AB0028 ACTIVATED STROMAL CELLS INDUCE CCL20 RELEASE AND T CELL MIGRATION

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Background: Although the role of IL-23/Th17 axis in psoriatic arthritis (PsA) is well known¹, little data is available on the contribution of stromal cells to this pathway². Enthesitis is a common feature of PsA and it may represent the site of onset^{3,4}, suggesting tendon stromal cells (tenocytes) may have an initiating role in Th17 driven pathogenesis.

Objectives: To assess the ability of stromal cells to produce CCL20, a chemokine able to recruit Th17 cells, and to induce T cell migration.

Methods: Healthy tenocytes cultured from hamstring tendons and fibroblast-like synoviocytes (FLS) from PsA patients were stimulated with human recombinant IL-1 β (1 ng/ml) and IL-17A (1, 10 and 100 ng/ml). Expression of CCL20 transcript and protein were assessed by quantitative PCR and ELISA, respectively. T cell migration assays were performed with magnetically enriched CD3⁺ cells from peripheral blood of PsA patients and healthy controls using a Transwell system. Following incubation with conditioned media from stimulated stromal cells, the migrated cells were harvested and analysed via light microscopy and flow cytometry.

Results: Both tenocytes and FLS were able to produce CCL20 following stimulation with IL-1 β . Furthermore, the addition of IL-17A induced a synergistic effect with IL-1 β . Following cytokine stimulation, diseased stromal cells produced greater levels of CCL20 compared to stimulated healthy tenocytes. In addition, conditioned media from stimulated tenocytes promoted T cell migration, compared with supernatants from unstimulated tenocytes.

Conclusions: We have shown that tendon and PsA synovium stromal cells are able to produce CCL20 and induce T cell recruitment, suggesting a role in the chemotaxis of Th17 cells. The positive feedback observed with IL1 β and IL-17A suggests a close relationship between stromal cells and Th17 cells.

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Acknowledgements: Giacomo Cafaro was supported by Partner Fellowship and EULAR Fellowship

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.4946

AB0029 CHARACTERISTIC PATTERNS OF HLA PRESENTATION AND T CELL DIFFERENTIATION IN ADULT-ONSET STILL'S DISEASE

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Background: The role of T cells in AOSD pathogenesis remains controversial. In autoimmune and auto-inflammatory diseases, such as rheumatoid arthritis (RA) and Behçet's disease, a human leukocyte antigen (HLA)-restricted T cell response to antigen has been shown to affect disease progression, with several HLA alleles strongly associated with disease severity.

Objectives: In this study, we investigated the frequencies of cells presenting HLA-DP, DQ, and DR, as well as differentiated T cell populations including naïve and effector memory T cells in peripheral blood leukocytes (PBLs) of patients with AOSD. Frequencies of the markers were then compared based on clinical outcomes and disease activity, to better understand the role of these cell populations in the pathogenesis of AOSD.

Methods: This study enrolled 14 active AOSD patients, 20 rheumatoid arthritis (RA) patients, and 20 healthy controls (HC). The percentage of surface-stained cells presenting HLA-DP, DQ, and DR alleles, and the proportions of differentiated T cell populations in peripheral blood leukocytes (PBLs) were measured by flow cytometry.

Results: Patients with AOSD exhibited significantly higher percentages of lymphocytes presenting HLA-DP and HLA-DR, and lower percentages of cells presenting HLA-DQ, than patients with RA or HC. The proportions of CD4⁺, CD4 +CCR7⁺, CD4 +CD62L⁺, and CD8 +CD62L⁺ cells in PBLs were decreased in

patients with AOSD relative to patients with RA or HCs. In contrast, AOSD patients exhibited increased proportions of CD8 +naïve T cells in whole blood relative to patients with RA or HC. The proportions of CD4 +effector memory T cells, CD8 +naïve T cells, and CD8 +effector memory T cells in whole blood cells and CD4 +effector memory T cell in lymphocytes were significantly associated with systemic score.

Conclusions: While the frequencies of CD4⁺, CD8⁺, CCR7⁺, CD4 +CCR7⁺, CD4 +CD62L⁺, and CD8 +CD62L⁺ cells were significantly decreased in patients with AOSD, the frequency of CD8 +naïve T cells was elevated in patients with AOSD, and correlated with systemic score. Additional studies in a larger cohort of patients will be necessary to evaluate the role of these markers in the pathogenesis of AOSD.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.6246

AB0030 TARGETING NF-KB SIGNALLING IN B CELLS: A POTENTIAL NEW TREATMENT MODALITY FOR ANCA-ASSOCIATED VASCULITIS

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Background: The pivotal role of B cells in the pathogenesis autoimmune diseases such as ANCA-associated vasculitis (AAV) is well-established and further substantiated by beneficial therapeutic effects of rituximab (anti-CD20 B cell targeted therapy). However, this results in prolonged B cell depletion while long-lived plasma cells are not targeted. Thus, there is a need for novel therapeutics targeting the B-cell lineage in AAV. NF- κ B signalling pathways that act downstream of various B cell surface receptors, including the B cell antigen receptor, CD40, BAFFR and TLRs, are crucially involved in B cell responses and may be suitable as novel targets.

Objectives: To identify whether inhibition of NF- κ B signalling by novel pharmacological inhibitors is effective in targeting B cell responses in general and more specifically blocks (auto)antibody production and plasmablast differentiation in B cells from AAV patients.

Methods: PBMC and sorted B cells from AAV patients and healthy donors were cultured with T cell-dependent (anti-IgM +anti CD40+IL-21) and T cell-independent (CpG +IL-2) stimuli. NF- κ B signalling was targeted in these cultures by small molecule inhibitors of NF- κ B inducing kinase (NIK, non-canonical NF- κ B signalling) and Inhibitor of κ B kinase β (IKK β , canonical NF- κ B signalling). Downstream NF- κ B signalling and nuclear NF- κ B translocation was determined by Western blot and confocal imaging. Effects on B cell proliferation and differentiation were determined by CFSE dilution assays and flow cytometric analysis of B cell markers. (Auto)antibody production was measured by ELISA.

Results: In B cells of AAV patients and healthy donors, targeting of NIK and IKK β effectively inhibited downstream non-canonical or canonical NF- κ B signalling, respectively. In a B cell stimulation assay, NIK and IKK β inhibition significantly reduced T cell-dependent (anti-IgM +anti CD40+IL-21) and T cell-independent (CpG +IL-2) B cell proliferation. In addition, B cell differentiation towards plasmablasts (CD27⁺/CD38⁺) and functional antibody production was attenuated by both NIK and IKK β inhibitors. Interestingly, the effects of NIK inhibition appeared to be B cell-specific as T cell proliferation was largely unaffected.

Conclusions: These data demonstrate that inhibition of NF- κ B signalling in AAV B cells results in the modulation of various B cell responses. Ongoing studies will indicate whether targeting of NF- κ B signalling in B cells may be an effective novel treatment modality for AAV.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.6339

AB0031 DEEP IMMUNE-PROFILING OF CD4+ T CELLS IN BEHÇET'S DISEASE

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Background: Functionality and immune-phenotypes of the human CD4⁺ T-cell compartment in Behçet's disease (BD) are under-investigated, but several lines of evidence point to its relevance in the pathogenesis, progression and remission of the disease.

Objectives: We aimed to apply an unbiased single cell approach to assess protein expression levels of phenotypic and functional markers within the human CD4⁺ T cell compartment in subjects with prototypical BD in order to identify cell populations of potential biologic relevance.

Methods: We determined single cell surface/intra nuclear expression levels of CD3, CD4, CD8, CD127, CD25, CD45RA, CCR7, FoxP3, HELIOS, Ki67, HLA-DR, CD38, CD39 in PBMC by flow cytometry and computed the representation of all mathematically possible cell populations within pre-defined starting populations. PBMC from BD subjects (n=13), healthy donors (HD) (n=25) and diseased subjects with non-BD auto-immune uveitis (n=11; VKH, Sarcoidosis, and HLA-B27 associated uveitis) were used. BD subjects met ISG criteria and were Arab or Chinese. 62% had pan-uveitis, 23% major vascular disease, and 7% parenchymal CNS disease. 46% were HLA-B51 carriers.

Results: Computation of all populations defined by CD127, CD25, CD45RA, CCR7, FoxP3, HELIOS, Ki67, HLA-DR within the CD4⁺ T cell compartment yielded a total of 6560 (3⁴8-1) cell populations out of which 45 reached a significance level of $p \leq 0.000001$ in ANOVA testing to differentiate 3 groups (BD, non-BD uveitis, and HD). All of these populations comprised sub-types of the human regulatory T (Treg) cell compartment with a strong predominance of non-proliferating, non-activated, FoxP3⁺HELIOS⁺ Treg carrying central-memory phenotypes (CD45RA⁻, CCR7⁺). Unpaired testing of BD vs non-BD revealed 43 distinct cell populations at a significance level of $p \leq 0.002$ representing CD25⁺ non-Treg; comparison of BD vs HD uncovered 58 populations at a significance level of $p \leq 0.0001$ representing FoxP3⁺HELIOS⁺ subpopulations, and non-BD vs HD identified 61 populations at $p \leq 0.001$, comprising CD25⁺CD127⁺ FoxP3⁺, but consistently HELIOS⁻ cells. A separate analysis using CD38, CD39, CD227, TIGIT, CD45RA, CCR7 within the CD3⁺CD4⁺CD8⁻CD127⁺CD25⁺ compartment that contains most human Treg, showed 48 populations ($p \leq 0.0001$) in 3-way comparison pointing to high significance of TIGIT and CD226 staining Treg subpopulations, and 18 populations with differential expression of CD39⁺ between BD and non-BD diseased subjects. TIGIT and CD226 co-expressing Treg (CD127⁺CD25⁺) subpopulations also reached significance ($p \leq 0.02$) in a longitudinal paired analysis of 7 BD subjects in active vs inactive disease states, as did 56 out of 6560 populations within total CD4⁺ T cells, mostly representing non-Treg cells in active BD.

Conclusions: Differential expression of CD4⁺ Treg and non-Treg cells shapes the immune-phenotype of BD vs states of health and non-BD autoimmune diseases that have phenotypic overlap with BD (uveitis). The populations with the highest significance for differentiating BD from healthy states seem to exist within the HELIOS⁺FOXP3⁺ compartment of non-activated, non-proliferating Treg, suggesting relevance of a true Treg phenotype. This is likely not specific to BD. Non-Treg CD25⁺ cell populations seem more indicative of BD vs non-BD uveitic disease as well as of clinically active BD while populations with high CD39 expression may signify non-BD states of immune-mediated disease.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.7160

AB0032

B-CELL SUBPOPULATIONS IN NEWLY DIAGNOSED EORA AND YORA PATIENTS

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Background: B-cells are thought to have an important role in rheumatoid arthritis (RA). This is demonstrated by the success of B-cell depleting therapy as well as the negative prognostic value of anti-citrullinated protein antibodies (ACPA)¹. However, the pathogenesis of the disease is unclear. Studies have suggested that there are differences in disease characteristics between elderly-onset RA patients (EORA, defined by disease onset at ≥ 60 years of age) and younger-onset RA patients (YORA, with disease onset < 60 years of age).²

Objectives: Our aim was to study the B-cell subpopulations in newly diagnosed EORA and YORA patients. We investigated whether there were differences in B-cell subpopulations between the groups and whether there was a correlation between B-cell subpopulations and disease activity, autoantibody profile and inflammatory parameters in these two RA patient groups.

Methods: Treatment-naïve EORA (n=29) and Yora (n=31) patients with newly diagnosed RA were included at their first visit to the Rheumatology clinic. The patients' clinical response (DAS28), autoantibodies and B-cells were assessed. Flow cytometry was used for the analysis of cellular surface markers on leukocytes in peripheral blood: CD19, CD27, CD24, CD27, CD38, PD-1, PD-L1, IgG,

IgD and IgM. Non-parametric tests were used for comparing groups and Spearman's test was used for correlation.

Results: We found a correlation between the ACPA titers and the frequency of the CD27⁺ and CD27⁻ memory B cell populations in EORA patients but not in YORA patients. This was further supported by a correlation of the ACPA titer and IgG⁺ B cells in the EORA patients ($r=0.7$, $p=0.003$) and not in the YORA patients. There was neither a correlation between age and ACPA titer nor between age and memory B cell populations. We did not find any significant difference between the B cell subpopulations in the two patient groups.

Conclusions: Our results suggest that the memory B cell compartment in peripheral blood in EORA patients reflects the ACPA titer. This was not seen in the YORA patients. The mechanisms behind these findings need to be further elucidated.

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Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.6459

AB0033

B REGULATORY CELLS POSITIVELY CORRELATE WITH ANTIBODIES AGAINST SS-A RO52 IN SYSTEMIC SCLEROSIS

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Background: We and Japanese Investigators have shown that IL-10-producing regulatory B cells (B10 cells) are decreased in systemic sclerosis (SSc).^{1,2} Contrary to the Japanese, we did not find a negative correlation between B10 cells and SSc-specific autoantibodies (autoabs) against centromere, Scl-70 or RNA polymerase III.³ Since we found anti-Ro52 SS-A antibodies in approx. 30% of patients with SSc,⁴ this being the 3rd most frequent autoantibody in this disease, we considered what is the relation of B10 cells with anti-Ro52 antibodies.

Objectives: We examined the number and function of Bregs in SSc in relation to anti-Ro52 autoabs.

Methods: Serum samples and PBMCs were collected from 40 SSc patients (15 anti-Scl-70, 20 anti-CEN and 5 anti-RNA pol III) and were further divided according to anti-Ro52-positivity into 22 anti-Ro52(+) and 18 anti-Ro52 (-). All serum samples were tested for the presence of disease-specific autoantibodies against Scl-70, CENP, RNA-pol, and against Ro52 using a line assay (Euroimmun Germany). The function of Bregs was determined by the ability to express IL-10 following activation with ODN 2006 (TLR-9). Percentages of transitional (CD19⁺CD24^{high}CD38^{high}) and memory (CD19⁺CD27⁺CD24^{high}) Bregs were assessed by flow cytometry using fluorochrome conjugated antibodies (BD Biosciences).

Results: IL-10(+) Bregs (B10) were significantly elevated in SSc patients (6.7% $\pm$ 2.6% n=15) with high-titre antibodies against Ro52 (mean anti-Ro52 arbitrary units AU >100; positivity cut-off AU >20) compared to patients (4.2% $\pm$ 1.9%, n=22) totally negative for Ro52 (mean AU <10) ($p=0.03$). Transitional Bregs were also significantly increased in all SSc patients tested positive for anti-Ro52 autoantibodies (7.5% $\pm$ 1.9%) compared to SSc patients negative for anti-Ro52 autoantibodies (3.7 $\pm$ 0.8, $p=0.02$). Furthermore, transitional Bregs positively correlated with anti-Ro52 antibody levels ($r^2=0.39$ $p=0.01$). In contrast, memory Bregs were not significantly different between anti-Ro52-positive (14.1% $\pm$ 2.7%) and -negative SSc patients (11.8% $\pm$ 2.2%) ($p>0.05$).

Conclusions: IL10-producing Bregs are higher in SSc patients with high anti-Ro52 antibodies and transitional Bregs correlated with antiRo52 antibodies in patients with SSc suggesting that this autoantibody could be a potential marker of Breg efficiency.

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