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Compartmentalized clonal expansions of CD8⁺ T cells in CSF during acute HIV infection

Julie Mitchell[1], Caroline Subra[2,3], A. Julian Pacheco Mendez[1], Faria Fatmi[2,3], Blake Colton[1], Supranee Buranapraditkun[4,5], Suteeraporn Pinyakorn[2,3], Carlo Sacdalan[6], Somporn Tipsuk[6], Nittaya Phanuphak[6], Denise Hsu[2,3], Sandhya Vasan[2,3], Serena Spudich[7], Lydie Trautmann[1,2,3], on behalf of the SEARCH010/RV254 Study group

[1] Vaccine and Gene Therapy Institute, Oregon Health and Science University, Beaverton, OR, USA [2] U.S. Military HIV Research Program, Walter Reed Army Institute of Research, Silver Spring, MD, USA [3] The Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., Bethesda, MD, USA. [4] Department of Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. [5] Chulalongkorn Vaccine Research Center, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. [6] Institute of HIV Research and Innovation (IHRI), Bangkok, Thailand. [7] Yale University School of Medicine, New Haven, CT.

BACKGROUND

Activated CD8⁺ T cells infiltrate the central nervous system (CNS) early in acute HIV infection (AHI). Whether CD8⁺ T cells in cerebral spinal fluid (CSF) are CNS specific or recirculate from the peripheral blood is not yet known. We characterized the CD8⁺ T cells in CSF and blood in different stages of HIV infection prior to and after initiation of antiretroviral therapy (ART) by sequencing their T cell receptor (TCR) and measuring frequencies of HIV-specific CD8⁺ T cells.

METHODS

- Participants enrolled in the Thai RV254 and RV304 cohorts who consented to optional lumbar puncture were studied. Peripheral blood mononuclear cells (PBMCs) and CSF samples were collected immediately prior to ART initiation during AHI (n=15) or chronic HIV infection (CHI; n=6), and after 24 and 96 weeks of ART.
- The 4th generation immunoassay (4GIA) was used to categorize participants who initiated ART in the earliest stages of AHI: Stage 1 (PCR+ 4GIA-), Stage 2 (PCR+ 4GIA+). Participants in Stage 3-5 (PCR+ 4GIA+ 3GIA+) were further categorized by western blot (WB): Stage 3 (WB-), Stage 4 (WB+/-), Stage 5 (WB+).
- The pelleted cellular fractions of CSF samples were polyclonally expanded for 2 weeks, then total CD8⁺ T cells were FACS purified before a second polyclonal expansion. For PBMCs, activated (CD38⁺HLA-DR⁺) memory cells (pre-ART AHI) or memory CD8⁺ T cells (all other samples) were FACS purified before undergoing a single round of polyclonal expansion.
- Genomic DNA was purified from polyclonally expanded CD8⁺ T cells for sequencing of the TCRβ chain (Adaptive Biotechnologies).
- Repertoire clonality within a sample was measured by the Simpson clonality index (0 = equal number of each clone present, 1 = monoclonal sample). Repertoire diversity between two samples was measured by the Morisita index, which measures both the number of sequences shared between two samples as well as the contribution of shared sequences to the repertoire (0 = no overlap between samples, 1 = identical repertoires in terms of the number and proportion of sequences).
- HIV-specificity was measured in polyclonally expanded CD8⁺ T cells by intracellular staining for IFNγ, TNFα, and IL-2 after stimulation with autologous EBV-transformed B cells pulsed with CRF01_AE peptide pools.

Increased clonal expansion of CD8⁺ T cells in the CSF during acute HIV infection was associated with persistence of HIV-specific CD8⁺ T cell responses in the CSF after ART.

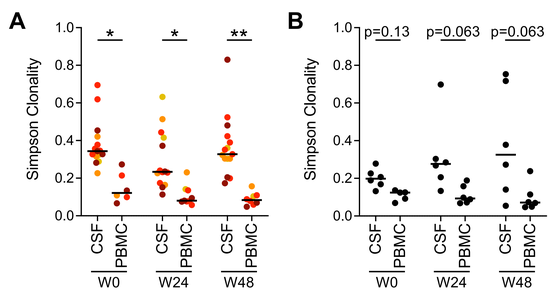

Figure 1. The CD8⁺ T cell repertoire is more clonal in CSF than PBMCs. Simpson Clonality Index of the CD8⁺ T cell repertoire in the CSF and PBMCs was calculated prior to ART initiation (W0) and after 24 and 96 weeks of ART in participants who initiated treatment in AHI *(A)* and CHI *(B)*. *p<0.05, **p<0.01 Wilcoxon matched pairs test.

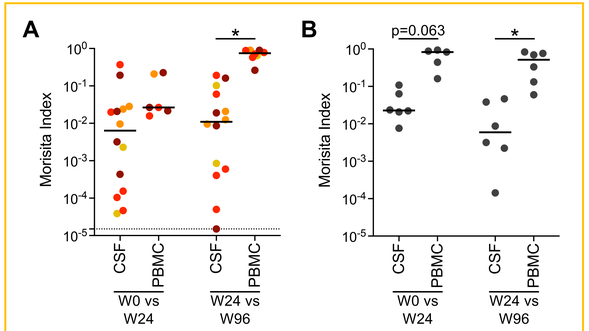

Figure 2. Higher turnover of the CD8⁺ T cell repertoire in CSF than PBMCs. Morisita Index of the CD8⁺ T cell repertoire in the CSF and PBMCs between consecutive visits for participants who initiated ART in AHI *(A)* or CHI *(B)*. *p<0.05 Wilcoxon matched pairs test.

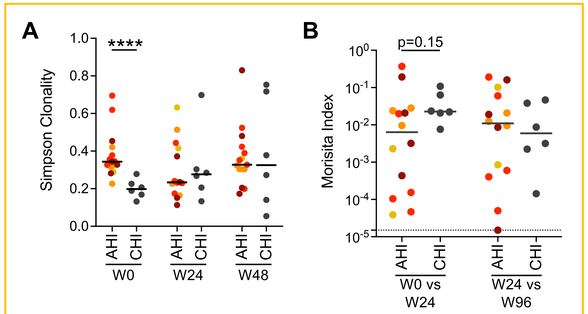

Figure 3. Higher CD8⁺ T cell repertoire clonality and turnover in the CSF at AHI compared to CHI. Comparison of the Simpson Clonality Index *(A)* and Morisita Index *(B)* of CSF samples collected from participants who initiated ART in AHI or CHI. ****p<0.0001 Mann-Whitney test.

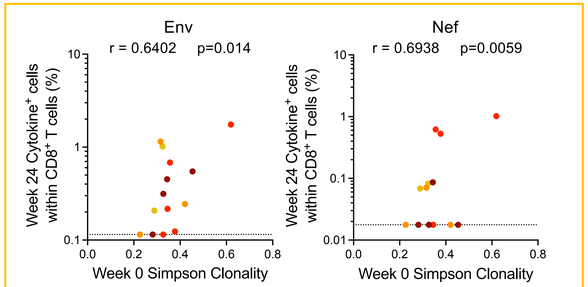

Figure 4. Correlation between TCR clonality and HIV-specific CD8⁺ T cell frequencies in CSF. The frequencies of HIV-specific CD8⁺ T cells in the CSF were measured by intracellular staining after stimulation with HIV peptide pools. Correlations between the Simpson Clonality Index at ART initiation and frequencies of HIV-specific CD8+ T cells after 24 weeks of ART are shown. Spearman correlation.

● AHI Stage 1 ● AHI Stage 2 ● AHI Stage 3 ● AHI Stage 4/5 ● CHI

CONCLUSIONS

- The CD8⁺ T cell repertoire is more clonal in the CSF than in PBMCs, particularly in participants who initiated treatment in AHI.
- CD8⁺ T cell turnover between time points was significantly greater in the CSF than PBMCs regardless of when ART was initiated.
- The CD8⁺ T cell repertoire in the CSF prior to ART initiation is more clonal and tends to have higher turnover in AHI compared to CHI.
- Higher CD8+ T cell clonality in the CSF at ART initiation was associated with increased frequencies of Env-, Nef-, and Rev/Tat-specific CD8⁺ T cells in the CSF after 24 weeks of ART.
- These data suggest that, while there is high turnover of the CD8⁺ T cell repertoire in the CSF over time, there remains a persistent HIV-specific CD8⁺ T cell response in the CSF after early initiation of ART.
- Further studies are needed to determine if these cells are indicative of a protective, tissue-resident HIV-specific CD8⁺ T cell population present in the CNS or of ongoing production of viral antigens in the CNS during ART.

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INHCC International NeuroHIV Cure Consortium

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MHRP U.S. MILITARY HIV RESEARCH PROGRAM

WRAIR Walter Reed Army Institute of Research