Molecular Determinants of Sarcoidosis

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Background

Sarcoidosis is a multi-system inflammatory disease that is characterized by non-caseating granulomas. Although the etiology remains unclear, it is hypothesized that granulomagenesis is provoked by an unknown antigen in those innately susceptible. The sarcoidosis granuloma is comprised of a core of macrophage-derived multinucleated giant cells (MGCs) and epithelioid cells surrounded by lymphocytes, fibroblasts, and blood vessels (1,2). More research is needed to better understand sarcoidosis granulomagenesis to develop targeted therapies and improve clinical outcomes. We sought to establish an *in vitro* model of sarcoidosis using human induced pluripotent stem cell-derived macrophages (iMacs). We also aimed to identify a circulating sarcoid-like cell (CSLC) using single-cell profiling of peripheral blood mononuclear cells (PBMCs) from patients with sarcoidosis.

Methods

Human induced pluripotent stem cells (iPSCs) from the DU11 line were differentiated into unpolarized macrophages over 3 weeks (Figure 1) (3). The macrophages were then exposed to various agents known to cause MGC formation *in vitro*, including mammalian target of rapamycin (mTOR) activator, Kveim reagent, concanavalin A (Con A), soluble CD40 ligand (CD40L), and interferon gamma (IFN-γ) (4-6).

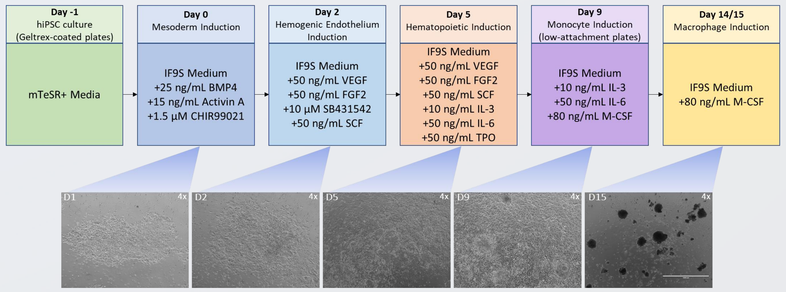

Figure 1: Differentiation protocol and representative bright field images of cells at each step of differentiation.

We also recruited 7 patients from the Duke Cardiac Sarcoidosis clinic with active sarcoidosis. From each subject, blood was processed to isolate PBMCs, which were sent for single cell profiling using the 10X Multiome platform. Data from this cohort were pooled with single cell data from 12 skin samples with sarcoidosis and 12 unaffected skin samples, along with a reference dataset of normal PBMCs (7).

Results

After unpolarized (M0) macrophages were treated with sCD40L and IFN-γ along with Con A, mTOR activator, or Kveim reagent for 3 days, no MGCs were observed (Figure 2).

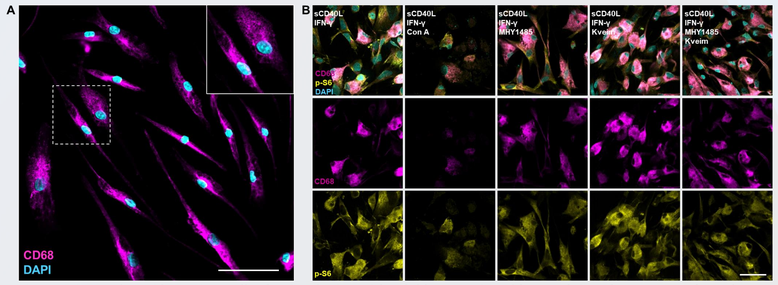

Figure 2: iMacs do not form giant cells after incubation with soluble CD40 Ligand (sCD40L), interferon gamma (IFN-γ), Concanavalin A (Con A), mTOR activator (MHY1485) and/or Kveim. A. Representative image of Day 29 plated iMacs stained for the macrophage marker CD68 (magenta) and DAPI (cyan) to mark nuclei. **B.** Cells treated on D59 with 30 ng/mL sCD40L and 10 ng/mL IFN-γ in M0 media (IF9S with 80 ng/mL macrophage colony-stimulating factor) along with 5 mg/mL Con A, 10 μM mTOR activator, or Kveim reagent (1:1,000 dilution of stock) are stained with CD68 (magenta), marker of mTOR activation p-S6 (yellow), and DAPI (cyan). Scale bar = 50 μm.

From the pooled single-cell sequencing data, 24 unique clusters of cells were identified (Figure 3A-C). Cluster 1 cells were enriched in unaffected skin and cells from clusters 6, 16, and 21 were enriched in skin with sarcoidosis (Figure 3D). Three CSLCs populations were identified: CD68^{+} cells in cluster 6 (CSLC^{Macro}), MS4A1/CD20^{+} cells from cluster 16 (CSLC$^{B\text{-}cell}$), and NCAM1/CD56^{+} NK cells from cluster 21 (CSLC^{NK}) (Figure 3E).

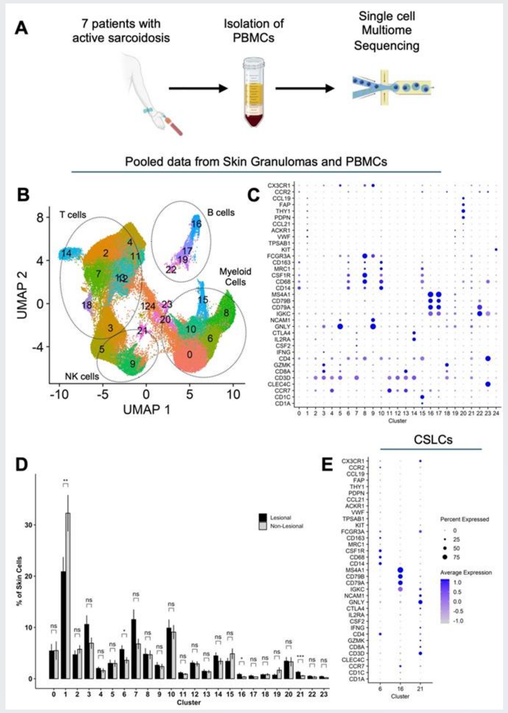

Figure 3: Identification of circulating sarcoid-like cells (CSLCs). A. Overall schematic. **B.** Cluster plot of single-cell RNA sequencing data from PBMCs pooled with reference samples of affected skin, unaffected skin, and a reference PBMC dataset. **C.** Dot plot of select markers for each cluster identified in panel A. **D.** Plot showing the prevalence of cells from lesional skin and non-lesional skin to determine which clusters are enriched for cells associated with sarcoidosis. Error bars are SEM. P<0.05, **P<0.01 (two-tailed Wilcoxon rank-sum test). **E.** Dot plot of PBMCs from patients with sarcoidosis that are found in clusters enriched for skin sarcoidosis. Panel A was made with BioRender.

Conclusions

We sought to establish a model of sarcoidosis using human iPSCs. We differentiated iPSCs into macrophages over the course of 3 weeks and then subjected these cells to agents known to induce multinucleated giant cell formation. We did not observe any characteristic giant cells after exposing unpolarized iMacs to a mammalian target of rapamycin (mTOR) activator or Kveim reagent. Furthermore, we did not observe any giant cell formation after simulating T-cell interactions with iMacs by exposure to soluble CD40 ligand, interferon gamma, and concanavalin A. Considering the growing evidence that M2 macrophages play an important role in both mouse and human sarcoidosis granulomas, it is possible that polarization of iMacs towards an M2 phenotype may be necessary for giant cell formation *in vitro*. Furthermore, single cell profiling data revealed a distinct population of CD68^{+} CSLCs (CSLC^{Macro}). Future directions include functional testing of CSLC^{Macro} pathways for their ability to induce a sarcoidogenic phenotype in iMacs.

Learn more:

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