Aging in Women With HIV: Single-Cell Transcriptome Analysis of the Immune System

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BACKGROUND

Reproductive aging in women with HIV (WWH) creates significant therapeutic challenges due to the post-menopausal decline in blood levels of estrogen, a known negative regulator of the latent HIV reservoir. The impact of age-related hormonal changes on the immune system of WWH requires detailed studies at a single-cell level, which will consider heterogeneity of the immune cells populations. ***We hypothesize that the shifts in innate immunity transcriptome signatures during the menopause transition have an effect on HIV pathogenesis.***

METHODS

Our work was made possible by the availability of extensive longitudinal data and biorepository samples across various reproductive stages from cisgender women with HIV from the Chicago Women's Interagency HIV Study (WIHS). Blood samples were collected during a ten-year period, carefully staged for reproductive aging, and their levels of sex hormones and relevant cytokines were determined. In this group, the inducible HIV RNA reservoir, as measured by the EDITS assay, increased in all women after menopause. To identify differentially expressed (DE) genes, surface protein markers and pathway enrichment (PE) along the timeline of aging, we used the BD Rhapsody scRNAseq-ABseq platform to generate cross-sectional datasets for PBMC from eight WWH, four pre- and four postmenopausal. To account for donor effect, 3 longitudinal samples (pre-, peri-, and postmenopausal) from 2 WWH were sequenced. For cell typing, we performed ABseq using 30 antibodies to major diagnostic surface antigens of immune cells.

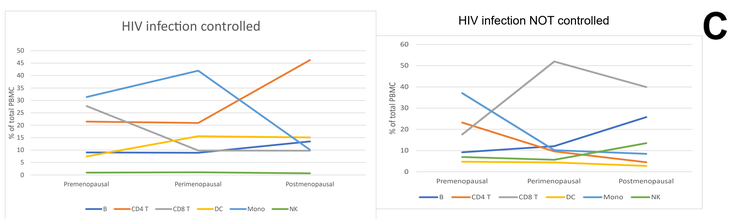


Figure 1. Variations in HIV infection control contribute to the donor effect in participants with HIV. Relative percentages of PBMC cell types in Pre-, Peri- and Postmenopausal blood samples from Participant 979 (left) and Participant 712 (right). Note the decline in CD4 T-cell percentage over time in participant with uncontrolled HIV infection.

The latent HIV reservoir expands in women with HIV during reproductive aging. The reservoir expansion is accompanied by a shift of CD4 T-cells towards a cytotoxic pro-inflammatory state during the pre- to peri-menopausal transition.

RESULTS

The resulting integrated datasets consisted of >100,000 transcriptome (>500 genes/cell) and ABseq profiles. We performed detailed DE gene and PE analyses in CD4 and CD8 T-cells, monocytes, NK, DC and B-cells, and their sub-populations (CD4 Naïve, TCM, TEM and CTL). We noticed the most significant changes between pre- and perimenopausal participants and less so between peri- and postmenopausal. Thus, TNFα signaling via NFκB, Interferon-α, p53, and complement showed significant enrichment in the cells from perimenopausal participants. Premenopausal CD4 T cells showed enriched PI3K/AKT/MTOR and androgen pathways, whereas genes of the late estrogen response, KRAS signaling, and glycolysis mark postmenopause (Figure 2). We observed similar DE genes and pathways in cross-sectional and longitudinal datasets.

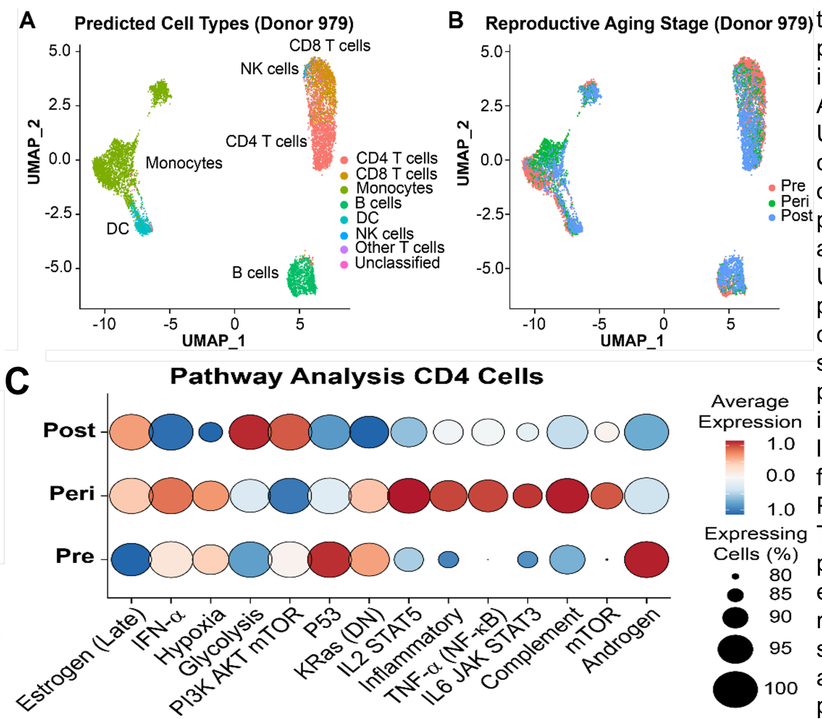


Figure 2. Immunophenotyping and differential pathway enrichment analyses in aging WWH by scRNAseq-ABseq. (**A**) An integrated UMAP plot of pooled single cell scRNAseq/ABseq profiles of total PBMC from participant 979 at pre-, peri- and post- menopause. (**B**) UMAP plot of that data from participant 979 highlighting cells obtained from each stage. (**C**) Dotplot showing pathway enrichment analysis in CD4 T-cells from longitudinal samples collected from three Pre-, Peri- and Postmenopausal participants. The dot size represents the percentage of cells expressing the pathway-related genes. The dot color shows the log-normalized average expression of each pathway.

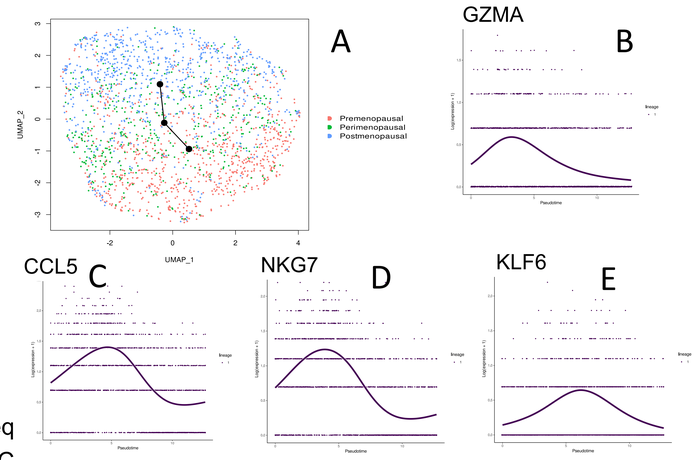


Figure 3. Trajectory analysis of CD4 T-cells progression from Pre- to Peri- and to Postmenopausal transcriptome signatures. CD4 T-cells were computationally isolated from the total PBMC dataset from longitudinal samples collected from participant 979 (Figures 1A and 2A-B). CD4 T-cells were typed based on the expression of 30 diagnostic immune cell markers using BD Rhapsody ABseq. Each dot in (**A**) represents a single cell transcriptome profile in the UMAP coordinates. Using the *tradeseq* package for R we identified a single lineage of CD4 T-cell progression (black line in (**A**)). Expression of each gene was fit in the negative binomial generalized additive model, and the genes whose expression was associated with pseudotime were identified. Representative progenitor genes that mark early transcriptome changes at Pre- to Perimenopausal transition are shown in (B) GZMA, (C) CCL5, and (D) NKG7. These genes are associated with cytotoxic pro-inflammatory T-cell phenotype. Transient expression of transcription factor KLF6 (E) was observed in the middle section of the lineage.

CONCLUSIONS

We conclude that reproductive aging in WWH shifts transcriptome signatures in the immune cells toward innate immune pathways and antiviral cytotoxicity in response to the rise of the inducible HIV reservoir. This phenomenon is especially pronounced at the Pre- to Perimenopausal transition. Future scRNAseq studies including expanded WWH cohort as well as matching males with HIV are necessary.

ADDITIONAL KEY INFORMATION

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