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Identification and characterization of CD4 T cells actively transcribing HIV RNA in peripheral blood

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Background: Determining the phenotype and molecular characteristics of cells within PBMC that are actively transcribing HIV will be critical to cure strategies. The goal of this study was to identify and characterize cells actively transcribing HIV in peripheral blood.

Methods: Live $CD8^-$ PBMCs from 6 HIV-infected individuals not on ART were sorted into CD4 bright, dim, and null populations. Antibodies to markers of T cell activation and HIV antigens were used to further identify cells expressing HIV. Spliced HIV RNAs were identified using primers and probes designed to span tat and rev mRNA splice sites; unspliced HIV RNA was identified using gag primers and probes. The frequency of T cells containing HIV RNA, and the quantity of HIV RNA in each population was determined using limiting dilution RT qPCR. HIV RNA copy number per cell was determined using values from wells likely to containing a single HIV RNA^+ cell. Measurable tat and/or rev RNA was used as evidence of active transcription from proviral DNA.

Results: The median frequency of cells containing unspliced RNA in the CD4 bright population was 0.054% significantly greater than the frequency of spliced HIV RNA in the same population, 0.019%. In the CD4 dim population there was routine co-expression of spliced and unspliced RNAs with a median frequency of 0.14%. Median spliced and unspliced RNA copy number increased with decreasing surface CD4 T cell expression. Median unspliced RNA copy number for cells transcribing proviral DNA was 113(20-325) in CD4 bright cells, 198(0-981) in CD4 dim cells and 703(191-2699) copies/cell in CD4 null T cells. Using markers of T cell activation a population of T cells was identified in which 1 in 30 (3.3%) were actively transcribing HIV. Broadly neutralizing env-specific monoclonal antibodies were used to identify HIV-infected cells directly ex vivo.

Conclusion: These data show viral RNA transcription in vivo is associated with strong down regulation of CD4 and identify methods of detecting cells within PBMC with a very high probability of active HIV transcription. Identifying genes expressed by cells actively expressing HIV will allow for the development of strategies to specifically target them for killing.