CASE WESTERN RESERVE UNIVERSITY
School of Medicine

Immune Dynamics During Reproductive Aging and Substance Use in Women With HIV

543

Konstantin Leskov[1], Anna Agaponova[1], Kathleen M. Weber[2], Elizabeth Daubert[2], Antoine Chaillon[3], Magali Porrachia[3], Jennifer Kinslow[2], Eileen Scully[4], Alan Landay[5], Saba Valadkhan[1], Victor DeGruttola[6], Alan Wells[3], Mary Ann Checkley[1], Benjamin Luttge[1], Sara Gianella[3], Jonathan Karn[1]

[1]Case Western Reserve University, Cleveland, OH, USA, [2]Hektoen Institute of Medicine, Chicago, IL, USA [3]University of California, San Diego, CA, USA, [4]The Johns Hopkins University School of Medicine, Baltimore, MD, USA, [5]UTMB Health, Galveston, TX, USA, [6]Harvard School of Public Health, Boston, MA, USA.

BACKGROUND

Estrogen depletion during reproductive aging in women with HIV (WWH) affects HIV reservoir burden and activity. Estrogen inhibits HIV transcription and modulates the immune system. Understanding the impact of hormonal changes on the immune system requires single-cell resolution to capture immune heterogeneity. Substance use disorder, prevalent among WWH, can further impact immune function.

METHODS

Plasma and PBMCs were evaluated across 3 reproductive stages (pre-, peri-, post-menopause) from 12 cisgender WWH in the Chicago Women's Interagency HIV Study (WIHS), spanning 2009-2019. Participant's average age at entry was 45 ± 5 years; 67% were Black and 58% reported substance use either close to the time of visit or in the past (e.g., crack/cocaine/heroin). All participants had sustained viral suppression and CD4>200 cells/μL. Reproductive stages were determined by sex hormones and self-report. Single-cell RNAseq and ABseq were performed using the BD Rhapsody platform to analyze CD4 and CD8 T-cells, monocytes, NK cells, DCs, B cells, and subpopulations. The inducible HIV RNA reservoir was measured by EDITS; integration site sequencing (Illumina) assessed HIV DNA clonal expansion in six women across reproductive stages

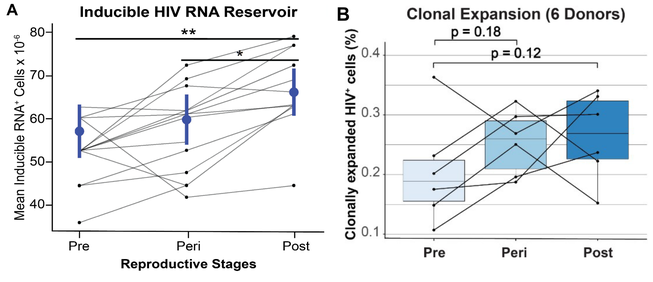


Figure 1. The HIV reservoir size increases in women during reproductive aging. **A**. Menopausal transition correlates with the expansion of inducible RNA reservoir as determined by EDITS, and **B**, clonal expansion of HIV+ cells.

From *Gianella et al. Clin Infect Dis. 2021 Oct 6:ciab873*

Reproductive aging in WWH shifts immune transcriptomes toward innate antiviral pathways during perimenopause. Substance use is the major factor contributing to single-cell transcriptome variance in T cells and monocytes and exacerbates immune dysregulation.

RESULTS

EDITS showed an 18% increase in the HIV RNA reservoir after menopause with evidence of increased clonally expanded HIV+ cells during perimenopause; these expanded clones persisted during postmenopause. Analysis of >900,000 single-cell transcriptome profiles identified a statistically significant ~5-fold enrichment in TNFα signaling via NFκB in premenopausal vs postmenopausal CD4 T-cells. In perimenopausal stages, IFNA and IFNG pathways were similarly enriched, indicating inflammatory responses. Ligand-receptor analysis predicted increased IFNA/IFNAR activity in CD4 T-cells as well as HLA-D/CD4 interactions between DCs and CD4 T-cells during the menopausal transition. Reported substance use was associated with unique transcriptomic changes, including the overexpression of MTRNR2L1 and other humanin paralogs in myeloid and T-cells. These markers decreased after reported substance cessation.

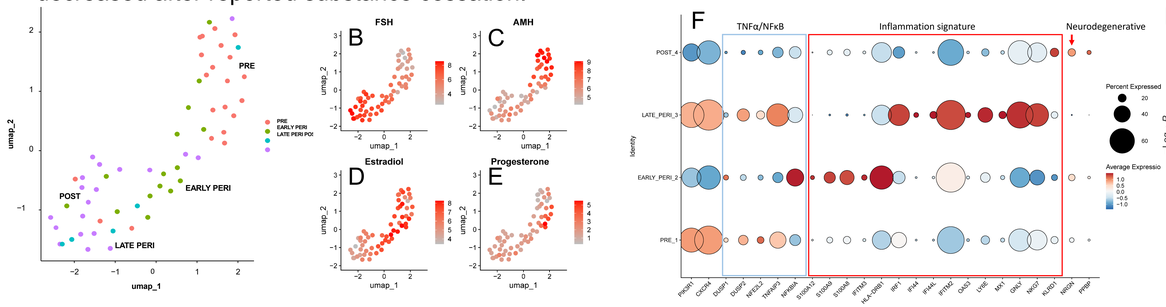


Figure 2. Markers of reproductive stages. **A**, UMAP based on serum levels of 10 sex hormones. **B-E**, Relative levels of sex hormones contributing to the variance. **F**. Top gene markers of reproductive stages based on scRNAseq of longitudinal PBMC samples of five WWH who do not use substances.

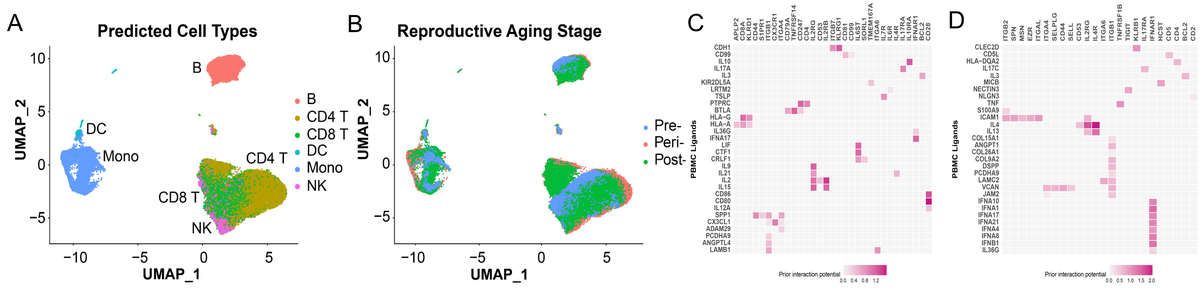


Figure 3. Reproductive aging shifts single-cell transcriptomes in immune cells. **A**. Representative UMAP of predicted cell types of PBMC from WWH. **B**. Reproductive stages indicated. **C** and **D**. Differentially expressed ligands and their receptors in WWH CD4 T cells in **C**, pre- vs peri and **D**, peri- vs postmenopause.

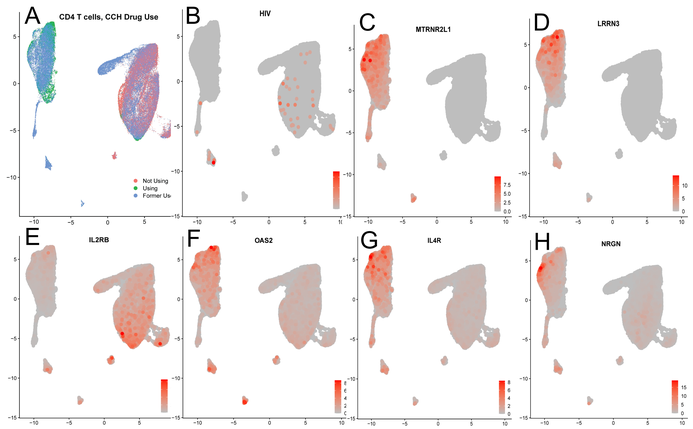


Figure 4. Substance use is the major factor contributing to the variance in CD4+ T cells transcriptomes. **A**, scRNAseq transcriptomes of CD4+ T cells from substance users and non-users are clustered separately. **B.** Cells expressing HIV. **C-H.** Relative levels of expression of top markers of substance use in the CD4+ T cells. MTRNR2L1 and LRRN3 have been previously associated with opioid and barbiturate use, respectively. OAS2 is an important marker of interferon response. IL2RB and IL4R are interleukin receptors. Neurogranin (NRGN) was associated with neurodegeneration.

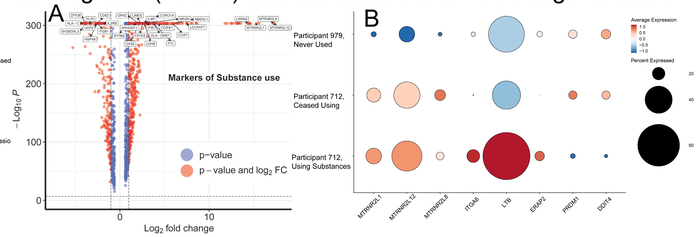


Figure 5. A. Statistically significant markers of substance use in CD4+ T cells. **B**. Some markers of substance use remain overexpressed after the cessation of use. Others return to the base level observed in the non-users.

CONCLUSIONS

Reproductive aging in WWH shifts immune transcriptomes toward innate antiviral pathways, likely due to the rise in the inducible HIV reservoir and proviral clonal expansion. Substance use exacerbates immune dysregulation.

ADDITIONAL KEY INFORMATION

Author contact Information: KSL9@CASE.EDU

Support

R01 AI181655 (Gianella & Karn)

Chicago MWCCS (Weber & Karn)

RUSTBELT CFAR
CENTER FOR HIV RESEARCH