pathway, played an important role in the development of RA. LY294002 could delay the onset and reduce the severity of arthritis in CIA mice through the promotion of neutrophils apoptosis. It might open a new door to the future clinical treatment of RA.

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Acknowledgements This work was supported by Youth Science Foundation of Guangdong Second Provincial General Hospital (No. YQ2016-002).

Disclosure of Interest None declared.

P138/O10 INVESTIGATING GENE EXPRESSION PATTERNS AND FUNCTION OF TOLEROGENIC DENDRITIC CELLS

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10.1136/annrheumdis-2018-EWRR2019.123

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

Introduction Dendritic cells (DCs) are professional antigen-presenting cells and play a major role in immune system responses and function. They express a repertoire of toll-like receptors (TLRs) that recognise pathogens. Tolerogenic dendritic cell (tolDC) therapy is a promising cellular therapy that aims to restore tolerance in autoimmune disease. Exposure to dexamethasone leads to the expression of TLR2; however, expression of this marker is not homogeneous. Some tolDCs have low TLR2 expression while some others have high TLR2 expression. Despite these differences in TLR2 expression, we hypothesised that tolDCs are homogenous in terms of their gene expression and function.

Objectives The study aimed to investigate the gene expression and function of tolDCs to determine if expression patterns and function seen in low TLR2 expressing tolDCs are similar to those of high TLR2 expressing tolDCs.

Methods Peripheral blood CD14⁺ monocytes, obtained from healthy individuals (n=8) were differentiated for 7 days into tolDCs using IL-4, granulocyte-macrophage colony-stimulating factor (GM-CSF), dexamethasone and Vitamin D3. As a control, mature DCs were differentiated using IL-4, GM-CSF and IFN- γ . TolDCs were stained for TLR2 and fluorescence activated cell sorted (FACS) as the 20% lowest TLR2 expressing tolDCs and the 20% highest TLR2 expressing tolDCs. TolDC phenotype was explored using flow cytometry, gene expression of pro-inflammatory, anti-inflammatory and migratory genes by quantitative PCR and CD40-Ligand re-stimulation and mixed lymphocyte reaction assay to investigate function.

Results Phenotypic marker expression was different between tolDCs and mature DCs, with tolDCs expressing low levels of CD83 and CD86 and high levels of HLA-DR, latency-associated peptide (LAP) and TLR2. In general, TLR2Low and TLR2High tolDCs showed similar phenotypic properties, gene expression patterns and tolerogenic functions. TLR2High tolDCs had increased LAP and HLA-DR expression. TLR2Low tolDCs had higher gene expression for CCR7 and TNF- α . Cytokine (IL-6 and IL-10) production for both TLR2Low and

TLR2High tolDCs was not significantly different upon re-stimulation with CD40-ligand and both populations had similar immunosuppressive capacity for CD4⁺ T cells compared to mature DCs.

Conclusions Both populations of tolDCs displayed similar gene expression profiles and phenotypic properties for the majority of characteristics investigated. The minor differences observed may be attributable to stochastic differences in dendritic cell exposure to, or uptake of dexamethasone. Despite these differences, all tolDCs function similarly, which is the most important factor when considering tolDCs as a therapy.

Disclosure of Interest None declared.

P140 IDENTIFICATION OF AFFIMERS THAT BIND TO THE IL-7R AND INHIBITS THE IL-7 SIGNALLING CASCADE

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10.1136/annrheumdis-2018-EWRR2019.124

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Introduction IL-7R is a heterodimer constituted by the IL-7R α chain (CD127) and the common gamma (γ) chain (CD132). IL-7 binding to IL-7R expressed on CD4⁺ T cells induces a survival signal. The IL-7/IL-7R signalling axis has been validated as a therapeutic target for treatment of both T-cell driven autoimmune diseases (AIDs)¹ and T Acute Lymphoblastic Leukaemia (T-ALL).² Affimers are small and stable artificial proteins which bind with nanomolar affinities to human proteins and can block protein-protein interactions.³ They are becoming widespread owing to their stability, ease of production and versatility.

Objectives Identify Affimers that recognise the IL-7R α and inhibits the IL-7 signalling cascade. This may result in an attractive approach for the treatment of both T-cell driven autoimmune diseases and T-ALL.

Methods The type-II Affimer library (10¹⁰) was interrogated by Phage display using fully glycosylated human IL-7R α ectodomain (ECD). PhageELISA and DNA sequencing were used to either obtain or elucidate the unique binders (Affimers), respectively. Affimers were produced as His-Tagged proteins (~13 kDa) in *E. coli* and purified using IMAC. Affimer binding to IL-7R α was confirmed by pull-down assays (soluble) and Flow cytometry (membrane). An IL-7 reporter assay using HEK-IL7R (HEK293 cells stably transfected with the IL-7R) was developed and the biological effect of the Affimers was elucidated.

Results We have screened an Affimer library using human ECD-IL7R α and after three consecutive panning rounds, 20 Affimers were raised as shown by PhageELISA and DNA sequencing. From these, 17 were able to pull-down the soluble ECD-IL7R α and 7 stained specifically HEK-IL7R cells (by flow cytometry using anti-His Tag Abs). Finally, we have identified 3 Affimers (1, 42 and 96) that showed inhibition of the IL-7 signalling cascade on HEK-IL7R cells.

Conclusions Our work demonstrates the possibility of screening an Affimer library for a cytokine-receptor target, and selecting specific binders, some of which showed the desired antagonist activity of the cytokine signalling cascade. IL-7 itself is a validated target, so this work offers an alternative to antibody-mediated protein interference. With further biological

validation including animal models, it may even offer a novel therapeutic tool for AIDs and T-ALL.

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Acknowledgements We would like to thanks Prof. S. Savvides, Dr. K. Verstraete (VIB, Belgium) and Dr. Joao Barata (IMM, Portugal) for their contribution on this work.

Disclosure of Interest None declared.

P141 DIFFERENTIAL DNA METHYLATION IN PERIPHERAL NAÏVE CD4+T-CELLS IN EARLY RHEUMATOID ARTHRITIS PATIENTS

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10.1136/annrheumdis-2018-EWRR2019.125

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

Introduction Alterations in DNA methylation patterns (epimutations) have been related to several diseases, including Rheumatoid Arthritis (RA). We hypothesise that such epimutations occur early in the RA disease process. T-cells are important cells in early pathogenesis, therefore we choose to analyse patterns of differential methylation (DM) of the DNA of CD4+ T cells.

Objectives To identify changes in DNA methylation pattern of naïve CD4 +T cells in early, drug naïve RA patients, towards understanding early events in disease pathogenesis.

Methods DNA methylation of 480,000 CpGs (Illumina methylation genome-wide array) were analysed in cell sorted, naïve CD4+ T cell from 6 healthy control (HC) and 10 RA patients. To priorities DM gene, we designed a scoring system.¹ We examined potential interactions between DM genes using the STRING-database of protein-protein network analysis. Flow cytometry was used to characterise subpopulations of naïve CD4+ T cells in early RA for 4 DM genes, coding for cell surface molecules.

Results We observed 648 DM-genes in naïve T-cells in early RA (354 hypo and 294 hyper-methylated). STRING analysis of these 648 genes/proteins revealed central of JAK1/STATs signalling, associated with an IL6/IL6R/STAT3 signalling node, a 2nd node for STAT2 linked to downstream interferon signalling, a 3rd node centred on IL2/IL15/STAT5, and many other associations notably related to TNF-alpha and TGF-beta signalling as well as a Th17 cell differentiation axis. Several genes were coding for cell surface proteins, notable CD4, IL2R, IL6R and CD62L. Analysis of CD4 and IL2R confirmed significant increased expression between RA and HC. CD62L identified 2 subpopulations of naïve T-cell in RA the classical naïve CD62L+ cells and CD62L- naïve T-cells as previously reported². IL6R expression was reduced in CD62L- naïve CD4+ T cell compared to CD62L+ cells.

Conclusions DNA methylation pattern in naïve CD4+ T cell point to a central role for JAK1/STAT3/IL6 signalling in very early RA pathogenesis, with a pivotal role in diversification towards other pathways (notably TNF, Th17, Th1), which

may already contribute to patient heterogeneity at this stage. Our data provides more understanding of early RA pathogenesis and should facilitate novel therapeutic strategies by targeting the right pathways.

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Disclosure of Interest None declared.

P142 ROS101, A NOVEL TARGETED METHOTREXATE PRODRUG, SELECTIVELY ACTIVATED BY REACTIVE OXYGEN SPECIES (ROS) SHOWS REDUCED TOXICITY IN RAT CIA MODEL OF RHEUMATOID ARTHRITIS

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10.1136/annrheumdis-2018-EWRR2019.126

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

Introduction The use of methotrexate (MTX) for the treatment of rheumatoid arthritis (RA) is limited by serious adverse effects. Some effects, such as stomatitis and gastric ulcer, can be alleviated by folate supplement. Others are folate-independent, e.g. fibrosis of liver and lung. Moreover, MTX further elevates the micronuclei count, which is generally already increased in RA.

In RA, inflamed tissue is characterized by up to 100-fold increased concentrations of reactive oxygen species (ROS, including hydrogen peroxide) compared to healthy tissue.

A novel MTX prodrug, ROS101 in development for the treatment of RA, releases MTX at exposure to ROS. This restricts MTX exposure to target tissues with increased ROS levels, e.g. the synovial membrane in RA.

Objectives To investigate the effect and toxicity of a targeted MTX prodrug in a rat CIA model of rheumatoid arthritis.

Methods ROS101 was tested in a collagen induced arthritis (CIA) model in dark agouti (DA) rats (10 per group) against vehicle and 0.3 mg MTX i.p./day at equimolar doses. Arthritis was induced by s.c. injection of an emulsion of collagen type II in complete Freund's adjuvant. Treatment was initiated at a mean arthritis severity score of 2/60. Blinded disease evaluation took place 3 times/week from day 10. Bone marrow cells for micronucleus test were obtained from the femur, pictures captured with LARS.4.8 software and analyzed blinded. Individual rat data were included until termination.

Results Mean and maximum arthritis severity score was reduced in rats in the ROS101 group compared to vehicle (mean score 6.3±3.1 vs. 14.1±3.3, mean±SEM); (max score 12.6±5.3 vs. 32.3±5.3, mean±SEM, p<0.05). All rats in the ROS101 group completed the study until day 32. Reduced arthritis severity in the MTX group after day 22 was accompanied by overt toxicity, causing all rats to be terminated from day 24 to 29. There was no significant difference in body weight between rats in the ROS101 and vehicle groups, whereas body weight in the MTX group decreased significantly from day 22 due to toxicity, including GI effects. Bone marrow micronucleus count was reduced in the ROS101