order to evaluate the diagnostic accuracy of miR-146a in PB and SF for distinguishing RA patients from healthy controls (HCs). Concentrations of IL-17A in matched serum and SF samples were determined by Human IL-17A ELISA kit (Gene probe, Diaclone). The results were compared to HCs as well as within the RA group.

Results miR-146a was overexpressed in 70.83% of RA SF ($p=0.007$) when compared to HCs SF. The ROC curve analysis showed diagnostic accuracy for miR-146a in SF with $AUC=0.769$ (95 CI: $0.600\div0.938$) with 75.0% sensitivity and 72.3% specificity ($p=0.006$). SF levels of miR-146a were overexpressed in 52.17% of the RA patients compared to its systemic levels. Levels of IL-17A were higher in RA SF compared to serum (8.645 pg/ml versus 0.315 pg/ml, $p=0.012$).

Conclusions The difference between the systemic and local levels of miR-146a and IL-17A in RA patients indicates that the local inflammatory process leads to their deregulation with a possible role of both molecules in the disease pathogenesis. The higher local levels of miR-146a and IL-17A suggest the possible relationship between miR-146a expression and the production of IL-17 from IL-17 expressing cells.

REFERENCES

- Gaffen SL. The role of interleukin-17 in the pathogenesis of rheumatoid arthritis. *Curr Rheumatol Rep*. 2009;**11**(5):365–70.
- Pauley KM, Satoh M, Chan AL, Bubb MR, Reeves WH, Chan EK. Upregulated miR-146a expression in peripheral blood mononuclear cells from rheumatoid arthritis patients. *Arthritis Res Ther*. 2008;**10**:R101.
- Niimoto T, Nakasa T, Ishikawa M, Okuhara A, Izumi B, Deie M, et al. MicroRNA-146a expresses in interleukin-17 producing T cells in rheumatoid arthritis patients. *BMC Musculoskelet Disord*. 2010;**11**:209.

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P101 SYSTEMIC AND LOCAL IL-17A AND MIR-155 LEVELS IN RHEUMATOID ARTHRITIS PATIENTS

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Career situation of first and presenting author Assistant.

Introduction Interleukin 17 (IL-17) is a proinflammatory cytokine, which overproduction promotes the autoimmune reaction in rheumatoid arthritis (RA). Posttranscriptional regulation of IL-17 by specific microRNAs (miRNAs) is of great interest in the recent years. Studies have shown that miR-155 enhances Treg and Th17 cells differentiation and IL-17A production by directly targeting the suppressor of cytokine signaling (SOCS) 1.^{1,2}

Objectives To examine a possible deregulation of the expression levels of miR-155 and levels of IL-17A in peripheral blood (PB) and synovial fluid (SF) of RA patients.

Methods Expression levels of miR-155 were determined in matched PB and SF samples of RA patients by relative quantitation method $2^{-\Delta\Delta C_t}$. As reference control for normalization RNU6B gene was used. Receiver operating characteristic (ROC) curve analysis using RQ values was constructed in

order to evaluate the diagnostic accuracy of miR-155 in PB and SF for distinguishing RA patients from healthy controls (HCs). Concentrations of IL-17A in matched serum and SF samples were determined by Human IL-17A ELISA kit (Gene probe, Diaclone). The results were compared to HCs as well as within the RA group.

Results miR-155 was overexpressed in 79.17% of RA SF ($p=1.63 \times 10^{-4}$) when compared to HCs SF. The ROC curve analysis showed diagnostic accuracy for miR-155 in SF with $AUC=0.858$ (95 CI: $0.757\div0.959$) with 81.3% sensitivity and 81.8% specificity ($p=2.3 \times 10^{-4}$). SF levels of miR-155 were overexpressed in 76.09% of the RA patients compared to its systemic levels. Levels of IL-17A were higher in RA SF compared to serum (8.645 pg/ml versus 0.315 pg/ml, $p=0.012$).

Conclusions The difference between the systemic and local levels of miR-155 and IL-17A in RA patients shows that the inflammatory process leads to their deregulation with a possible role of both molecules in the disease pathogenesis. The higher local levels of miR-155 and IL-17A confirm the data about the possible role of miR-155 in regulating the production of IL-17.

REFERENCES

- Khan D, Ansar Ahmed SA. Regulation of IL-17 in autoimmune diseases by transcriptional factors and microRNAs. *Front Genet* 2015;**6**:236.
- Yao R, Ma YL, Liang W, Li HH, Ma ZJ, Yu X, et al. MicroRNA-155 modulates Treg and Th17 cells differentiation and Th17 cell function by targeting SOCS1. *PLoS ONE* 2012;**7**(10):e46082.

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P102 HIGH DIMENSIONAL PROFILING OF REGULATORY T CELLS IN SPONDYLOARTHRITIS REVEALS CELLULAR HETEROGENEITY

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Career situation of first and presenting author Student for a master or a PhD

Introduction The Spondyloarthritides (SpA) are immune-mediated conditions characterised by spinal and joint inflammation. Numerous studies implicate Th17 lymphocytes in pathogenicity but few reports exist on the role played by regulatory T cells (Tregs) in the disease.

Objectives

- To describe Treg frequency and phenotype in SpA patients compared to controls.
- To identify differential expression of trafficking molecules and activation markers within Tregs at the inflammatory site.

Methods A total of 61 patients with SpA (38 with AS, 23 with PsA) and 16 age-matched healthy controls were recruited. Peripheral blood (PB) and paired synovial fluid (SF) mononuclear cells ($n=8$) were stained for with 3 multicolor flow cytometry panels, including a total of 35 surface and

intracellular protein markers. Manual gating was done in parallel with unsupervised data analysis using FlowSOM and SPADE.

Results No difference in PB Treg frequency was observed between SpA and healthy controls. In the SF Tregs were enriched relative to the blood memory (CD45RA-) compartment (mean: 11.7 vs 4.7%; $p=0.01$) with a higher expression of Foxp3 ($p=0.04$). The distribution of trafficking markers between patients and controls showed considerable heterogeneity, as visualised on SPADE analysis, but little difference in their relative frequency.

Intracellular cytokine staining demonstrated that Tregs from AS and PsA patients have higher potential to secrete IL-17A compared to controls (IL-17A+Tregs mean: 2.8 vs 1.2%; $p=0.02$). Production of this cytokine overlapped with expression of CD161 and CCR6, both markers associated with Th17. Interestingly, this subset, despite expressing normal levels of Foxp3, expressed lower levels of TIGIT ($p=0.04$) and Helios ($p<0.001$) when compared to conventional Tregs.

Conclusions High-dimensional immunoprofiling in SpA patients shows normal frequency of Tregs in the PB, but increased Tregs with activated phenotype in the inflammatory site. The presence of phenotypical markers commonly associated with Th17 in Tregs of non thymic origin are intriguing findings and require further evaluation.

REFERENCES

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P103/O14 INTERLEUKIN-17 IS PRODUCED BY CD4+ BUT NOT CD8+ T CELLS AFTER TCR ACTIVATION IN SYNOVIAL FLUID OF PSORIATIC ARTHRITIS PATIENTS

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Introduction Interleukin-17A (IL-17A) contributes to the pathogenesis of psoriatic arthritis (PsA) as evidenced by the success of biologics targeting IL-17 pathway in PsA patients. Both CD4+ and CD8+ T cells are reportedly producers of IL-17A in PsA with IL-17A+ CD8+ T cells specifically enriched in PsA synovial fluid (SF). However, most current findings regarding IL-17A-producing cells in PsA patients are coming from intracellular staining using flow cytometry.

Objectives To confirm and compare *ex vivo* production of IL-17A by CD4+ and CD8+ T cells from PsA synovial fluid using flow cytometry, enzyme-linked immunosorbent assay (ELISA) and RT-qPCR.

Methods Fresh SF of established PsA patients were collected and part of the cells were directly stained intracellularly for

IL-17A and IFN γ after 4 hours' (hrs) phorbol myristate acetate and ionomycin (PMA and iono) stimulation. Blood samples of early PsA patients and age/sex-matched healthy volunteers were included as controls for PsA SF. In addition, the rest of SF cells underwent density gradient separation and mononuclear cells (SFMCS) were stored till use. CD4+ and CD8+ T cells were sorted from SFMCs, and *ex vivo* cultured with soluble anti-CD3/anti-CD28 (aCD3 and 28), PMA and iono or without stimulation for 4 or 72 hours. Furthermore, SF CD4+ and CD8+ T cells were co-cultured with either allogeneic PsA fibroblast-like synoviocytes (FLS) or autologous PsA CD14+ monocytes with aCD3 and 28 activation for 72 hours. Culture supernatants were tested for ELISA and cells were analyzed with intracellular staining or RT-qPCR.

Results Accumulation of IL-17A+ CD8+ T cells was significantly higher in PsA SF compared to in blood of either PsA patients or healthy volunteers. Although both CD4+ and CD8+ T cells from SFMCs are IL-17A+ with flow cytometry, only CD4+ T cells produce measurable amounts of IL-17A in culture supernatants after 72 hours of aCD3 and 28 activation. Similar results were found after 3 days' co-culture of aCD3 and CD28 stimulated CD4+ or CD8+ T cells with PsA FLS. Also, when either co-cultured with autologous CD14+ monocytes, only CD4+ T cells showed mRNA IL-17A expression and IL-17A production. In contrast, if PMA and iono were used to stimulate CD4+ or CD8+ T cells, both produce IL-17A in supernatants after 4 or 72 hours.

Conclusions Although both CD4+ and CD8+ T cells show IL-17A positivity in PsA SF with flow cytometry, the use of strong stimuli such as PMA and iono during intracellular staining may account for the IL-17A positivity in CD8+ T cells. The contribution of CD8+ T cells to IL-17A production in local PsA joints might be limited as normal TCR activation, mimicked by aCD3 and 28, didn't induce IL-17A release in CD8+ T cells in contrast to CD4+ T cells.

Disclosure of Interest None declared.

P104 ANAEROPLASMA, A POTENTIAL ANTI-INFLAMMATORY PROBIOTIC FOR THE TREATMENT OF CHRONIC INTESTINAL INFLAMMATION

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Introduction The human intestine is colonized with billions of microorganisms, which form the gut microbiota, consisting of up to 1000 different bacterial species. Recent studies have implicated the intestinal microbiota in the pathogenesis of chronic inflammatory diseases, such as rheumatoid arthritis, systemic lupus erythematosus, ankylosing spondylitis, systemic sclerosis, and Sjögren's syndrome. Yet, we still lack the knowledge which bacteria of the gut microbiota induce, promote or inhibit chronic inflammatory inflammation.

Objectives The aim of our work is to identify members of the intestinal microbiota with pro- or anti-inflammatory properties