their diverse biological roles, proteins can reflect ongoing RA disease processes and may provide biomarkers of response; however, studying proteins in isolation can lead to challenges in separating the cause from the consequence of inflammation. If protein levels are under genetic control, then potentially stable, pre-treatment genetic biomarkers of etanercept response may be identified.

**Objectives:** To determine whether there is a genetic basis underlying protein expression in patients with RA treated with etanercept.

**Methods:** Participants were recruited from a UK-based prospective multi-centre study of patients fulfilling either the 1987 ACR or 2010 ACR/EULAR classification criteria for RA, starting etanercept as a first biologic. Quantitative proteomics were performed using Sequential Window Acquisition of all THeoretical fragment ion spectra mass spectrometry (SWATH-MS). Genotyping was carried out using the Illumina Infinium HumanCoreExome 12 BeadChip kit and genotype calling was carried out using GenomeStudio software (both Illumina, San Diego, CA, USA). Following standard genetic QC and imputation, a protein quantitative trait loci (pQTL) analysis was performed using a linear model adjusted for potential confounding covariates (age, biological sex, disease duration, concurrent DMARD use, seropositive status). A suggestive significance level of p<1e-05 was set for cis pQTLs; trans pQTLs were not considered due to modest sample size. Significance thresholds were adjusted for false discovery rate and subsequently, any adjusted result with p < 0.05 was considered to be significant. pQTLs were sought at baseline and after 3 months of treatment. Frequently, any adjusted result with p < 0.05 was considered to be significant. pQTLs; DMARD use, seropositive status). A suggestive significance level of p<1E-05 Objectives:

**Results:** 147 participants were recruited, with a median age of 56.39 years [IQR 49.34-64.73], median disease duration of 6 years [IQR 2-13] and of whom 108 (75.52%) were female. 482 unique proteins were available for analysis following proteomics and genetics data QC. At baseline (pre-treatment), 2,184 cis pQTLs were identified for 60 proteins (this may reflect many pQTLs in strong linkage disequilibrium with one another). After 3 months of treatment, 1,432 cis pQTLs were identified for 68 proteins, 2 proteins measured at pre-treatment had pQTLs disequilibrium with one another). After 3 months of treatment, 1,432 cis pQTLs were identified for 68 proteins, 2 proteins measured at pre-treatment had pQTLs disequilibrium with one another).

Conclusion: Collectively, our data demonstrate that AIRE expression, as well as the expression of the immunoregulatory molecules IDO and PD-L1 in eTACs and DCs can be induced through CD40 stimulation in a non-canonical NF-κB signaling dependent manner. These findings concerning the regulation of AIRE and immunoregulatory molecule expression may point towards a novel role of eTACs in peripheral tolerance.

REFERENCES: NIL.

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Disclosure of Interests: None Declared.

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**Innate Immunity in Pathogenesis of RMDs**

**Keywords:** Cell biology, Innate immunity

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**OP0105**

**SINGLE CELL RNASEQ AND FUNCTIONAL STUDIES SUGGEST A ROLE FOR ENTHESIS MESENCHYMAL STEM CELLS RATHER THAN TREGS IN ENTHESIS SOFT TISSUE AND BONE ANCHORAGE SITE IMMUNOMODULATION**

**Keywords:** Enthesitis, Inflammatory arthritis, Innate immunity

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**OP0104**

**NON-CANONICAL NF-κB SIGNALLING IS REQUIRED FOR EXTRATHYMIC AIRE EXPRESSION AND IMMUNOREGULATORY MOLECULES IN CELLS OF THE DENDRITIC LINEAGE**

**Keywords:** Cell biology, Innate immunity

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**Background:** The transcription factor Autoimmune Regulator (AIRE) is crucial for the establishment of central tolerance in the thymus. Although thymic expression is primarily restricted to medullary epithelial cells, recent studies have identified perihemical CD45+ extrathymic AIRE-expressing cells (eTACs), which share features with antigen presenting cells, such as dendritic cells (DCs). Although, nuclear factor (NF)-κB signaling has been implicated in thymic AIRE expression, stimuli and pathways that induce extrathymic AIRE expression are hitherto unknown.

**Objectives:** In this study we aimed to unravel the molecular mechanisms underlying the regulation of extrathymic AIRE expression in eTACs and DCs with a focus on NF-κB signaling.

**Methods:** Confocal and Vectra Polaris multiplex imaging was performed on secondary lymphoid tissues, including tonsils and lymph nodes to identify and characterize eTACs. Second, we employed a novel sorting strategy to isolate DCs and eTACs from tonsil tissue for RNA-sequencing (seq) to generate transcription profiles. To study the regulation of AIRE, monococyte-derived DCs (mDCs), DCs and eTACs were stimulated with anti-CD40 to induce NF-κB activation. Gene transcription and protein expression levels of AIRE, indoleamine 2,3-dioxygenase (IDO), programmed death ligand-1 (PD-L1) and non-canonical NF-κB signaling components were detected by real-time PCR, flow cytometry, Western blot and/or confocal microscopy. Activation of NF-κB signaling was modulated by siRNA-mediated silencing or using a small molecule inhibitor (SMI) of activating kinases.

**Results:** In human tonsil and lymph node tissue AIRE+ cells were identified, which displayed DC characteristics, including MHCIi and CD40, but were relatively low for the pan DC marker CD11c. Bioinformatic analysis on RNA-seq derived gene expression profiles of sorted eTACs and DCs from tonsil tissue revealed an increased expression of AIRE and immunoregulatory genes, including IDO and PD-L1 in eTACs compared to DCs. In addition, the expression of genes implicated in non-canonical NF-κB signaling, including NIK and CD40, were significantly higher in eTACs compared to DCs. CD40 stimulation has been shown to be critical for IDO production by mDCs. CD40-stimulation of mDCs resulted in the activation of non-canonical NF-κB signaling, which was accompanied by upregulation of AIRE, IDO and PD-L1 expression. Interestingly, the induction of AIRE expression was abrogated by siRNA-mediated silencing of NF-κB-inducing kinase (NIK). Likewise, CD40 stimulation of sorted eTACs and DCs resulted elevated AIRE, IDO and PD-L1 expression levels, albeit 5 fold higher AIRE transcription levels were detected in eTAC. Subsequently, modulation of NIK via an SMI resulted in the abrogation of AIRE, IDO and PD-L1 expression in eTACs and DCs. In contrast, specific modulation of canonical NF-κB signaling had no significant effects on AIRE, IDO and PD-L1 expression in CD40-stimulated eTACs and DCs.

**Conclusion:** Collectively, our data demonstrate that AIRE expression, as well as the expression of the immunoregulatory molecules IDO and PD-L1 in eTACs and DCs can be induced through CD40 stimulation in a non-canonical NF-κB signaling dependent manner. These findings concerning the regulation of AIRE and immunoregulatory molecule expression may point towards a novel role of eTACs in peripheral tolerance.
1:1, 2:1, 4:1, and 8:1 ratios for 5 days. T cells proliferation index was calculated and Treg related markers expressed on MSCs including CD73 and CD39 were measured in PEB and EST.

Results: Following quality control, the transcriptome of 25,635 single cells was analysed. The compositional analysis revealed different abundances, such as stromal cells being more abundant in the EST, and haematopoietic precursors almost exclusively in the PEB. Analysis of the main cell populations revealed: the presence of innate and innate-like lymphocyte subpopulations such as MAIT and γδT cells, and a transcriptional gradient in neutrophils, reflecting various stages of maturation. In the T cell compartment, a putative regulatory (Tr1-like) population characterised by TGFβ1 expression, was more abundant than conventional Foxp3+ Tregs. A number of stromal cells expressed the regulatory marker NT5E (CD73). Consistently with scRNAseq, EST-MSCs expressed CD73 significantly more than PEB-MSCs prior to co-culture (P<0.001), with significant upregulation with T-cell co-culture (P<0.05). EST and PEB derived MSCs showed trilineage differentiation to bone, cartilage and fat[3] and significantly upregulated CD4+ CD25+ proliferation in dose dependent manner compared to control co-culture (P<0.001). Interestingly, PEB-MSCs showed an increase in the CD39 expression after co-culture with T cells compared to PEB-MSCs with no T cells at ratio 1:4 and 1:8 (P<0.001). No EST-MSCs CD39 expression increase after T cells co-culture was noted but PEB-MSCs CD39 expression increased at 1:4 and 1:8 ratio (P<0.01, P<0.01 respectively).

Conclusion: Our single-cell transcriptomic analysis suggests that EST and PEB have few Tregs and that other T cells and MSCs possess immunomodulatory functions in these tissues. This immunomodulatory activity may be important at these sites of high mechanical stress to maintain homeostasis.

REFERENCES:

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SYNOVIAL IMPRINTING OF SKIN-DERIVED IMMUNE CELLS INITIATES SPREADING OF INFLAMMATION FROM SKIN TO JOINT IN PSORIATIC ARTHRITIS

Keywords: Psoriatic arthritis, Animal Models, -omics

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Figure 1. Healthy spinal enthesis dataset paired PEB and EST. A: cluster composition and anatomical distribution. B: Cluster abundance by tissue. C: Per sample cluster abundance

Acknowledgements: NIL.
Disclosure of Interests: None Declared.
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