Spatial Profiling Reveals Immunometabolic Pathways Involved in Trained Immunity in PWH With CVD

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BACKGROUND

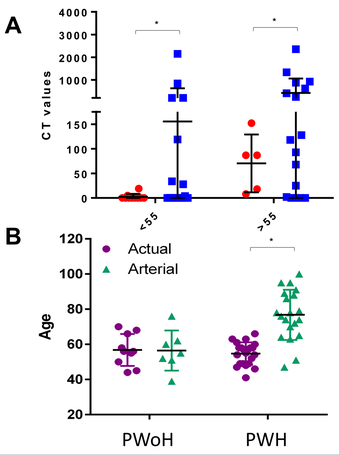
People with HIV (PWH) are at increased risk for cardiovascular disease (CVD). Chronic exposure of innate immune cells to microbial products and pro-inflammatory lipids may drive trained immunity in these cells through transcriptomic and metabolism changes. Markers of myeloid cell activation, including soluble CD14 (sCD14) and sCD163, are also elevated in PWH and have been linked to atherosclerotic plaque development. We have previously shown that macrophage maturation from blood monocytes is altered and linked to activation indices in people with HIV (PWH) . We hypothesize that monocytes/macrophages from PWH may contribute to accelerated CVD progression in this population and that monocyte derived macrophages (MDM) from PWH are primed to have excessive pro-inflammatory responses to TLR ligand exposure. We measured increased levels of coronary artery calcification (CAC) among PWH, found linkages between increased CAC and increased immune activation, and further defined alterations in macrophage signaling, metabolic activity and interactions with aortic endothelial cells that may contribute to vascular inflammation and elevated CVD risk in this population.

METHODS

Coronary artery calcium (CAC) scores among people with HIV (PWH) and people without HIV (PWoH) (N=25,43) were used to assess atherosclerosis and vascular age. Blood was collected from participants for assay of serum markers of immune activation and differentiation of monocyte-derived macrophages (MDMs). Response to TLR ligands was assessed in MDMs exposed to bacterial products including LPS by bulk RNAseq. Spatial profiling of FFPE arterial blood vessels from PWH and people without HIV was done via NanoString's GeoMx DSP to validate these signatures in vivo. Cells within vessels were stained for CD14 and CD163.

RESULTS

Figure 1. Coronary artery calcification levels are increased in PWH and correlate with markers of immune activation and inflammation. PWH had increased CAC scores (350 vs 18; p=0.04) compared to PWoH, and PWH vascular ages were higher than biological ages (77y vs 55y; p<0.0001). Increasing CAC was associated with elevated inflammatory markers (osteopontin, CRP, IL6, sCD14; P<0.02).

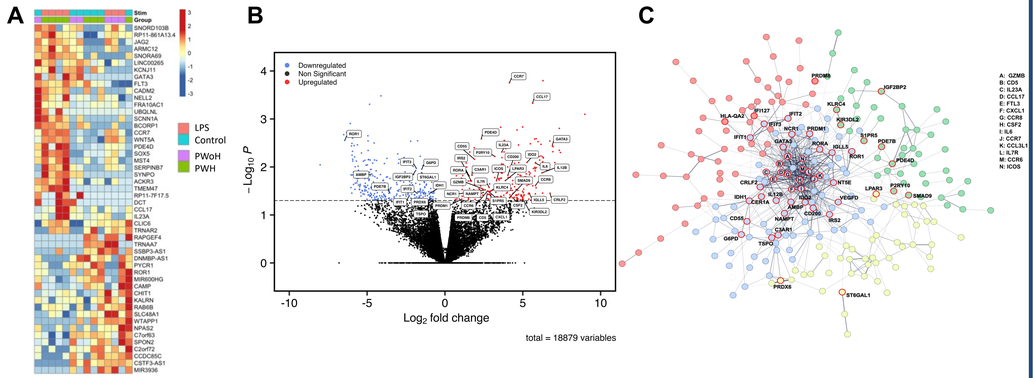


Biomarker/CAC Correlations

	R-value	P-value
Age	0.310	0.043
CRP	0.551	0.0001
D Dimer	0.517	0.007
sCD14	0.368	0.015
IL-6	0.434	0.004
TNF-R1	0.440	0.003
TNF-R2	0.301	0.050
VCAM-1	0.408	0.007
Endothelin-1	0.481	0.001
Osteopontin	0.390	0.021

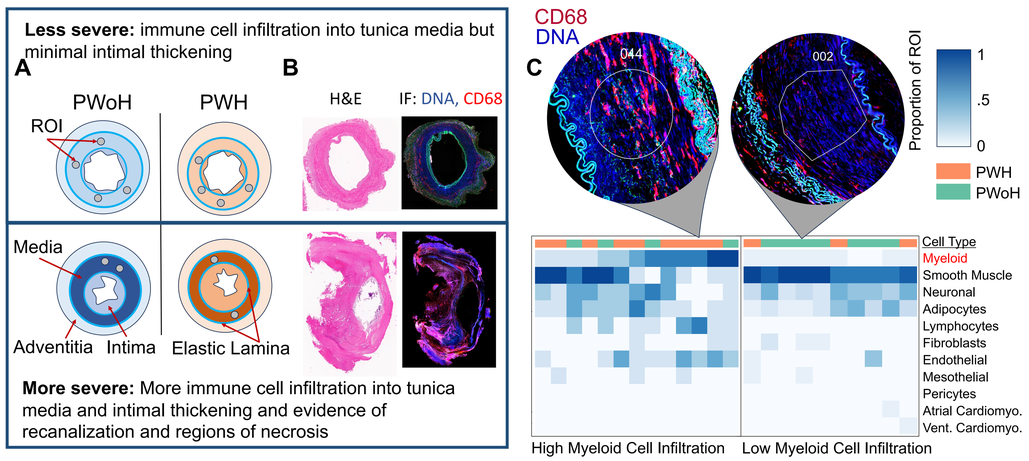
RESULTS

Figure 2. MDMs from PWH display differential responsiveness to TLR ligands. A) Heatmap of top 50 Differentially Expressed Genes (DEG) from MDMs from PWH vs. PWoH exposed to the TLR4 ligand LPS during differentiation. B) Volcano plot of significantly differentially expressed genes from MDMs from PWH vs. PWoH exposed to the TLR4 ligand LPS during differentiation. C) Protein-protein interaction network of significantly differentially expressed genes.



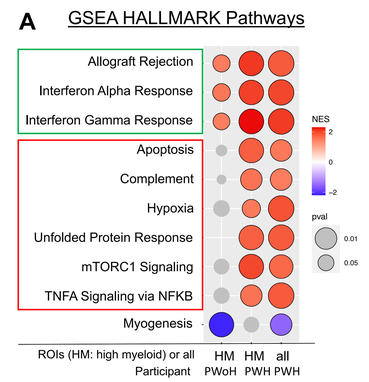
Spatial profiling of atherosclerotic plaques in PWH reveals enrichment of trained immunity and senescence pathways that may play a role in driving the enhanced severity of CVD.

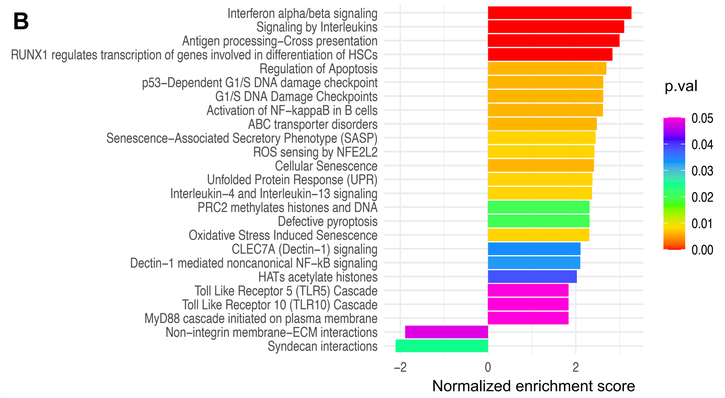
Figure 3. Calcified atherosclerotic plaques from PWLH and control group showed a range of immune cell infiltration and disease severity. A) Diagram of an artery. ROIs were collected from tunica media. **B)** H&E and IF staining of example sections **C)** Spatial deconvolution was used to identify and quantify cell types within ROIs. The proportion of myeloid cells in ROIs across arteries is significantly higher in atherosclerotic plaques from PWH (p=0.046). **C)** ROIs within arterial media were separated into two groups: high myeloid cell infiltration (>10%), low myeloid cell infiltration (<10%).



RESULTS

Figure 4. Identification of pathways uniquely associated with increased tunica media myeloid infiltration in PWH. A) Common pathways associated with high myeloid infiltration in both ROIs from PWH and PWoH were identified (green box). These pathways are likely associated with atherosclerosis and CVD and are not necessarily specific to mechanisms driving CVD in PWH. Pathways enriched in the following conditions may be associated with atherosclerosis specifically in PWH (red box): enriched in high myeloid ROIs from PWH, enriched in ROIs from PWH (both high and low myeloid groups combined), **not** enriched in high myeloid ROIs from PWH. **B)** Additional examples of pathways enriched only in media ROIs from PWH. Several pathways of interest involved in trained immunity, senescence, and epigenetic remodeling are indicated.





CONCLUSIONS

- Coronary artery calcification is increased and associated with inflammatory biomarkers in PWH
- Enhanced responsiveness of MDMs from PWH to TLR ligands, may exacerbate vascular inflammation and atherosclerosis.
- Signatures of enhanced TLR signaling, senescence, cell cycle, DNA damage, ROS, epigenetic remodeling, cell death, dectin signaling and trained immunity are evident in macrophages from PWH treated with TLR ligands and are also evident in situ in the arterial blood vessels of PWH and CVD.
- Increased CVD risk in PWH may be driven by trained immunity as evidenced by immune functional profiling of blood-derived MDMs and spatial profiling of macrophages within blood vessel walls.

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