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.

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P103/014 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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Career situation of first and presenting author Student for a master or a PhD.

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