staining were performed. Immunohistochemistry using anti-Runx2, anti-Sox9, anti-Collagen II and X, anti-CD31 and CD68 antibodies has been performed. We used also anti-TNAP (Tissue Nonspecific Alkaline Phosphatase) and ENPP1 (Ectonucleotide Pyrophosphatase/Phosphodiesterase 1) antibodies. Indeed, these two enzymes are essential in the physiological mineralization: extracellular inorganic pyrophosphates are provided by ENPP1 then hydrolyzed by TNAP to promote mineralization.

Results

Five calcified samples were collected On HE staining, voluminous calcium deposits were encapsulated by a fibrocartilaginous tissue. In one sample, we observed an intra-tendinous osseous metaplasia. This fibrocartilaginous area presented a red coloration (proteoglycan specific) on SO/FG staining but was collagen II negative whereas the fibrocartilage at the tendon attachment was strongly positive. Within this area, cells with round nuclei and pericellular lacunae were observed as previously described (Uthoff, 1975). These cells expressed Runx2 and Sox9 suggesting a chondrocyte differentiation but only a small number of them expressed type X collagen, hypertrophic chondrocytes-specific marker. These cells also expressed ENPP1 and TNAP. Interestingly, extracellular TNAP deposits were also present at the periphery of the deposits. We identified vessels surrounding the deposits on 4 of the 5 calcified samples. Finally, no CD68 positive cells or TRAP positive cells were detected around the deposits.

Conclusions Histological analyses of whole calcified tendon tissues showed a fibrocartilaginous area surrounding the calcium deposits with chondrocyte-like cells expressing ENPP1 and TNAP suggesting their crucial role in the deposition of apatite crystals. Further analyses are necessary to understand the origin of these cells and the regulatory factors involved in their differentiation.

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Disclosure of interest None declared

P040

INVOLVEMENT OF THE ANTI-AGEING PROTEIN KLOTHO IN CHONDROCYTE AUTOPHAGY AND APOPTOSIS DURING OSTEOARTHRITIS

¹E Le Tilly, ¹T Ong, ²J Abadie, ^{1,3}J Guicheux, ¹L Beck, ¹C Vinatier*. ¹INSERM UMR 1229 – RMeS, Regenerative Medicine and Skeleton, STEP Team, University of Nantes, UFR Odontology; ²Animal Cancers as Models for Research in Comparative Oncology (AMaROC), ONIRIS; ³PHU 4 OTONN, CHU Nantes, Nantes, France

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Introduction The pathogenesis of OA is not fully characterised, but is thought to be due to perturbation of chondrocytes homeostasis including an impairment in the autophagy process particularly during ageing. Among the anti-ageing factors, Klotho has been shown to regulate autophagy in a variety of cell types, and Klotho polymorphisms have been associated with higher risks of OA.

Objectives The aim of this project was to investigate the role of Klotho in OA.

Methods The expression of Klotho and autophagy markers (LC3b and Beclin-1) and OA onset were evaluated in ageing C57BL/6 mice as an age-related spontaneous model of OA. The cartilage integrity, autophagy and apoptosis status in

Klotho-deficient mice knee joints were also analysed. In parallel, to investigate the relationship between Klotho and autophagy, immature murine articular chondrocytes (iMACs) were stimulated with increasing doses of soluble recombinant Klotho. The effect of Klotho on autophagy was also evaluated in a pathological context, i.e. following IL1- β stimulation (10 ng/ml) for 24 hours. Bax/Bcl-2 ratio, a marker of the intrinsic apoptotic pathway, was also evaluated in IL1- β -treated chondrocytes.

Results In the knee joints of mice from escalating ages, the expression of Klotho correlated with LC3b and Beclin-1 expression and gradually decreased with age while OA features appear. Articular cartilage of KL-/- mice revealed an increase in the OARSI score, associated with increased chondrocyte death and LC3b expression, as well as caspase 3 and TUNEL expression. In IL1- β -treated chondrocytes, the autophagy markers and Bax/Bcl-2 ratio were overexpressed, while the addition of Klotho counteracted this effect.

Conclusions In summary, we described an early articular cartilage degradation in the absence of Klotho, suggesting a potential protective role of Klotho in OA development. The increase in autophagic process in Klotho mutant mice associated with the decrease in autophagy and Klotho expression with age clearly indicate intimate relationships between these two players. *In vitro* experiments suggested that Klotho may not have a direct effect on autophagy but rather through reducing apoptosis induced by pro-inflammatory cytokines such as IL1- β . Our study revealed the close relationship between the anti-ageing Klotho protein and chondrocyte death in articular cartilage, unveiling Klotho as a potential target to enhance chondrocyte survival.

Disclosure of interest None declared

P041

TISSUE GENE PROFILING UNCOVERS CADHERIN 11 RELATED SIGNATURES IN RHEUMATOID ARTHRITIS PATIENTS

¹K Hatje, ¹T Kam-Thong, ²D Hartl, ¹G Duchateau-Nguyen*. ¹Roche Pharma Research and Early Development, Pharmaceutical Sciences; ²Roche Pharma Research and Early Development, Immunology, Inflammation and Infectious Diseases, F. Hoffmann-La Roche Ltd, Basel, Switzerland

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Introduction Cadherin 11 is selectively expressed by synovial fibroblasts and plays a role in the pathogenesis of rheumatoid arthritis (RA).¹ Consequently, blocking cadherin 11 function in inflamed tissues may represent a potentially effective and novel therapy for RA.

Objectives Here we interrogated publicly available transcriptomics datasets from RA patients synovial tissue and healthy controls using pre-defined primary cell gene signatures to better understand the heterogeneity of the underlying pathology. In addition, we analysed the association of these gene signatures with the expression of cadherin 11 gene to narrow down the underlying mechanistic network on which targeted treatments and biomarkers can be developed.

Methods We used two publicly available transcriptomics studies from NCBI Gene Expression Omnibus² performed on synovial tissue of RA patients and healthy controls: GSE7307 (RA=5, healthy=7) and GSE77298 (RA=16, healthy=7). The pre-defined gene signatures were derived from ENCODE primary cell expression data³ and signature enrichment was applied using the BioQC package.⁴ Principal component

analysis (PCA) was applied on the BioQC enrichment scores to (i) identify potential clusters of samples and to (ii) identify the gene signatures responsible for the clustering. In addition, associations between cadherin 11 gene expression and gene signatures were tested.

Results In both studies, cadherin 11 was higher expressed in RA patients compared to healthy controls. PCA performed on gene enrichment scores showed RA patients clustered apart from the healthy controls. Moreover, fibroblast, macrophage, lymphocyte and osteoclast gene signatures were significantly enriched in the RA patients compared to healthy controls in both studies. Unexpectedly, an adipokine-related signature was significantly enriched in RA patients from one transcriptomics dataset (GSE77298), while only showing a trend in the second set (GSE7037). In addition, in both studies Cadherin 11 was associated positively with fibroblast and lymphocyte signatures and negatively with adipokines-related signature.

Conclusions The identified cadherin 11 related gene signatures expand our knowledge on cadherin 11 biology in human RA and may serve as potential biomarkers for RA studies in the future.

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P042

TARGETING NF-KB SIGNALLING IN B CELLS: A POTENTIAL NEW TREATMENT MODALITY FOR ANTIBODY MEDIATED AUTOIMMUNE DISEASES

¹JP Van Hamburg, ²P Tuijnburg, ¹B Helder, ¹L van Keep, ¹K Wesenhagen, ³P Kucharzewka, ²MH Jansen, ¹A Al-Soudi, ¹PL Klarenbeek, ³H Olsson, ¹N de Vries, ²T Kuijpers, ¹SW Tas*. ¹Amsterdam Rheumatology and Immunology Centre, Academic Medical Centre/University of Amsterdam; ²Department of Experimental Immunology, Academic Medical Centre/University of Amsterdam, Amsterdam, Netherlands; ³Respiratory, Inflammation and Autoimmunity IMED Biotech Unit, AstraZeneca, Gothenburg, Sweden

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Introduction The pivotal role of B cells in the pathogenesis autoimmune diseases such as ANCA-associated vasculitis (AAV) is well-established and further substantiated by beneficial therapeutic effects of rituximab (anti-CD20 B cell targeted therapy). However, this results in prolonged B cell depletion while long-lived plasma cells are not targeted. Thus there is a need for novel therapeutics targeting cells in the B-cell lineage in AAV. Novel targets might be encountered in the NF-κB signalling pathway, which acts downstream of various B cell surface receptors, including the B cell antigen receptor, CD40, BAFFR and TLRs, and is crucially involved in B cell responses.

Objectives To identify whether inhibition of NF-κB signalling by novel pharmacological inhibitors is effective in targeting B cell responses in general and more specifically blocks (auto) antibody production and plasmablast differentiation in B cells from AAV patients.

Methods PBMC and sorted B cells from AAV patients and healthy donors were cultured with T cell-dependent (anti-IgM +anti CD40+IL-21) and T cell-independent (CpG +IL-2) stimuli. NF-κB signalling was targeted in these cultures by

small molecule inhibitors of NF-κB inducing kinase (NIK, non-canonical NF-κB signalling) and IKKβ (canonical NF-κB signalling). Downstream NF-κB signalling and nuclear NF-κB translocation was determined by Western blot and confocal imaging. Effects on B cell proliferation and differentiation were determined by CFSE dilution assays and flow cytometric analysis of B cell markers. (Auto)antibody production was measured by ELISA.

Results In B cells, targeting of NIK and IKKβ effectively inhibited non-canonical or canonical NF-κB signalling, respectively. In a B cell stimulation assay, NIK and IKKβ inhibition significantly reduced T cell-dependent (anti-IgM +anti CD40+IL-21) and T cell-independent (CpG +IL-2) B cell proliferation, plasmablast differentiation (CD27+/CD38+), and antibody production. The effects of NIK inhibition appeared to be B cell-specific as T cell proliferation was largely unaffected. Currently, studies are ongoing to investigate the effect of IKKβ inhibition on B cell responses and to explore the effects of targeting NF-κB signalling in AAV B cells.

Conclusions These data demonstrate that inhibition of NF-κB signalling in B cells results in the modulation of various B cell responses. Ongoing studies will indicate whether targeting of NF-κB signalling in B cells may be an effective novel treatment modality for AAV.

Disclosure of interest None declared

P043

INVESTIGATING IL-6 INTRACELLULAR SIGNALLING IN PERIPHERAL BLOOD CELL SUBSETS IN PATIENTS AT EARLY AND LATER STAGES OF RHEUMATOID ARTHRITIS (RA)

^{1,2}L Ouboussad*, ^{1,2}L Hunt, ^{1,2}C Wong, ¹P Emery, ^{1,2}M McDermott, ^{1,2}A Aslam, ^{1,2}M Buch. ¹Leeds Institute of Rheumatic and Musculoskeletal Medicine, University of Leeds; ²NIHR-Leeds Biomedical Research Centre (NIHR-LBRC), Leeds Teaching Hospitals NHS Trust, Chapel Allerton Hospital, Leeds, UK

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Introduction Rheumatoid arthritis (RA) is a chronic, inflammatory arthritis that evolves along an immunological and inflammatory disease continuum. The era of targeted biological therapies has been transformative; however, a significant unmet need is the effective tailoring of therapy to deliver optimal treatment responses. In addition, the concept of a window of opportunity is well-recognised whereby early commencement of treatment confers improved outcomes compared to delayed treatment. The importance of pro-inflammatory cytokines TNF and IL-6 in particular, is well recognised; but high, homogeneous response in early RA (ERA) compared to later RA remains unexplained.

Objectives The present project focuses on measuring the phosphorylation of STAT3 (p-STAT3) levels as an indication of the activation of IL-6/JAK-STAT signalling pathway at different disease stages (early and established/late). The main aim is to evaluate the variation in cell-subset IL-6 signalling and its association with response to treatment which included IL-6 targeted therapy (Tocilizumab-TCZ) as well as other bDMARD.

Methods Phosphorylation of IL-6/JAK-STAT key transcription factor STAT3 (p-STAT3) was measured using multiparameter phosphoflow cytometry (phosflow) in T-, B- cells and monocytes isolated from peripheral blood of RA patients. Patients cohorts represented groups at different stages of RA: Treatment-naïve Early RA (ERA group) n=20. Later RA group