Disentangling mononuclear phagocyte heterogeneity in rheumatoid arthritis synovial fluid through single cell transcriptomics

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Introduction:

The mononuclear phagocyte (MP) system comprises monocytes, macrophages and dendritic cells (DCs). It is responsible for a broad range of functions, including tissue healing, pathogen clearance, and the induction of adaptive immune responses. This flexibility is in part the result of functionally distinct MP subsets but also an inherent plasticity that allows its components to adapt to their environment. However, this makes it challenging to identify and profile MPs in tissue and inflammation using standard approaches such as flow cytometry. In this study, we performed an unbiased high-dimensional analysis of matched blood and synovial fluid (SF) using single cell RNA sequencing to align MPs between tissues and perform functional analysis of the infiltrating populations.

Methods:

Matched peripheral blood and synovial fluid was isolated from three donors with seropositive rheumatoid arthritis. Samples were flow sorted to comprehensively sample lineage$^-$HLA-DR$^+$ cells with subsampling to enrich for monocyte and dendritic cell subsets using established blood surface markers. In total 1140 cells were isolated for single cell RNA sequencing, with 977 passing quality-control.

Results:

Our study reveals SF equivalents of peripheral blood CD14$^+$ monocytes, CD1c$^+$ DCs, CLEC9A$^+$ DCs, plasmacytoid DCs (pDCs) as well as the recently identified Axl$^+$ DCs (ASDCs) but no equivalent to non-classical CD16+ monocytes suggesting they fail to infiltrate the joint. Of the SF populations CD1c$^+$ DCs and CD14$^+$ monocytes exhibit most significant variation between donors and tissues reflecting their plasticity. In contrast the transcriptional profile of tissue pDCs and CLEC9A$^+$ DCs is mostly conserved. We identify a novel population of activated cDC2 that lack expression of standard DC markers (CD1c/CD11c/CD14) but have high levels of transcripts associated with maturation (LAMP3, CD83) and lymph node homing (CCR7/S1PR1).

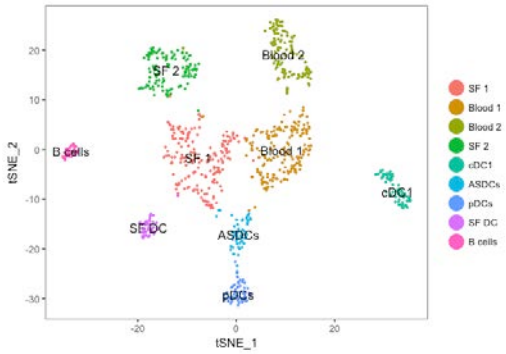

Figure 1: Peripheral blood and matched synovial fluid was obtained from three donors and flow sorted to isolate mononuclear phagocyte subpopulations. cDNA was generated according to the SmartSeq2 protocol and sequenced. Reads were aligned to the reference genome using Salmon and data was analysed using the Seurat package in R. This resulted in nine clusters across two tissue (top).

Conclusion:

This study highlights conserved transcriptional signatures in SF inflammation that supports the identification of known MP subsets as well as the use of an unbiased approach such as single cell RNA sequencing to identify novel MP populations.