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 a longitudinal paired analysis 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

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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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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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