Monocyte Dysregulation in PASC With Ongoing Pulmonary Symptoms

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BACKGROUND

Studies have shown that excessive monocyte activation is involved in the development of a cytokine storm and subsequent acute lung injury induced by SARS-CoV-2 infection. However, long-term consequence of COVID-19 on immune dysregulation and its contribution to PASC development has not been fully explored. Here we characterized transcriptomic changes in circulating monocytes associated with pulmonary PASC (PPASC) and these alterations may affect immune response post-infection and the potential role monocytes may play in the persistence of pulmonary symptoms.

We hypothesized that monocytes isolated from individuals with PPASC would have dysregulation of inflammatory pathways compared to those of individuals that are fully recovered with no long-term symptoms (RC) and uninfected, healthy individuals (HC). Based on our previous work, we also expected to see higher levels of circulating monocytes expressing activation markers such as CD169 in PPASC patients. Our previous single cell RNA-sequencing of monocytes from COVID19 convalescent patients suggested that glycolytic pathways were significantly altered; we hypothesize that metabolic pathways in long-COVID monocytes may be disrupted due to chronic inflammation, activation, and mitochondrial dysregulation.

METHODOLOGY

Monocytes were isolated from cryopreserved PBMCs from participants of the ongoing Ola Study Hawaii Center for AIDS Long-COVID study in collaboration with the RECOVER program. Patients with self reported long-COVID symptoms persisting >3 months since previous infection were enrolled into the PPASC group; HC individuals were confirmed COVID19 negative using serological assays. On average, PPASC patients self reported an infection >440 days before entry into the study. Bulk RNA-seq was conducted on isolated monocytes from 31 samples (PPASC, n=14; RC, n=8; HC, n=8) at the University of Hawaii Cancer Center Genomics Core. Differentially expressed gene (DEG) data was analyzed utilizing Qiagen's Ingenuity Pathway Analysis (IPA). PBMCs were stained for various monocyte activation, motility, maturation, M1/M2, and senescence markers and analyzed on an Attune NxT flow cytometer and subsequently analyzed using FloJo software (Figure 4A).

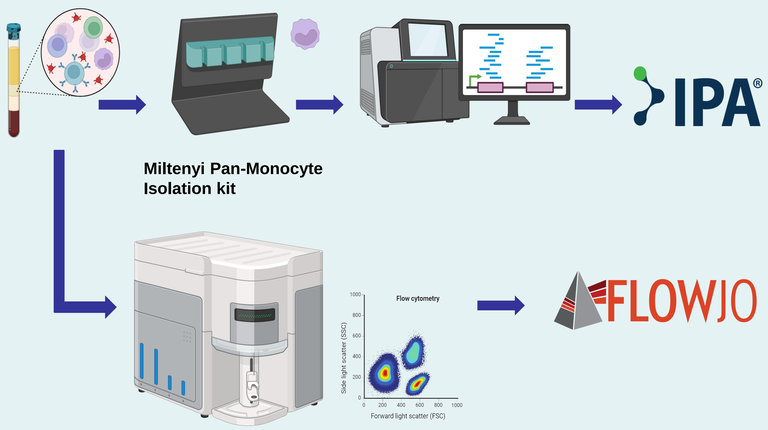


Figure 1 Experimental workflow of monocyte isolation, RNA-seq data analysis, and flow cytometry. Figure made using BioRender.

MAIN FINDINGS

- Chronic **upregulation** of pro-inflammatory pathways as shown by RNA-seq, including **IL-8 signaling**, **ROS production** in macrophages, and **IL-6 signaling** (Fig 1A)
- **Downregulation of IL-10 pathway**, indicating potential reduction in anti-inflammatory response (Fig 1A)
- Upregulation of inflammatory genes, including **S100A8/9,** ROS genes including **NRROS** and **SYK,** and monocyte immune response gene (Fig 1 B,C)
- Significantly higher percentage of classical monocytes expressing Programmed Cell Death Protein 1 **(PD-1)** with decreased expression of cyclic ADP ribose hydrolase **(CD38)** in PPASC compared to RC

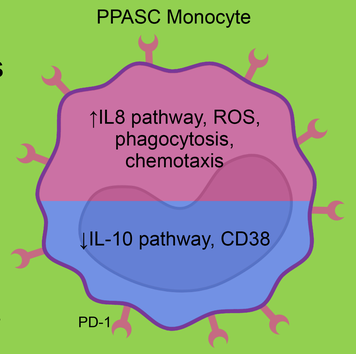


Figure 2 Graphical schematic of preliminary findings based on RNA-seq and flow cytometry analysis of PPASC monocytes compared to RC/HC. Red indicates upregulation with blue indicating downregulation. Figure made using BioRender.

RESULTS

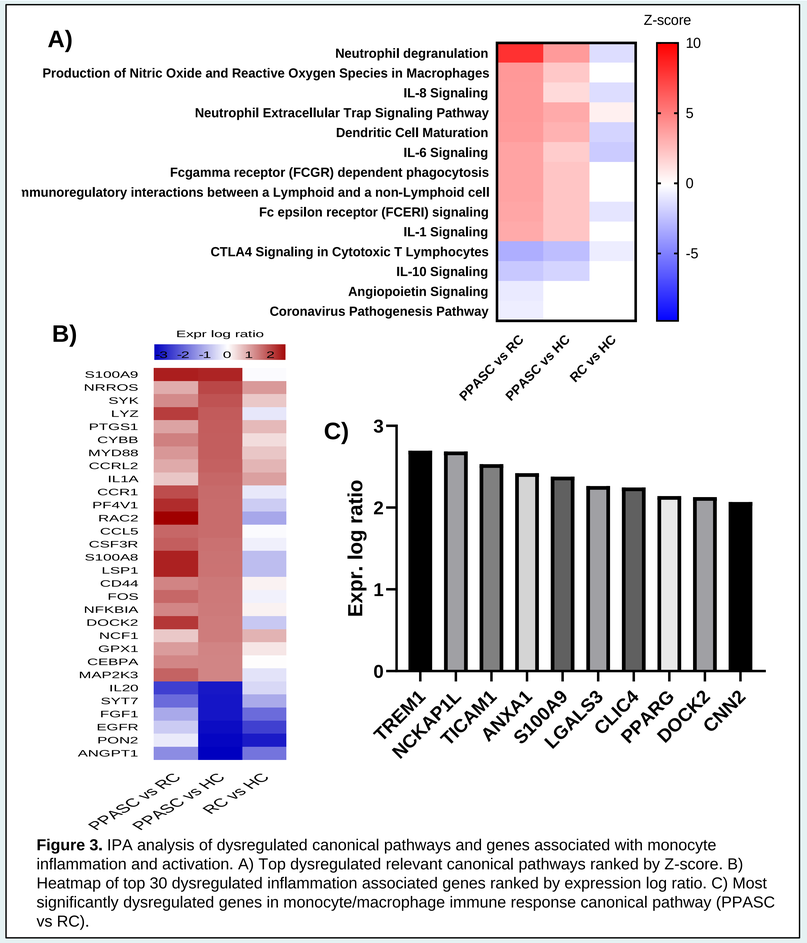


Figure 3. IPA analysis of dysregulated canonical pathways and genes associated with monocyte inflammation and activation. A) Top dysregulated relevant canonical pathways ranked by Z-score. B) Heatmap of top 30 dysregulated inflammation associated genes ranked by expression log ratio. C) Most significantly dysregulated genes in monocyte/macrophage immune response canonical pathway (PPASC vs RC).

RESULTS

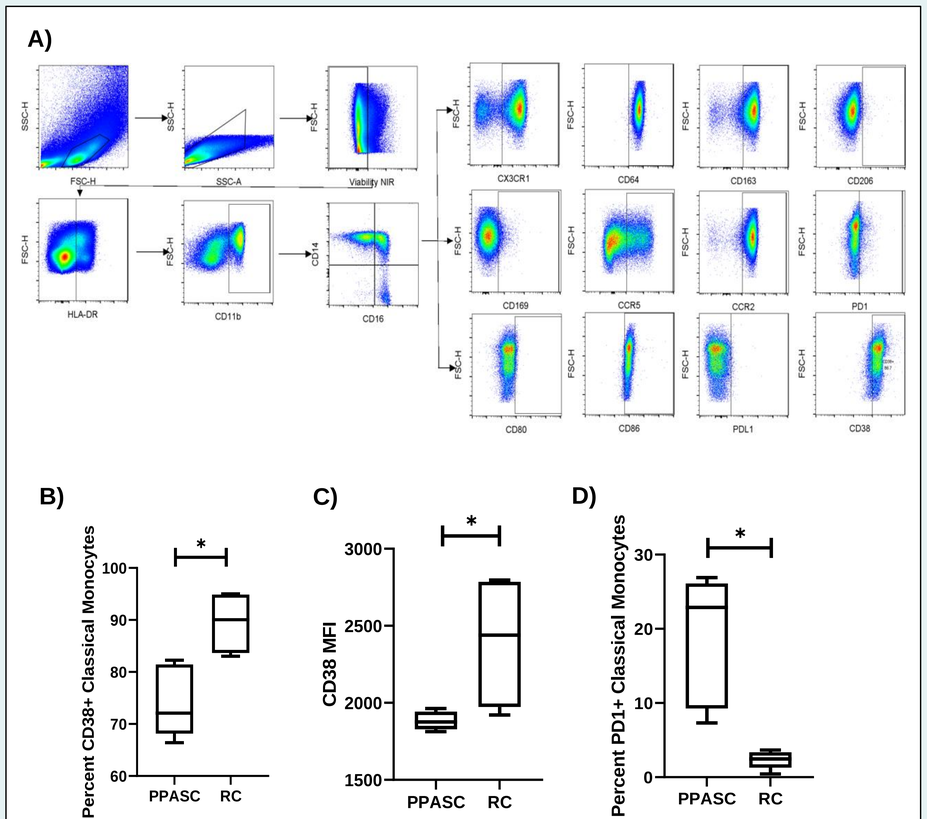


Figure 4. Flow cytometry analysis of monocytes stained for activation associated markers. A) Flow gating strategy for identifying classical, nonclassical, and intermediate monocytes positive for various activation associated markers. B) Percentage of total classical monocytes (CD14+, CD16-) expressing CD38 in PPASC is significantly downregulated compared to RC. C) Overall expression of CD38 as measured by mean fluorescence intensity (MFI) is significantly lower on PPASC classical monocytes compared to RC. D) Percentage of total classical monocytes expressing PD1 is significantly downregulated compared to RC.

CONCLUSIONS & FUTURE RESEARCH

Our findings suggest chronic dysregulation in pathways related to monocyte immune activation, ROS production, and general inflammation in PPASC monocytes. Additionally, we observed that overall senescence as indicated by PD1 levels, is upregulated. This may suggest that monocytes play an active role in the persistence of inflammation in those with PASC, resulting in chronic disease.

We plan to conduct monocyte-associated cytokine multiplex analyses to determine circulating levels of inflammatory cytokines in our patient population. We are continuing to acquire and analyze flow cytometry data to identify any additional dysregulated monocyte subpopulations, and plan on conducting qPCR/Western blotting experiments to validate our RNA-seq findings.

ACKNOWLEDGEMENTS

This work was supported by NIH/NIMHD (U54MD007601) and the *Molecular and Cellular Immunology Core through the funding of the Centers of Biomedical Research Excellence (COBRE) program (P30GM114737).* Special thanks to Dr. Vedbar Khadka at the Bioinformatics Core Facility for assistance with RNA-seq analysis and Dr. Alexandra Gurary at the Flow Cytometry Core who provided technical guidance for flow cytometry experiments. *We are also grateful to the study participants who generously volunteered their time for this study.*