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NO EVIDENCE FOR ONGOING HIV REPLICATION IN LYMPH NODES DURING SUPPRESSIVE ART

Basic Science: (D) HIV Reservoirs, Latency, and All Curative Strategies Including Therapeutic Vaccines and Gene Therapy

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Background: Lymph nodes have been implicated as potential sanctuary sites of ongoing HIV replication during ART due to inadequate drug penetration. To investigate this possibility, we characterized HIV proviral populations, their levels of expression, and their sites of integration in paired lymph node (LNMC) and peripheral blood (PBMC) samples collected after long-term ART.

Methods: PBMC and LNMC were obtained from five donors: four had <40 cps/ml on ART for 4.3-12.9 years and one was ART-naïve. Pre-ART samples were obtained for three of the treated patients. Longitudinal on-ART LNMC were obtained from two patients one year apart, including sampling from two different inguinal nodes in both individuals. Proviral populations and expression were characterized by cell associated RNA- and DNA- single genome sequencing of p6-PR-RT. Proviral sequences were compared phylogenetically and by testing for panmixia. Infected cell clones were identified by integration sites assay (ISA) in PBMC and LNMC from one donor.

Results: Comparisons of the proviral sequences on ART in PBMC (n=176) and LNMC (n=234) showed no increase in branch length, diversity, or divergence from pre-ART plasma or PBMC due to ongoing viral replication in either location. A test for panmixia of proviral sequences in PBMC and LNMC and across two separate lymph nodes sampled at the same time point showed no evidence for compartmentalization (probability of panmixia p>0.3). Proviruses with identical sequences were found in LNMC and PBMC and were transcriptionally active at both sites, although a greater fraction of infected cells in LNMC was expressing HIV RNA than in PBMC (13% vs. 6%). High-expressing cells (>20 HIV RNA copies/cell) were observed in samples obtained prior to but not during ART, with the exception of one LNMC. In one patient, forty clones of infected cells were identified by ISA. There were no differences in the locations of these clones in PBMC vs. LNMC (p=0.8).

Conclusion: Comparison of proviral populations, including clones of infected cells, and their expression in LNMC and PBMC, showed that populations of infected cells were well-mixed. There was no evidence of tissue compartmentalization. There was also no evidence for divergence from pre-ART populations in PBMC or in LNMC whether ART was initiated in acute or chronic infection, which is not consistent with the HIV reservoir being maintained by ongoing cycles of viral replication in either PBMC or LNMC during suppressive ART.