Conclusion: Persistence and spontaneous resolution of synovial inflammation are accompanied by characteristic compositional and mechanistic signatures that can be observed in key cellular niches during active early inflammation. There is potential to exploit such signatures with the aim of disrupting persistent inflammation.

REFERENCES: NIL.

Acknowledgements: NIL.

Disclosure of Interests: Andrew Filer Consultant of: Abbvie, Amgen, Eli Lilly, Novartis, Astra Zeneca., Grant/research support from: UCB, Bristol Myers Squibb, Caboletta, Compugen, MoonLaker, Pfizer, and Astra Zeneca., Consultant of: Janssen, Sonoma, Grant/research support from: BMS, Roche, UCB, Nascient, Mostag, GSK, Janssen, Jason D Turner: None declared, Cesar Prada-Medina: None declared, Moustaﬁ Attar: None declared, Christopher D Buckley: None declared, Stephen Sansom: None declared, Karim Raza Consultant of: Abbvie and Sanofi, Grant/research support from: Bristol Myers Squibb.

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OP0122 MACHINE-LEARNING-ASSISTED ANALYSIS IDENTIFIES DISCRETE PHOSPHORYLATION SIGNATURES IN T AND B LYMPHOCYTES ASSOCIATED WITH EARLY RHEUMATOID ARTHRITIS

Keywords: Rheumatoid arthritis, Biomarkers, Disease-modifying drugs (DMARDs)

M. Nyrenda1, M. Akbär2, A. Gilmour3, C. Wallace4, C. Paterson5, D. Porter6, D. Reid7, J. Liversedge8, I. McInnes9, C. Goodyear10, University of Glasgow, School of Infection and Immunity, Glasgow, United Kingdom; University of Aberdeen, School of Medicine, Medical Sciences and Nutrition, Aberdeen, United Kingdom; University of Glasgow, College of Medical Veterinary and Life Sciences, Glasgow, United Kingdom

Background: Delineating systemic immune cell signatures in rheumatoid arthritis (RA) has the potential for understanding pathogenesis and stratifying therapeutics accordingly.

Objectives: To evaluate the phosphoprotein signatures in circulating lymphocytes of early RA patients compared with healthy controls across disease trajectory over one year.

Methods: We used phospho-flow cytometry to measure the phosphorylation state of phosphoproteins in basal (unstimulated), and ex vivo stimulated circulating lymphocytes from 55 early RA patients before and after 6- and 12-month treatment with methotrexate (MTX) and compared these to 37 age- and gender-matched healthy controls. Flow cytometry datasets were analysed using FlowSOM, an unbiased clustering algorithm that partitions cell populations based on marker expression patterns.

Results: Multiple differential phosphorylation signatures are expressed across CD4+ and CD8+ T-cells and CD19+ B-cells that differentiate baseline RA from healthy controls; the signatures normalised towards a healthy state following treatment with MTX. Stimulation of cells from RA patients at baseline is refractory to further stimulation-induced phosphorylation, which was recovered following MTX treatment (p<0.01). A comprehensive unbiased analysis identified discrete clusters of cells exhibiting unique phosphoprotein signatures associated with early RA that differentiates the systemic immune response from healthy controls. Methyltransferase treatment recovers this phosphoprotein signature towards a healthy state.

REFERENCES:

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Disclosure of Interests: Mukanthu Nyrenda: None declared, Moeded AKBAR: None declared, Ashley Gilmour: None declared, Carol Wallace: None declared, Caron Paterson: None declared, Duncan Porter Consultant of: Abbvie and Eli Lilly, Grant/research support from: Abbvie and Eli Lilly, Janet Liver- sidge: None declared, Iain McInnes Consultant of: Abbvie, Amgen, Eli Lilly, Novar- tis, Janssen, UCB, Bristol Myers Squibb, Cabaletta, Compugen, MoonLaker, Pfizer, and Astra Zeneca., Grant/research support from: UCB, Bristol Myers Squibb, Novartis, Astra Zeneca, and Eli Lilly., Carl Goodyear: None declared.

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Table 1.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>T</th>
<th>B</th>
<th>Plasma</th>
<th>DC</th>
<th>Myeloid</th>
<th>Fibroblast</th>
<th>Mural</th>
<th>Vascular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upregulated resolving</td>
<td>43</td>
<td>300</td>
<td>4</td>
<td>378</td>
<td>270</td>
<td>11</td>
<td>153</td>
<td></td>
</tr>
<tr>
<td>Upregulated persisting</td>
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<td>82</td>
<td>3</td>
<td>205</td>
<td>223</td>
<td>7</td>
<td>229</td>
<td></td>
</tr>
</tbody>
</table>

Persistence of arthritis was associated with (i) the presence of plasma cells per se and (ii) a phenotypic shift in fibroblast, vascular, myeloid, Th, and B-cell populations with enrichment of SPP1+ Myeloid cell populations. Patients with active arthritis destined to resolve exhibited enrichment of Treg cells. Persistent and resolving states were characterised by significant compositional differences in key linings and sublining fibroblast subpopulations including sublining MMP9+ positive cells, and by gene expression programmes related to matrix remodelling and fibroblast/macrophage interactions.

OP0121 SYNOVIAL CELLULAR NICHES ARE DETERMINANTS OF ARTHRITIS PERSISTENCE VERSUS RESOLUTION IN EARLY UNTREATED ARTHRITIS

Keywords: -omics, Inflammatory arthritides, Rheumatoid arthritis

A. Filer 1, J.D. Turner2, C. Prada-Medina3, M. Attar4, C. D. Buckley5, S. Sansom6, K. Raža7,8,9,10, University of Birmingham, Institute of Inflammation and Ageing, Birmingham, United Kingdom; University Hospitals Birmingham, Dept of Rheumatology, Birmingham, United Kingdom; University of Oxford, The Kennedy Institute Of Rheumatology, Oxford, United Kingdom; Sandwell and West Birmingham NHS Trust, Dept of Rheumatology, Birmingham, United Kingdom

Background: Published synovial research largely focuses on persistent clinical syndromes such as RA and PsA. However spontaneously resolving synovitis is a common manifestation of viral infection (parvovirus, influenza), post-bacterial reactive states and metabolic disease such as gout. The study of synovial tissue taken from such patients using advanced single cell methodologies may provide biomarkers of outcome in early disease but is also a valuable resource to understand mechanisms underlying the subversion of healthy mechanisms of resolution resulting in persistent disease.

Objectives: To use single cell RNA sequencing to understand the cellular interactions governing resolution versus persistence of disease in untreated patients with active arthritis.

Methods: Synovial tissue biopsies were obtained using ultrasound guidance from treatment naïve patients in the Birmingham BEACON early arthritis cohort presenting with at least one clinically swollen joint and a joint atraumatic to ultrasound guided biopsy. Tissue samples from patients who went on to develop RA (n=15) or PsA (n=7) according to ACR criteria or CASPAR criteria at 24-month follow-up, and patients whose arthritis spontaneously resolved (n=5) underwent enzymatic disaggregation and were processed through a multimodal single-cell sequencing approach including the characterisation of the transcriptome and 58 surface-protein-panel profile. Sequenced gene libraries were integrated according to batch and sample using the Harmony integrative algorithm before clustering and annotating cell states using canonical marker genes. Seurat-based automated pipelines were used for variable gene identification and clustering. We used DESeq2 to identify per cell type genes, and weighted gene co-expression network analysis (WGCNA) to identify gene programmes associated with persistence (RA and PsA) versus resolving groups. NAMTI was used to identify unique cellular niches associated with persistence or resolution.

Results: ~90,000 viable cells were sequenced and data normalised, clustered and annotated describing over 80 cellular states across eight main synovial cell types (T, B, Plasma, Plasmacytoid DC, myeloid, fibroblast, mural and vascular). A total of 1945 genes varied significantly with prognosis, distributed unequally across cell types (Table 1).

Table 1.