Macrophages are known to exhibit remarkable phenotypic plasticity and understanding the role of this characteristic in regulating inflammation and pathology remains a major challenge. As does the characterization of factors in the microenvironment such as the synovium that control such macrophage characteristics. Importantly, whether the infiltrating, inflammatory macrophages of the RA ST similarly exhibit such phenotypic plasticity, and whether this occurs during the process of reaching remission, remains to be studied.

**Objectives:** We investigated the phenotypic plasticity of inflammatory synovial macrophages from patients with RA in vitro, investigating their ability to convert from an inflammatory macrophage population into ‘regulatory’ CD206+/MerTK+ macrophages. These findings will provide a proof-of-concept as to the utility of these macrophages for a cell-based therapy in resolving inflammation in patients with RA, and will likely extend our understanding of the mechanisms of action of currently used therapeutics.

**Methods:** Synovial fluid (SF) mononuclear cells were obtained from patients with active early RA (<1 year; fulfilling 2010 ACR/EULAR classification criteria). Cryopreserved SFMCs were cultured for 48 hr in the presence of 10 ng/mL interferon (IFNγ), 50 ng/mL dexamethasone, 10 μg/mL Inflimab, or diluent. Following culture, cells were immonostained and analysed using a Beckman Coulter CytoFLEX flow cytometer and FlowJo software. SF macrophages were characterised by expression of CD14, CD45, CD68 (Figure 1A), and proportions of CD206+/MerTK+ macrophages measured.

**Results:** Prior to culture, the CD68+ macrophage populations present in SF were found to be predominantly CD206−/MerTK−. After 48 hours of culture, in the absence of any stimulus, there was an increase in proportions of CD206+/MerTK+ macrophages. Treatment with either dexamethasone or anti-TNF (Infliximab) resulted in a further increase in proportions of CD206+/MerTK+, M2-like macrophages. In contrast, culture with IFNγ induced a reduction in this population. Importantly, we found that the generated CD206+/MerTK+ macrophages were phenotypically stable in culture following removal of these differentiating agents.

**Conclusion:** Our findings demonstrate that inflammatory SF cells are indeed able to polarise to regulatory, CD206+/MerTK+ macrophages in vitro. The findings provide further mechanistic insights into the role for the therapeutic benefit of glucocorticoids and TNF inhibitors as well as providing initial proof-of-concept of the use of regulatory macrophages as a cellular-based therapy or therapeutic target for patients with RA.

**REFERENCES:**

**Disclosure of Interests:** None declared

**DOI:** 10.1136/annrheumdis-2022-eular.4129

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**Figure 1. Synovial fluid CD68+ macrophage plasticity in vitro.** (A) Gating strategy depicting CD68+ CD45+CD14+ SF macrophage determination. (B) Proportions of CD206+ and MerTK+expressing SF macrophages after 48hr culture in the presence of 10 ng/mL IFNγ, 50 ng/mL dexamethasone or 10 μg/mL Inflimab, or absence. Data are representative of 5 individual experiments. Data were analysed by two-way ANOVA followed by Dunnett’s multiple comparison test, *p<0.05.*

**POS0059**

**COMPLEMENT PROTEINS ARE ELEVATED IN PATIENTS WITH AXSPA COMPARED WITH RELATIVE CONTROLS OF PATIENTS WITH LOW BACK PAIN AND SPA-FEATURES WITHOUT AXSPA.**

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**Background:** Axial spondyloarthritis (axSpA) is associated with a certain genetic predisposition, i.e., with the presence of human leukocyte antigen (HLA)-B27. However, the pathogenesis remains largely unexplained. Animal models of ankylosing spondylitis have shown inhibition of complement to be beneficial in terms of limiting structural damage (1). The lecit pathway of complement activation serves as a key component in innate immune system and plays a pivotal role in both homeostasis and development. The influence of complement in axSpA is mainly unexplored. We have, however, previously reported elevated plasma levels of the lectin pathway proteins L-ficolin and H-ficolin in patients with axSpA compared with blood donors (2).

**Objectives:** Our aim was to investigate plasma levels of lectin pathway proteins in a clinical cohort of patients with axSpA and compare them to relevant controls that do not often experience spinal challenges in differentiating from axSpA.

**Methods:** Plasma samples were obtained from individuals in a cohort of patients suffering from low back pain (LBP) including: 1) 23 patients with axSpA, 2) 55 patients without axSpA experiencing SpA-features/symptoms, and 3) 64 patients...
with nonsppecific LBP without SpA-features or MRI findings suggestive of axSpA. Diagnosis of axSpA was based on multidisciplinary team conference consensus after 3.5 years of follow-up (3). Plasma levels of 10 lectin pathway proteins (MBL, H-ficolin, M-ficolin, MASP-1, MASP-2, MASP-3, MASP-4, and MAP19) were measured by immunoassays developed in-house.

Results: Patient characteristics are shown in Table 1. Plasma levels of lectin pathway proteins L-ficolin, M-ficolin and CL-L1 differed significantly in the patient groups (p = 0.03). L-ficolin and M-ficolin were elevated in axSpA-patients compared with patients with SpA-features without axSpA and nonsppecific LBP patients (Figure 1). CL-L1 was elevated in axSpA-patients and patients with SpA-features without axSpA compared with nonsppecific LBP patients (Figure 1).

No significant differences were observed for MBL, H-ficolin, MASP-1, MASP-2, MASP-3, MAP44, and MAP19. L-ficolin levels correlated with CRP in axSpA-patients (Spearman’s rho=0.58, p=0.004). M-ficolin levels correlated weakly with CRP in nonsppecific LBP patients (Spearman’s rho=0.36, p=0.003). Lechti pathern protein levels did not correlate with disease activity (ASDAS).

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>axSpA</th>
<th>Not axSpA</th>
<th>Non-specific low back pain</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age, years (range)</td>
<td>32 (19-40)</td>
<td>39 (19-41)</td>
<td>32 (19-39)</td>
<td>0.75</td>
</tr>
<tr>
<td>Males, n (%)</td>
<td>10 (43)</td>
<td>17 (40)</td>
<td>11 (41)</td>
<td>0.01</td>
</tr>
<tr>
<td>HLA-B27 positive, n (%)</td>
<td>17 (74)</td>
<td>26 (70)</td>
<td>23 (71)</td>
<td>0.02</td>
</tr>
<tr>
<td>Inflammatory back pain, n (%)</td>
<td>16 (78)</td>
<td>28 (78)</td>
<td>23 (71)</td>
<td>0.11</td>
</tr>
<tr>
<td>Good response to NSAID</td>
<td>14 (61)</td>
<td>27 (69)</td>
<td>20 (59)</td>
<td>0.01</td>
</tr>
<tr>
<td>Saccritolysis at MRI, acc. ASAS, n (%)</td>
<td>22 (96)</td>
<td>45 (82)</td>
<td>31 (67)</td>
<td>0.01</td>
</tr>
<tr>
<td>Elevated CRP, n (%)</td>
<td>3 (13)</td>
<td>7 (13)</td>
<td>6 (14)</td>
<td>0.97</td>
</tr>
<tr>
<td>ASDAS (range)</td>
<td>2.5 (1.3-3.7)</td>
<td>2.3 (0.8-3.8)</td>
<td>2.4 (1.8-3.9)</td>
<td>0.52</td>
</tr>
</tbody>
</table>

Figure 1. Plasma levels of L-ficolin, M-ficolin and CL-L1.

Conclusion: L-ficolin and M-ficolin are increased in patients with axSpA when compared with relevant control cohorts of patients with LBP or with SpA-features without axSpA. Our findings support a potential pathogenic role for complement in axSpA, however, further studies are needed to elucidate the diagnostic potential of the specific complement proteins.

REFERENCES:

Disclosure of Interests: None declared

MEASUREMENTS OF COMPLEMENT ACTIVITY SPECIFICALLY THOUGH THE LECTIN OR THE CLASSICAL PATHWAY IN PATIENTS WITH SLE

Background: In systemic lupus erythematosus (SLE), the complement system (CS) is activated and thought to occur primarily through the classical pathway (CP) (1). Traditionally, when diagnosing SLE or assessing disease activity, measurements of low C3 or C4 are used as proxies for complement activation (2). However, measurement of C3 and C4 does not differentiate which complement pathway initiated the activation (i.e., the lectin pathway (LP), the CP, or the alternative pathway) (1, 3).

C1-esterase inhibitor (C1inh) is one of the key regulators of the CS. C1inh is the exclusive inhibitor of the active CP enzymes C1r and C1s (4), and the major inhibitor of active LP enzymes MASP-1 and MASP-2 (5). A possible way of assessing complement activation through a specific pathway, is by measuring activated enzymes complexed with C1inh in plasma, as these complexes only exist after complement enzyme activation.

Objectives: Our aim was to investigate and unravel LP and CP complement activation in SLE, by measuring the protein complexes MASP1/C1inh (LP specific activation) and C1r/C1inh (CP specific activation). Furthermore, we aimed to investigate whether there is a association between complement activity, disease activity (SLEDAI) and disease manifestations (lupus nephritis (LN)).

Methods: A cross sectional cohort of 150 patients with SLE fulfilling the 1997 ACR classification criteria for SLE were included from the outpatient clinic at the department of Rheumatology, Aarhus University Hospital (AUH), Denmark. Disease manifestations and disease activity using SLEDAI score was assessed at inclusion. Fifty healthy individuals included at the Blood Bank, AUH, were used as controls. Both C1s/C1inh and MASP1/C1inh complexes were measured in all samples using two newly developed sandwich ELISAs (C1s/C1inh: cat#: HK399; MASP1/C1inh: Cat#:3001, Hycult Biotech, Uden, The Netherlands), EDTA-samples from both SLE patients and controls were measured in duplicates.

Results: When comparing SLE patients to controls, we observed a difference in complement activation through the LP, where a lower mean MASP1/C1inh plasma concentration was observed (p=0.01). C1s/C1inh concentrations were significantly increased in active SLE patients (SLEDAI >6) when compared to SLE patients with low disease activity (SLEDAI <6, p=0.01) and correlated with SLEDAI score (r=0.285, p=0.01). C1s/C1inh concentrations were increased in SLE patients with active LN compared to non-active LN (p=0.09).

No differences in MASP1/C1inh plasma concentrations were observed between active SLE patients and patients with low disease activity (p=0.11), nor did we observe a significant correlation with disease activity (r=0.12, p=0.13). In active LN, plasma concentrations of MASP1/C1inh were significantly elevated compared to non-active LN (p=0.02).

Conclusion: Our data suggest that the CP and the LP is activated in SLE. CS is generally activated in active SLE disease, whereas activation of the LP might be more specific to particular disease manifestations like LN.

Our findings warrant further research into activation of the specific pathways in relation to specific disease manifestations in SLE.

REFERENCES:

Figure 1. Measurements of plasma concentrations of C1r in complex with C1 inhibitor (C1r/ C1inh) (a1-a4) and MASP1 in complex with C1 inhibitor (MASP1/C1inh) (b1-b4) in Systemic Lupus Erythematosus (SLE) patients and controls. A1 and b1 show measurements of SLE patients versus controls. A2 and b2 show measurements in SLE patients with active disease (SLEDAI >6) versus low disease activity (SLEDAI<6). A3 and b3 show correlation analysis between measurements and SLEDAI score in SLE patients. A4 and b4 show measurements in SLE patients with active lupus nephritis (LN) versus non-active LN.

Disclosure of Interests: Mads Christian Lamm Larsen: None declared, Anne Trolldborg: None declared, Erik Toonen Employee of: ET is an employee of Hycult Biotech, Lisa Hurler: None declared, Zoltán Prohászka: None declared, László Cervenak: None declared, Annette G Hansen: None declared, Steffen Thiel: None declared