bulk RNA-seq deconvolution analysis, cell clusters with high expression of type 1 interferon-related genes, such as ISG15+ SMCs, CD4+ISG15+ T cells, FCN1+ISG15+ macrophages and KLRK1+XCL1+ NK cells, were specifically cloned in DM. Cell subpopulations involved in mitochondrial changes, such as Mito_high_CD8+ TFEFs, were expanded in anti-Jo1 ASS group. TMP1+ SMCs and Mito_high_NKs have the highest proportion in anti-SRP IMM. Mito_high_NKs were positively correlated with serum creatine kinase (CK) in IIM patients. scRNA-seq and spatial transcriptomics combined analysis indicated ligand-receptor pairs MIF→(CD74+CD44)/(CD74+CXCR4) play key roles in the interactions of ANKRD2+ SMCs and inflammatory cells. The levels of CD44 and CXCR4 in muscle tissues were positively correlated with CK, lactate dehydrogenase, aspartate aminotransferase and alanine aminotransferase.

Conclusion: Our results demonstrate previously unrecognized SMCs and immune cells phenotypes in IIMs subgroups, and indicate the interactions of SMCs and immune cells are key pathological mechanisms driving muscle damage.

REFERENCES: NIL.

Disclosure of Interests: None Declared.

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**OP019**

**MUSCLE FIBRE PLAYS A CRUCIAL ROLE IN THE THERAPEUTIC RESPONSE OF MYOSITIS TO GLUCOCORTICOIDS THROUGH THE PARACRINE EFFECT OF EPINEPHRINE ON THE IMMUNE SYSTEM**

**Keywords:** Animal Models, Myositis

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**Background:** Glucocorticoids (GC) are the first line treatment in myositis. GC treatment is empirical. Both GC therapeutic and iatrogenic effects are mediated by the glucocorticoid receptor (GR), which is ubiquitously expressed. Our team has recently shown that muscle fibres immuno-metabolic modifications participate to muscle weakness and perpetuation of the disease[1]. Thus, myofibers could be a therapeutic target of GC.

**Objectives:** To unravel the mechanism of GC therapeutic effect in order to optimize myositis care.

**Methods:** Experimental myositis (EM) was induced in 8 to 10 week-old C57BL/6J mice through the immunization against a polypeptide from skeletal muscle fast-type C protein. In order to investigate whether GC target skeletal muscle fibres to elicit their therapeutic response in EM, we generated mice in which GR can be selectively ablated in skeletal muscle fibres in a temporal manner. To this end, EM was induced at day (D) 0 in HSA-Cre-ERT2tg(td)GRL2/GRL2 mice (pre-mutant mice), which express the tamoxifen-dependent CreERT2 recombinase selectively in skeletal muscle fibres and bear two LoxP-flanked GR alleles, as well as in HSA-Cre-ERT2tg(td)GRL2/GRL2 littermates, which do not express the recombinase (GRL2/GRL2 mice). Thus, tamoxifen treatment from D9 to D13 induced GR inactivation in the former mice only, termed hereafter GR(−)/skm−/− mice. Mice of both lines were treated by prednisone (PDN) from D14 to D20 at the dose of 1 mg/kg/day by gavage. Grip test was performed at D0, before the 1st PDN administration (D14) and the day before sacrifice (D20). Creatine-kinase (CK) activity assay in serum, muscle histology, immune-cell phenotyping using flow cytometry and gastrocnemius transcriptomic analysis were run. Transcriptomic data were validated in independent mice cohorts, in vitro and on human muscle biopsies.

**Results:** In pre-mutant EM mice at D0 as well as in EM GR(−)/skm−/− at D14, muscle strength was similar to that of EM GRL2/GRL2 mice. At D20, muscle strength was still comparable between V-treated EM GRL2/GRL2 and GR(−)/skm−/− mice. Conversely, PDN treatment did not induce a regain of muscle strength in EM GR(−)/skm−/− mice (143.9 vs 175.7 ± 75 kg in PDN-treated GRL2/L2, p=0.0007). V-treated EM GRL2/L2 and GR(−)/skm−/− mice showed a similar increase in serum CK levels at sacrifice. CK decreased only in GRL2/L2 and not in GR(−)/skm−/− mice after PDN treatment (146.6 ± 10.9 vs 234.5 ± 10.9 UI/L, p=0.002). Although no major differences in the histological inflammatory infiltrate score among the four experimental groups, at muscle flow cytometry, the percentage of proinflammatory macrophages, F4/80-Ly6c-positive, was greater (76% vs 67%, p=0.5) and that of anti-inflammatory macrophages, F4/80-CD11b-double negative T cells, that inhibit the immune response by killing effector T cells, slightly increased in PDN-treated EM GRL2/L2 compared to V-treated GRL2/L2 mice (35% vs 24%, p=0.2). This effect of PDN was suppressed in PDN-treated EM GR(−)/skm−/− mice. Transcriptomic and functional analyses of muscles in vivo and in vitro demonstrated the importance of epinephrine secreted by the myofiber relaying the effects of GC. The expression of epinephrine by the myofiber in response to GC has been validated in patients with myositis.

**Conclusion:** Skeletal muscle fibres play a critical role in the GC therapeutic response in myositis through an epinephrine-mediated polarization of inflammatory infiltrate toward an anti-inflammatory phenotype.

**REFERENCE:**


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**Predictors of outcome in early rheumatoid arthritis**

**OP0120**

**IDENTIFICATION OF EARLY RISK FACTORS IN A NOVEL PREDICTION MODEL FOR ACRA POSITIVE RA**

**Keywords:** Diagnostic Tests, Rheumatoid arthritis, Autoantibodies

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**Background:** Individual testing positive for anti-cyclic-citrullinated-peptide-antibodies (Anti-CCP) and musculoskeletal (MSK) complaints are at risk for developing rheumatoid arthritis (RA).

**Objectives:** We aim to identify factors involved in arthritis progression in a population considered at risk for RA.

**Methods:** Anti-CCP-positive individuals with MSK complaints referred to rheumatologist in the Region Stockholm were recruited. Individuals lacked arthritis at clinical and ultrasound examination and were followed for ≥3 years or until arthritis diagnosis was made. Blood samples from inclusion were analyzed for 9 selected anti-citrullinated-protein-antibody (ACPA) reactivities (citrullinated α-1-enolase, fibrinogen, filaggrin, histone, vimentin and tenasin peptides) as well as a panel of 92 inflammation-associated proteins and HLA-SE alleles. Cox regression was applied to the data and a predictive multivariate model was identified. Results are shown with a confidence interval (CI) of 95 percent.

**Results:** 267 individuals were recruited. 101 (38%) developed arthritis in median after 14 months (IQR: 6-27). In the multivariate analysis: ACPA reactivity (HR 8.0, CI 2.9-22, p<0.0001), IL15Rα levels (HR 0.6, CI 0.4-0.9, p 0.006), IL6 levels (HR 1.5, CI 1.2-1.8, p<0.001) and the presence of tenosynovitis as detected by ultrasound (HR 3.4, CI 2.0-5.5, p=0.001) were significantly associated with arthritis. Diagnostic accuracy for ACPA reactivity test had a sensitivity of 96% (CI 92-99.8), a specificity of 30% (CI 30-46), a positive predictive value of 51% (CI 43-58) and a negative predictive value of 94% (CI 87-99.7). Diagnostic accuracy for ultrasound assessed tenosynovitis had a sensitivity of 17% (CI 9-24), a specificity of 99% (CI 97-100), a positive predictive value of 89% (CI 74-100) and a negative predictive value of 64% (CI 57-70).
Conclusion: We propose a high-risk RA phase characterized by the presence of certain ACPA reactivities, IL15-R, IL6, and tenosynovitis, parameters that could be used to identify individuals at particular low risk and high risk for arthritis progression.

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

Keywords: -omics, Inflammatory arthritides, Rheumatoid arthritis

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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 aminul in synovial-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.

<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>
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<td>378</td>
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<td>11</td>
<td>153</td>
</tr>
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<td>3</td>
<td>1</td>
<td>205</td>
<td>223</td>
<td>7</td>
<td>228</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 and 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 lining and sublining fibroblast subpopulations including sublining MMP9 positive cells, and by gene expression programmes related to matrix remodelling and fibroblast/macrophage interactions.

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.

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OP0121

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)

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Background: Delineating systemic immune cell signatures in rheumatoid arthritis (RA) has the potential for understanding pathogenesis and stratifying thera peutics 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 stimulus-induced phosphorylation, which was recovered following MTX treatment (p<0.01).

Conclusion: This comprehensive and unbiased analysis identified discrete clusters of cells exhibiting unique phosphoprotein signatures associated with early RA that differentiates the systemic immune response from healthy controls. Methotrexate treatment recovers this phosphoprotein signature towards a healthy state.

REFERENCES: NIL.

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OP0122

Delineating systemic immune cell signatures in rheumatoid arthritis (RA) has the potential for understanding pathogenesis and stratifying thera peutics 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 stimulus-induced phosphorylation, which was recovered following MTX treatment (p<0.01).

Conclusion: This comprehensive and unbiased analysis identified discrete clusters of cells exhibiting unique phosphoprotein signatures associated with early RA that differentiates the systemic immune response from healthy controls. Methotrexate treatment recovers this phosphoprotein signature towards a healthy state.

REFERENCES: NIL.

Disclosure of Interests: Mukanthu Nyirenda: None declared, Moed 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, David Reid: None declared, Janet Liversidge: None declared, Iain McInnes Consultant of: Abbvie, Amgen, Eli Lilly, Novartis, Janssen, UCB, Bristol Myers Squibb, Cabaletta, Compugen, MoonLake, 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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