentage (45%) of effector memory T-cells (EM) contained an integrated HIV genome. The majority of the ENV sequences were genetically identical in EM cells. One clonotype accounted for 60% of the TCR sequences of the EM cells. Chromosome 14 was the only integration site shared across all sorted memory CD4+ T-cell subsets and represented the highest frequency in EM cells. Using forward primers in chromosome 14 and reverse primers in the 3'LTR of HIV, we sequenced the expanded provirus in EM cells. The majority (83%) of these proviruses belonged to an identical sequence expansion which matched the ENV region from our earlier sequence analyses but lacked the 5'LTR-pol region.

Conclusion:

These results indicate that a massive expansion of a single defective provirus can occur *in vivo*. These proliferative events contribute to the weak association between DNA measurements and replication competence in some individuals.