Single Cell Transcriptomics of HIV Heart Tissue Identifies a Unique NK Cell Population

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BACKGROUND

Cardiac disease, in particular heart failure, is elevated among people with HIV (PWH)[1]. Systemic inflammation and immune activation has been postulated to play a role in the development of heart disease. We hypothesized that single nuclei RNA-sequencing could provide improved understanding of immune cell subtypes that underlie cardiovascular disease among PWH.

METHODS

Left ventricle tissue samples were obtained from three participants:

1. Person without HIV with normal heart - CTRL (rejected UNOS transplant heart)
2. PWH with normal heart – HIV-CTRL (tissue isolated post-mortem as part of the Last Gift rapid autopsy study among PWH[2])
3. PWH with end-stage non-ischemic heart failure - HIV-NICM (tissue sample obtained during left ventricular assist device placement)

Samples were immediately flash frozen to -80C upon isolation and later processed for single-nuclei RNA sequencing. Briefly, samples were thawed and nuclei isolated. Single nuclei were captured and barcoded using 10X chromium microfluidics and mRNA poly-A capture. cDNA was formed such that mRNA from each nuclei carried a common unique barcode. PCR amplification was used to generate the cDNA library with sequencing using Illumina Nextseq.

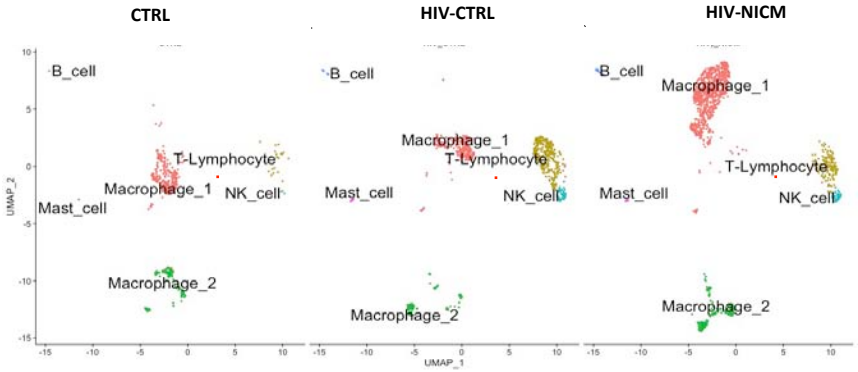
Deduplication of reads and alignment was performed using Cellranger 3.1.0. Initial quality assessment and clustering of cells was performed using Seurat 3.1 (R package, Satija Lab). Doublet cells were identified and removed using DoubletFinder (R package).

Unique Natural Killer Cell population identified in cardiac tissue from people with HIV

RESULTS

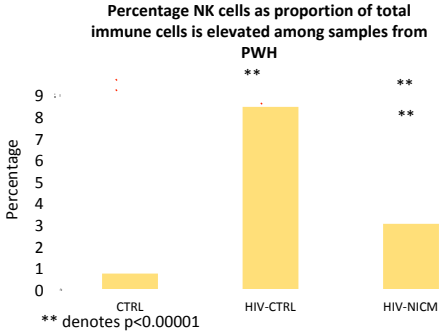
Single-nuclear transcriptomic data were obtained from 9008, 8746 and 8176 nuclei for CTRL, HIV-CTRL and HIV-NICM respectively. All PWH were virally suppressed on antiretroviral therapy. Cluster analysis was performed and clusters expressing high PTPRC (CD45+) were selected for further analysis. NK Cells were identified using markers NCAM1 (CD56), GNLY (Granulysin), TBX21, NKG7 and CD3 negative. Cluster allocations for each sample are shown in **Figure 1**.

Figure 1: Cell clustering of left ventricle tissue from two HIV+ (HIV-CTRL, HIV-NICM) and one HIV- (CTRL) persons demonstrating increased NK cell population (highlighted in red box) in cardiac tissue from PWH



RESULTS – continued

The proportion of immune cells in each sample that were identified as NK cells is shown in **Figure 2.** As a proportion of total immune cells, **the NK cell population in cardiac tissue from PWH was elevated compared to the control HIV-negative sample**: 8.4% and 3.0% for samples HIV-CTRL and HIV-NICM vs 0.7% for sample CTRL ($p<0.00001$).



CONCLUSION

We used single nuclear RNA sequencing to evaluate the immune cell composition of cardiac tissue from PWH and identified a **prominent NK cell population present in cardiac tissue obtained from PWH**.

Dysregulation of the immune system, including NK cells, has been associated with cardiac fibrosis, myocarditis and cardiac transplant rejection in the HIV uninfected population. The role of NK cells in cardiac disease among PWH remains to be explored further.

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References:

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