inflammation research, Göteborg, Sweden; ³Karolinska Institute, Department of Biosciences and Nutrition, Solna, Sweden; ⁴the Sahlgrenska Academy at University of Gothenburg, Department of Chemistry and Molecular Biology, Gothenburg, Sweden

Background: The oncogenic protein survivin is a marker of severe rheumatoid arthritis (RA). High serum levels of Survivin predict progressive joint damage¹ and poor treatment response².

Objectives: To study the role of survivin in the transcriptional regulation of phenotype in CD4⁺ T cells.

Methods: CD4⁺ T cells of RA female patients were isolated from the peripheral blood. Activated CD4⁺ cells were treated with survivin inhibitor YM155. Transcriptional analysis was done by RNAseq (Illumina) and conventional qPCR. Chromatin of CD4 cells was immunoprecipitated using polyclonal antibodies to survivin and subjected to deep sequencing (survivin ChIPseq, Hiseq2000, Illumina) and aligned to GRCh38. Statistical analysis of differentially expressed genes (DEG) was done in R-studio using Benjamini-Hochberg adjustment for multiple testing (Bioconductor, DESeq2 package).

Results: Survivin ChIPseq of the activated CD4⁺ T cells was enriched with the genes engaged in regulatory transcription factor specific DNA binding (GO:0000987, adj p=0.0005) and RNA polymerase II regulatory transcription (GO:0000978, adj p = 0.0004). Among survivin targets were the genes of HOX-B cluster and TALE family proteins MEIS, PKNOX and PBX1 controlling early leukopoiesis and T cell maturation. Inhibition of survivin in PBMC resulted in significant upregulation of PBX1 (p=0.023), MEIS3 (p=0.0036), similar tendency was observed for HOXB6 and HOXC4 genes. RNAseq analysis CD4 cells of RA patients with different transcription of PBX1, identified 1636 genes (adj p<10⁻⁵). BIRC5, coding for survivin, was 8.3 folds higher in CD4⁺ cells with low PBX1 (p=0.0005). Among the core transcription factors of T helper cell differentiation, we identified NF-κB1 and NF-κB2, TBX21, IRF4, IRF8 and STAT3, BATF and BATF3. This followed by significantly higher TNF, IFNγ and IL17A and IL17F in PBX1lo CD4 T cells. The pathway enrichment analysis of DEG identified strong over-representation of cytokine-specific genes (GO:005125, GO:0005126, GO:0048018, GO:0030545, FDR q-values 10⁻¹²-10⁻⁹). The genes of IL4, IL5, IL13, IL9, IL3 and CSF2 located within the chromosome 5 were common for all GO-lists, and were higher in PBX1lo, but none of those genes was identified by survivin-ChIPseq or PBX1-ChIPseq. Analysis of ChIPseq data identified the genes of STAT3, IRF4, IRF8 and BATF as common targets for PBX1 and survivin.

Conclusion: This genome-wide analysis indicates that survivin regulates transcription of the TALE family protein PBX1 in CD4⁺ T cells, which has essential effect for differentiation and phenotype of Th subsets. Low PBX1 in RA patients is associated with terminally differentiated effector CD4⁺ T cells.

References:

- [1] Svensson, B. *et al.* Smoking in combination with antibodies to cyclic citrullinated peptides is associated with persistently high levels of survivin in early rheumatoid arthritis: a prospective cohort study. *Arthritis Res Ther* **16**, R12 (2014).
- [2] Levitsky, A. *et al.* Serum survivin predicts responses to treatment in active rheumatoid arthritis: a post hoc analysis from the SWEFOT trial. *BMC Med* **13**, 247 (2015).

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2020-eular.5426

THU0038 DIFFERENTIAL ROLES OF TNFRI AND TNFRII IN THE MORPHOLOGY OF SECONDARY LYMPHOID ORGANS

K. Jeucken^{1,2}, J. P. Van Hamburg^{1,2}, S. Tas^{1,2}. ¹Amsterdam UMC, Experimental Immunology - Clinical Rheumatology and Immunology, Amsterdam, Netherlands; ²Amsterdam Infection and Immunity Institute, Amsterdam, Netherlands

Background: Tumour necrosis factor (TNF) induced signaling events are important in lymphoid organ development and function, both in health and in immune-mediated inflammatory diseases such as arthritis. Important receptors involved in this process include TNF receptors (R) I and II that exert distinct functions. Mice overexpressing transmembrane (tm)TNF develop various features of chronic inflammation, including arthritis, that are mediated via TNFRI and/or TNFRII.

Objectives: To investigate the importance of TNFRI and TNFRII in spleen and peripheral lymph node (PLN) morphology in mice overexpressing tmTNF.

Methods: Spleen and PLN were collected from 13 week old mice with the following genotypes: WT, tmTNF transgenic heterozygotes (tmTNF tg HET), WT x TNFRI^{-/-}, tmTNF tg HET x TNFRI^{-/-}, WT x TNFRII^{-/-} and tmTNF tg HET x TNFRII^{-/-}. Spleens were cut and stained by immunofluorescence for B and T cell markers, imaged by confocal microscopy and analyzed using FIJI software. PLN were used for whole mount tissue staining for B and T cell markers,

cleared, 3D imaged by light sheet microscopy and analyzed using Imaris software.

Results: Although no macroscopic differences were observed, spleens of tmTNF tg HET animals exhibited an altered morphology characterized by smaller follicles (69,73 ± 72,25 μm²) compared to spleens of WT mice (139,3 ± 71,36 μm²), which was accompanied by a decrease in central T cell areas as measured by % of the total follicle (15,22 ± 9,685; WT 26,60 ± 10,46). WT mice lacking either TNFRI or TNFRII had a morphology comparable to WT (follicle size: TNFRI 234,0 ± 166,9 μm²; TNFRII 154,2 ± 87,92 μm²; % of T cell area: TNFRI 27,81 ± 11,03; TNFRII 28,14 ± 19,66). tmTNF tg x TNFRI^{-/-} exhibited a normal spleen architecture (follicle size: 239,2 ± 250,5 μm²; % of T cell area: 29,92 ± 11,46), whereas the spleen follicles of tmTNF tg x TNFRII^{-/-} mice had T cell areas (17,31 ± 8,88) that were comparable to tmTNF tg HET mice.

The size of PLN of tmTNF tg HET mice (3,74 ± 1,31 mm³) was increased compared to WT (2,41 ± 0,60 mm³), also in combination with deficiency of TNFRI (3,98 ± 2,07 mm³) or TNFRII (4,01 mm³). tmTNF tg HET mice had an increase in absolute B cell volume (0,86 ± 0,33 mm³) vs WT (0,51 ± 0,07 mm³) but no change in B cell area as % of total PLN volume. The increased B cell volume was critically dependent on TNF-RII (tmTNF tg HET x TNFRII^{-/-} (14,87). Interestingly, whereas B cell area morphology of tmTNF tg HET, tmTNF tg HET x TNFRII^{-/-} and WT x TNFRII^{-/-} PLN was similar to WT, TNFRI deficiency caused profound alterations in the morphology of the B cell areas that appeared as one peripheral layer of B cells covering a central T cell area rather than properly developed follicles.

Conclusion: The current study demonstrates that overexpression of tmTNF leads to an aberrant spleen architecture, characterized by smaller follicles and a decrease in central T cell areas, which is critically dependent on TNFRI. In addition, overexpression of tmTNF results in enlarged PLN and increased total B cell volume, which is dependent on TNFRII, whereas TNFRI is more important in the proper organization of B cell follicles. Overall, this study employing different state-of-the-art (3D) imaging techniques highlights the importance of tmTNF-TNFR-induced signaling events in secondary lymphoid organ morphology and function, and reveals distinct roles for TNFRI and II. Advancing our knowledge in this field might provide a better understanding of the pathophysiology of TNF-associated diseases such as arthritis, which may be important to develop new or improved treatment strategies.

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2020-eular.5873

THU0039 DECIPHERING DISEASE-RELEVANT T CELL SUBSETS IN RHEUMATOID ARTHRITIS IDENTIFIES A NOVEL CELLULAR SUBSET OF PATHOGENETIC IMPORTANCE IN THERAPEUTIC RESISTANCE.

M. Nyirenda¹, I. McInnes¹, C. Goodyear¹. ¹Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, Glasgow, United Kingdom

Background: Aberrant T cell responses are key in driving autoimmunity and are commonly associated with rheumatoid arthritis (RA). Unravelling pathways of importance in therapeutic partial response and failure is of critical importance, as this will potentially provide new insights into key drivers of immune-mediated pathogenesis.

Objectives: To delineate disease-relevant T cell subsets in RA and assess their potential to act as cellular markers amenable to precision medicine approaches, particularly in the context of therapeutic partial or non-response.

Methods: FACS-based immunophenotyping and ex-vivo functional response profiles of CD4⁺CD161⁺CCR2⁺CCR5⁺ T cells were performed in peripheral blood mononuclear cells (PBMC) obtained from patients with RA and healthy controls, using previously characterised methodologies. RA patients fulfilled the 2010 ACR/EULAR criteria for RA. All samples were obtained after written consent, with the appropriate ethical approvals in place.

Results: RA patients harboured a higher frequency of CCR2⁺CCR5⁺ cells within the CD4⁺CD161⁺ T cell compartment compared with healthy controls. In RA patients this T cell subset had a higher proportion of cells that secrete pro-inflammatory cytokines such as IL-17A, GM-CSF, IFN-γ, and TNF. Importantly, the CD4⁺CD161⁺CCR2⁺CCR5⁺ T cell subset was significantly increased in DMARD non-responders compared to both responders and healthy controls. Moreover, in DMARD non-responders, these cells had a propensity to express increased proportions of pro-inflammatory cytokines. Notably, there was also a significant increase in the ratio of effector: regulatory T cell (Teff: Treg) compared to both responders and healthy controls. In addition, the CD4⁺CD161⁺CCR2⁺CCR5⁺ T cell subset was less responsive to suppression by Tregs. In further support of a role for this T cell population in disease pathogenesis, the frequency of CD4⁺CD161⁺CCR2⁺CCR5⁺ T cells significantly correlated with disease activity, as measured by the DAS28 (R² = 0.65; p = 0.003; n=11).