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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Disclosure of Interest None declared.

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

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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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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