Conclusions In addition to proinflammatory and anti-inflammatory cytokines MTX treatment regulates the SLAP and the CD3 ζ expression of human T-cells.

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Acknowledgements OTKA K77537.

Disclosure of Interest None declared.

P030

TRANSCRIPTIONAL LANDSCAPES OF MEMORY T CELLS FROM PATIENTS WITH JUVENILE IDIOPATHIC ARTHRITIS

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10.1136/annrheumdis-2018-EWRR2019.22

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

Introduction Juvenile idiopathic arthritis (JIA) is a chronic inflammatory disease (CID) of unknown origin and is characterized by joint inflammation in children and young adults.¹ Evidence suggests a strong contribution of memory T cells to disease pathogenicity in JIA. While few markers for T cells adapted to chronic inflammation have been identified,^{2,3} a comprehensive analysis of what distinguishes pathogenic memory T cells in inflamed tissues of chronic inflammation from protective, circulating memory T cells is still lacking.

Objectives To characterize the transcriptional profiles of memory T cells that putatively maintain chronic inflammation in JIA patients.

To identify biomarkers that are associated with autoantigen-specific clonotypes among memory T cells in JIA.

Methods Memory T cells were isolated from the synovial fluid (SF) and the peripheral blood (PB) of oligoarticular JIA patients and purified by fluorescence-activated cell sorting (FACS). Subsequently, single cell sequencing including T cell receptor (TCR) sequencing was performed on ~18.000 memory T cells of each JIA patient.

Results Memory T cell populations both from the blood and from the SF are heterogenous populations according to their transcriptional expression patterns. The SF harbored a larger population of enriched T memory cell clonotypes than the blood. In addition, enriched memory T helper cell clones in the SF showed a transcriptional pattern of activation compared to non-enriched clonotypes. Finally, small subpopulations of enriched memory T helper cell clones in the SF show a transcriptional signature that resembles transcriptomes obtained by bulk sequencing. Thus, a rather small subpopulation of antigen-specific cells might be responsible for the overall transcriptional character of T cells found at the inflamed sites of CIDs.

Conclusions Single cell sequencing combined with TCR sequencing is a powerful tool to identify and characterize subsets of T memory cells in chronic inflammation. The obtained data might be useful to better understand how T cell subsets contribute to disease pathogenicity in CIDs and reveals putative targets that could be therapeutically exploited in order to selectively deplete pathogenic memory T cells.

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Acknowledgements This work is supported by the European Regional Development Fund (ERDF 2014–2020 and EFRE 1.8/11).

Disclosure of Interest None declared.

P031/O12

SIALIC ACIDS NEGATIVELY AFFECT THE BONE RESORPTIVE CAPACITY OF OSTEOCLASTS

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10.1136/annrheumdis-2018-EWRR2019.23

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Introduction In rheumatoid arthritis the imbalance between osteoblasts and osteoclasts leads to an increased bone resorption, resulting in extensive bone destruction. Osteoclasts are multinucleated cells formed after fusion of macrophages. Macrophages are known to acquire an anti-inflammatory phenotype upon engagement of the carbohydrate sialic acid¹, however whether triggering by sialic acids affects osteoclastogenesis is unknown. Therefore, we investigated the effect of sialic acids on osteoclastogenesis and their expression of the sialic acid binding receptors, called Siglecs.

Methods Human monocytes were plated on either plastic, glass or bone slices and stimulated with M-CSF and RANK-L to generate osteoclasts. We designed a novel sorting methodology to separate the osteoclasts and the residual macrophages in our osteoclast-derived cultures. Purified osteoclasts were reanalyzed by microscopy and assessed for gene expression of osteoclast-specific genes by RT-PCR. Flow cytometry was used to obtain a full expression profile of the different siglec receptors in the osteoclasts, macrophages and monocytes precursor cells. Stimulation of pre-osteoclasts with sialic acids was done at day 7 and 10 of the culture. Supernatants were assessed for IL-6 and IL-10 production.

Results Our novel osteoclast purification method resulted in pure osteoclasts expressing the classical genetic osteoclast markers such as cathepsin K and TRAP, while being negative for CD14. During the transition to osteoclasts, expression of siglec-1, -5/14 and -7 was lost, while siglec-6, -9 and -15 expression was maintained or gained. Osteoclasts are known for their effectively bone resorption, however after adding sialic acids to the pre-osteoclasts, both osteoclast formation and bone resorption were reduced, while an increase of IL-6 and IL-10 cytokines was observed.

Conclusions Pre-osteoclasts stimulated with sialic acids display reduced osteoclast formation and bone resorption. Based on the siglec profiling we hypothesize that sialic acids engage siglec-9 on osteoclasts. Macrophages stimulated via siglec-9 produce IL-10 cytokine,¹ similarly as we observed in our stimulation experiments. Currently, we are addressing the role of siglec-9 in osteoclastogenesis.

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Disclosure of Interest None declared.