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/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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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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Career situation of first and presenting author Post-doctoral fellow.

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

for targeted and therapeutic manipulation of the microbiota in chronic inflammatory diseases.

Methods We have developed high-resolution microbiota flow cytometry which allows us to analyze the microbiota on a single cell level. This provides a non-invasive, fast and efficient diagnostic tool to visualize dramatic changes of microbiota composition in inflammatory diseases, fast and efficiently, and isolate distinct bacteria for functional and molecular analyses.

Results We have identified bacteria belonging to the genus *Anaeroplasm*, which enhances the levels of mucosal IgA. Adoptive transfer of *Anaeroplasm* increases the numbers of IgA+ germinal center B cells in the Peyer's patches and of IgA-secreting plasma cells in the lamina propria of the small intestine leading to significantly enhanced mucosal IgA levels. *Anaeroplasm* controls IgA expression presumably its ability to induce expression of the regulatory cytokine TGF- β in T cells, as we show here.

Conclusions The anti-inflammatory properties of *Anaeroplasm* to induce the anti-inflammatory cytokine TGF- β , thereby also strengthening the intestinal barrier by enhancing mucosal IgA, qualify *Anaeroplasm* as potent probiotic for the prevention and treatment of chronic inflammation.

Disclosure of Interest None declared.

P105

IDENTIFICATION OF RARE CODING VARIANTS IN IL-1-RELATED PATHWAYS IN PATIENTS WITH ADULT-ONSET STILL'S DISEASE

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Introduction Adult-onset Still's disease (AOSD) is a rare auto-inflammatory disease characterized by fever, arthritis, and multi-organ involvement. Inflammation in AOSD is mediated by interleukin (IL)-1 β , as confirmed by the clinical efficacy of selective blockers. The genetic predisposition to this IL-1-driven inflammation remains nevertheless elusive. Previous studies failed to identify associations between polymorphisms in the IL-1 genes and AOSD, thus pointing at more complex genetic mechanisms. These cannot be investigated with traditional techniques for genetic partitioning, such as GWAS, which only assess common variants and polymorphisms. Studies focusing on highly penetrant rare variants or different types of mutations (i.e. small copy-number variations; insertions/deletions) are warranted.

Objectives We hypothesized that genetically determined changes in IL-1-related pathways resulting in excessive IL-1 β activity lead to the development of autoinflammation in AOSD. Scope of this study was to unravel the combined mutational variation of a network of IL-1-related receptors, pathways, counter-regulators, and cellular processes possibly involved in the pathogenesis of IL-1-mediated inflammation.

Methods We collected clinical and genetic data from a large cohort of 76 AOSD patients and developed an innovative platform based on molecular inversion probes technology, which enables highly multiplexed targeted-resequencing of the coding sequence of 48 genes related to the IL-1-pathway, and allows studying rare and common variants in one assay. We have also screened 500 healthy controls, and 1000s of samples with other disorders using the same assay.

Results We identified rare and unique (i.e. private variants) in the IL-1 pathway in several individuals with AOSD. Whether any of these are involved in a strong predisposition to AOSD is currently followed-up. Rare genetic variants have been identified in six IL-1-pathway 'clusters':

1. Inflammasomes;
2. IL-1 pathway;
3. IL-1 family;
4. IL-18 pathway;
5. Autophagy;
6. ROS production.

Conclusions Unraveling the genetic bases of inflammation in AOSD deepens our understanding of the human innate immunome. This study platform may now serve for the genetic analysis of other IL-1-mediated conditions (i.e. gout and other autoinflammatory diseases), whose genetic predisposition remains elusive. Equally important, the identification of pathways amenable to targeting with small molecules or biologics may translate into remarkable clinical implications.

Disclosure of Interest None declared.

P106/O25

DNA METHYLATION IN LYMPHOCYTE SUBSETS AS A MEDIATOR OF GENETIC RISK IN EARLY RHEUMATOID ARTHRITIS

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

Introduction Genome-wide association studies (GWAS) have identified over 100 RA-associated risk loci, whose enrichment for lymphocyte-specific enhancer elements is consistent with a regulatory function of many causal variants in these cells. Epigenetic modifications have also been strongly implicated in RA pathogenesis.

Objectives To investigate the role of DNA methylation as a mediator of RA genetic risk.

Methods CD4+ T lymphocyte-specific DNA and RNA were extracted from freshly isolated blood of 43 RA and 60 disease control patients, along with equivalent material from B-lymphocytes of 46 RA and 73 controls. Comparator groups were drug-naïve and matched for age, sex, and acute phase response. Genotyping, gene expression and methylation