

## Fibroblast activation in rheumatic diseases

OP0094

### SPATIAL POSITIONING AND MATRIX PROGRAMS OF SYNOVIAL FIBROBLASTS UNDERPIN IMMUNE CELL ORGANISATION AND SYNOVIAL TISSUE PATHOTYPE

**Keywords:** Synovium, -omics, Rheumatoid arthritis

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**Background:** Rheumatoid arthritis (RA) is highly heterogeneous with distinct cellular and molecular tissue pathotypes that associate with disease outcome[1]. The synovial membrane is the primary target tissue and undergoes significant architectural re-modelling in response to inflammation. While fibroblasts are known to shape immune cell compartmentalisation in secondary lymphoid organs, by producing distinct sets of chemokines and remodelling extracellular matrix (ECM)[2], the mechanisms underlying immune cell localisation in the synovium are poorly understood.

**Objectives:** To define the functional zonation of the inflamed synovium at the single cell and regional tissue level using spatial transcriptomics and multiplex imaging.

**Methods:** We performed oligonucleotide probe-based spatially resolved transcriptomics on synovial tissue samples obtained by minimally invasive, ultrasound guided synovial tissue biopsies performed in patients with both rheumatoid arthritis (RA) and osteoarthritis (OA) using the 10x Visium platform. A total of 27 tissue biopsies processed sequencing 34170 spot transcriptomes. Each tissue section was composed on 1-8 fragments of synovial tissue from the same donor biopsy sample within each capture area, to maximise tissue representation and account for sampling variability. Cellular deconvolution of spot transcriptomes was performed using single cell reference datasets. Multiplex imaging was obtained to confirm gene profiles and cell types associated with the specific zonation patterns.

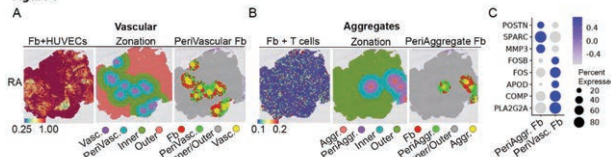
**Results:** We identified six spatially resolved tissue niches (or zones) within synovial membrane defined by gene expression and comprised of distinct populations of stromal and immune cells. Specific patterns of gene expression were observed in each of these sub-synovial tissue zones that link to functions of immune infiltrate recruitment and organisation. This zonation pattern is maintained across pathotypes (Fibroid, Lymphocytic) and spatial gene expression analysis revealed that RA is associated with specific synovial re-modelling and the formation of lymphocytic niches when compared to OA synovial tissue. We next defined zonation areas by manually annotating immune infiltrates (as aggregates) and vasculature, based on histology. From these, we selected fibroblasts specific to immune aggregate zones and peri-vascular zones (Figure 1). We have identified differences in gene expression between these fibroblasts based on their anatomical location. Peri-vascular fibroblasts have gene expression profiles related to myofibroblast differentiation, ECM deposition and modulation (COMP), but also immune cell recruitment (PLA2G2A), cell activation (FOS & FOSB), and lipid metabolism (APOD), also associated with OA synovium. These fibroblasts display either a TGF- $\beta$  response or NOTCH3 activated[3] gene expression program and are expanded in the fibroid pauci-immune tissue pathotype. Immune interacting (peri-aggregate) fibroblasts in contrast, show specific gene expression profiles linked to ECM regulation (POSTN), degradation (MMP3) and co-ordination of ECM remodelling to recruit immune cells (SPARC), and permit aggregate formation. These fibroblasts display a T-cell interacting[4] and IFN- $\gamma$  gene expression program and along with IL-1 $\beta$  and TNF- $\alpha$  activated lining layer fibroblasts dominate the stromal cell landscape of the lymphoid tissue pathotype.

**Conclusion:** We demonstrate that cytokine directed fibroblast-immune cell crosstalk, positional identity, and matrix specific, fibroblast gene expression programs are responsible for localising infiltrating immune cells to specific niches within the tissue and defining functional tissue zonation. These data identify novel therapeutically tractable disease pathways that underpin the pathogenicity of synovial tissue fibroblasts in RA.

#### REFERENCES:

- [1] Lewis et al. Cell Reports 2019;28:2455-2470
- [2] Bajénoff M et al. Immunity 2006;25:989-1001
- [3] Wei K et al. Nature 2020;582:259-264.
- [4] Korsunsky I et al. Med (N Y) 2022;3:481-518.e14

Figure 1



**Figure Legend:**  
(A) Fibroblast (Fb) gene signature upon co-culture with HUECs mediated by NOTCH3 (Wei et al, 2020) projected as spatially resolved gene expression on RA synovial tissue. Definition of zonation areas around vascular cells and selection of peri-vascular fibroblasts.  
(B) Fb gene signature upon co-culture with T cells (Korsunsky et al, 2022) projected as spatially resolved gene expression on RA synovial tissue. Definition of zonation areas around aggregates and selection of peri-aggregate fibroblasts.  
(C) Expression of key markers genes in peri-aggregate fibroblasts compared to peri-vascular fibroblasts.

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**Disclosure of Interests:** None Declared.

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## Autoinflammation – the next chapter

OP0095

### MONOSODIUM URATE AND CALCIUM PYROPHOSPHATE CRYSTAL-INDUCED INFLAMMATION RELIES ON CELL VOLUME REGULATION AND LRRC8/VRAC CHANNEL ACTIVATION

**Keywords:** Inflammatory arthritides, Gout, Crystal Arthritis

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**Background:** Monosodium urate (MSU) and calcium pyrophosphate (CPP) crystals are responsible for interleukin (IL)-1 $\beta$  dependent acute arthritis. The release of mature IL-1 $\beta$  is dependent on the NLRP3 inflammasome, which can be activated by other sterile stimuli such as hypo-osmolarity and ATP. During extracellular hypotonic stress, there is cellular swelling secondary to water influx. The cell sets up a defence mechanism, called regulatory volume decrease (RVD), allowing it to return to its initial volume. RVD depends on the anion channel VRAC, a hetero-hexamers composed of members of the LRRC8 family. LRRC8A is the obligatory key protein required to form active VRAC channel. Activation of LRRC8/VRAC channel results in an efflux of anions (mainly chloride) and osmolytes leading to water efflux and cell volume reduction.

**Objectives:** To evaluate the role of the LRRC8/VRAC channel in cell volume regulation and IL-1 $\beta$  release induced by MSU and CPP crystals.

**Methods:** In-vitro, primed THP-1 monocytes were stimulated by synthetic sterile MSU and CPP and cytokine production was quantified by ELISA. Cell volume variations were visualized by live video recording and cell surface was measured using ImageJ software. The role of the LRRC8/VRAC channel was evaluated using a pharmacological inhibitor DCPIB or by silencing the LRRC8A subunit (shLRRC8A RNA) in these cells. In vivo, MSU and CPP crystals were injected into air pouches created subcutaneously (mimicking synovial cavity) in wild-type mice and conditional LRRC8A Knock-out mice in the macrophagic lineage (Cxcr3Cre\_Lrrc8aflox/flox). Supernatants and pouch lavages were used to measure cytokine production by ELISA.

**Results:** MSU- and CPP-induced NF- $\kappa$ B activation was reduced in WT THP-1 cells treated with DCPIB and in THP-1 cells where LRRC8A expression was silenced. Similarly, IL-1 $\beta$  production induced by MSU and CPP crystals was substantially decreased in WT THP-1 treated with DCPIB (MSU 5200 vs 1080 pg/ml; CPP 11500 vs 4980, p<0.0001) and in shLRRC8A THP-1 cells compared to crystal-treated WT cells. MSU and CPP crystals exposure induced a biphasic cell volume change characterised by a significant increase followed by a RVD-like phenomenon. These cell volume changes were abolished in the presence of DCPIB and not observed in shLRRC8A THP-1 cells. In vivo, inflammation induced by MSU and CPP crystals assessed in lavage fluid and conventional histology was lower in Cxcr3Cre\_Lrrc8aflox/flox mice as compared with wild-type mice in terms of IL-1 $\beta$  production and cell infiltrate.

**Conclusion:** These results suggested that MSU and CPP crystal-induced inflammation involved and cell volume variation regulated by VRAC/LRRC8 channel.

**REFERENCES:** NIL.

**Acknowledgements:** NIL.

**Disclosure of Interests:** None Declared.

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