Background: Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease of unknown and complex etiology with severe detrimental effects for the patient’s quality of life. While rheumatoid factors (RF) and anti-citrullinated protein antibodies (ACPA) have been used extensively for the diagnosis of RA, no clear mechanism of action towards disease pathogenesis and progression has been identified. Importantly, both seropositive and seronegative RA patients experience significant improvement in disease severity following B cell depletion. Therefore, we hypothesised that B cells have a central role in ACPA+ and ACPA- RA irrespective of their capacity to produce auto-antibodies.

Objectives: The characterisation of B and T cell populations in the peripheral blood and synovium of ACPA+, ACPA- and arthralgia patients. The identification of non-antibody-mediated B cell function under the hypoxic conditions of the inflamed joint.

Methods: Peripheral blood, synovial fluid and tissue was obtained from ACPA+, ACPA- and arthralgia patients. Following enzyme digestion of the tissue, several 15-colour panels were used for the flow cytometric analysis of T and B cell populations of ACPA+, ACPA- and arthralgia patients compared to healthy subjects. Activation and function of healthy, sorted B cells, cultured in vitro and stimulated by CD40 and BCR mediated signals under normoxic (21% O2) and hypoxic (1% O2) conditions was examined.

Results: Pro-inflammatory cytokine production by peripheral blood CD4+ T cells is not significantly different between ACPA+, ACPA- and arthralgia patients when compared to healthy controls. However, a significant reduction in CD27+ switched memory B cells was observed between healthy subjects and ACPA+ RA patients. The aforementioned decrease in memory B cells is potentially a result of increased susceptibility to FAS induced apoptosis since healthy B cells cultured with RA patient plasma showed increased activation, CD80/CD86 and FAS expression. In the synovial fluid and synovial tissue, CD4 T cell pro-inflammatory cytokine production was increased when compared to peripheral blood CD4 T cells. Interestingly, ACPA+ RA patient CD4+ T cells produced reduced amounts of pro-inflammatory cytokines when compared to ACPA- RA patient CD4+ T cells. Despite accumulated expression of both negative (DN) memory B cells in the synovial fluid and tissue, compared to peripheral blood, no differences in synovial B cell subpopulation composition between ACPA+ and ACPA- RA patients was observed. Interestingly, sorted B cells from healthy subjects showed increased sensitivity to in vitro stimulation with increased expression of CD80 and CD86 when cultured under hypoxic conditions, while co-culture with RA patient synovial fibroblasts did not enhance this effect.

Conclusions: The increased capacity of ACPA+ compared to ACPA- RA patient synovial CD4+ T cells to produce pro-inflammatory cytokines, could be responsible for the more severe disease progression of ACPA+ compared to ACPA- RA. The accumulation of memory B cells in both ACPA+ and ACPA- RA, underlines a common, antibody independent, contribution of B cells in synovial inflammation. While B cell activation under hypoxic conditions and increased CD80/CD86 expression is potentially an important mediator for the emergence of auto-reactive T cells and disease progression in RA.

Disclosure of Interest: None declared


OP0316

INCREASED EXPRESSION OF MICRONA-142-3P IS ASSOCIATED WITH THE FUNCTIONAL DEFECT OF REGULATORY T CELLS IN ANTI-NEUTROPHIL CYTOPLASMATIC ANTIBODY ASSOCIATED VASCULITIS

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Background: Circulating regulatory T cells (Tregs) in anti-neutrophil cytoplasmatic antibody associated vasculitis (AAV) are frequently functionally deficient. The mechanism behind their impaired function is however unknown. Small non-coding microRNA (miR) are post-transcriptional regulators of protein synthesis and previous microarray studies have shown that differently expressed miRs in T cells are associated with autoimmunity.

Objectives: To investigate whether the dysfunctionality of Tregs in AAV is due to altered microRNA (miR) expression.

Methods: Tregs (CD4+CD45RO+CD25+CD127-) of healthy controls (HC) and AAV patients in remission without treatment (AAV-REM) were FACS-sorted, and total RNA was isolated. Samples from 8 HCs and 8 AAV-REMs were subjected to microRNA microarray analysis. Based on relative expression and fold change, 5 differentially expressed miRs were validated in an independent cohort using qRT-PCR and a database and literature search was performed to identify potential targets.

Results: Nineteen miRs differentially expressed were detected by microarray analysis, of which Let-7g, miR-20a-5p, miR-26a-5p, miR-142-3p, and miR-146a-5p were validated in an independent cohort. Of these, miR-142-3 p was confirmed to be significantly upregulated (2.0 fold, p=0.03) in Tregs from AAV-REM patients compared to HC Tregs (n=23, n=22). To study the functional impact of miR-142-3 p overexpression, HC Tregs were transfected using either a mimic-miR-142-3 p or a scrambled (SCR)-control. After transfection, HC Tregs were co-cultured with T effectors (CD4+CD25+) in a suppression assay to test their suppressive capacity. Transfection with mimic-miR-142-3 p significantly increased the miR-142-3 p levels (2.4 fold, p=0.03) and reduced the suppressive capacity compared to SCR-transduced Tregs (1.9 fold reduction, p=0.02). Moreover, miR-142-3 p levels tended to correlate to the suppressive function of Tregs (p=0.06, rho=−0.591). A database and literature search identified adenylly cyclase 9 (AC9) as a promising target of miR-142-3 p. miRNA levels of AC9 tended to be lower in AAV-REM patients compared to HC (3.8 fold, p=0.07). In addition, CAMP
levels, which are partly produced by AC9, were significantly lower in Tregs from AAV-REM patients after 48 hour of stimulation with anti-CD3 and anti-CD28 (1.7 fold, p=0.003).

Conclusions: Increased expression of miR-142–3p in Tregs of AAV-REM patients is associated with their functional impairment, potentially by targeting the AC9/cAMP mediated suppression.

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SCREENING OF AN AUTOANTIBODY SIGNATURE OF EARLY KNEE OSTEOARTHRITIS: DATA FROM THE OSTEOARTHRITIS INITIATIVE

Abstract OP0317 – Table 1

<table>
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<th>Acc number</th>
<th>Symbol</th>
<th>Wilcoxon test (p value)</th>
<th>Specificity at 95%</th>
<th>AUC</th>
<th>AUC (p value)</th>
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<tr>
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<td>VPS4B</td>
<td>0.005</td>
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<td>0.855</td>
<td>0.4732</td>
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<tr>
<td>Q9NZL9</td>
<td>MAT2B</td>
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<tr>
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<tr>
<td>P60763</td>
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<td>76.30%</td>
<td>0.88</td>
<td>0.0496</td>
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<td>76.90%</td>
<td>0.885</td>
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</table>

Conclusions: This work is the first to screen a large number of human proteins for the discovery of OA-associated AAbs. We define a panel of six AAbs, which are increased prior to the development of symptomatic knee OA. These results suggest that a serum AAbs signature can facilitate the discovery of early OA biomarkers useful for clinical diagnosis.

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Disclosure of Interest: None declared

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NETOSIS-INHIBITING T-ACPA THERAPY FOR USE IN DIFFERENT NET-DRIVEN HUMAN AUTOIMMUNE DISEASES

Abstract OP0318

Background: Aberrant Neutrophil Extracellular Trap (NET) formation contributes to the induction and propagation of inflammation and plays a key role in causing tissue damage in conditions like sepsis, SLE, RA and vasculitis. Cytokullination of proteins is involved in the formation of NETs, autoimmunity, and the breaking of tolerance in NET-driven autoimmune diseases. In SLE and RA, neutrophils undergo enhanced NETosis, and NET components are observed in blood, inflamed tissues and joints.

Objectives: Our objective is to develop a novel first in class NET-inhibiting therapeutically-citrullinated protein antibody (tACPA) targeting citrullinated histones 2A and 4, for the treatment of human diseases in which aberrant NET formation add to the severity of the pathology with an initial focus on autoimmune diseases. Here, we demonstrate the utility of tACPA for different NET-based diseases beyond RA, including SLE, vasculitis, gout and idiopathic pulmonary fibrosis (IPF).

Methods: Previously, using two RA animal models, the therapeutic properties of tACPA have been demonstrated. Chirivi et al., 2013. In the current studies, neutrophils from RA and SLE donors, as well as biological NET-inducing stimuli, such as RA synovial fluid (SF), gout SF and activated platelets, have been used to demonstrate the NETosis-inhibiting properties of tACPA in different human disease contexts. We have further expanded tACPA’s therapeutic utility by testing it in a surrogate model for NET-mediated organ damage (sepsis) and IPF.

Results: NETosis in human RA and SLE neutrophils have been induced with a calcium ionophore and could be inhibited by tACPA treatment (40%–100% reduction). Similar results were obtained using RA and gout SF or activated platelets as NETosis inducers in combination with neutrophils from healthy donors. These observations have been confirmed with multiple NET readouts such as MPO activity, MPO/DNA ELISA, DNA quantification as well as imaging readouts. In addition, we demonstrated that in an LPS-induced sepsis model 30% of tACPA-treated mice survived (compared to 0% in placebo controls), showing protection against organ failure. In a bleomycin-induced IPF mouse model, tACPA protected mice from the development of lung fibrosis (compared to placebo controls). When determining neutrophil counts in bronchoalveolar lavage samples, we found that in tACPA-treated mice, neutrophil levels were normal, while levels in placebo-treated mice were elevated.

Conclusions: In a sepsis and IPF mouse model, tACPA prevented NET-mediated organ damage, providing evidence that tACPA could be a promising therapeutic strategy for diseases where NET-mediated endothelial toxicity causes organ damage like SLE, vasculitis and IPF. Central to our strategy for generating a preclinical data package supporting clinical testing, is to demonstrate that patient NETOSIS can be significantly inhibited ex vivo. We will present data that confirm that tACPA can block human SLE NETosis as well as human NETOSIS induced by activated platelets or gout SF.

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