protein or 1000ng/ml of human IgG1 as a control for 12h. Gene expressions were detected by microarray assay.

Results: Microarray data analysis revealed that DR3 up-regulated or down-regulated the expression of various genes in RA-FLS (Figure). The function of regulated genes included protein-L-isoaspartate (D-aspartate) O-methyltransferase activity, carboxyl-O-methyltransferase activity, protein carboxyl O-methyltransferase activity, regulation of cilium assembly, O-methyltransferase activity, regulation of plasma membrane bounded cell projection assembly, regulation of cell projection assembly, regulation of organelle assembly, protein methyltransferase activity, and S-adenosylmethionine-dependent methyltransferase activity. The most up-regulated 2 genes by DR3 were KIAA1109 (KIAA1109), and adhesion G protein-coupled receptor A3 (ADGRA3). The most down-regulated 2 genes by DR3 were RNA exonuclease 2 (REXO2), and family with sequence similarity 120A (FAM120A).

Conclusion: In this study, we first revealed the expression profiles of genes regulated by DR3 in RA-FLS. KIAA1109/TENR/IL2/IL21 gene is strongly associated with RA in European descent populations [3]. ADGRA3 is a member of G protein-coupled receptors (GPCRs). GPCRs associates with the regulation of cytoskeletal organization, the cell adhesion and migration, cell proliferation and apoptosis, and cell differentiation [4]. Loss of REXO2 affects cell growth and morphology [5], and REXO2 was identified as a target gene for inflammatory bowel disease-associated variants [6]. FAM120A regulates activity of Src kinase to protect cells from oxidative stress-induced apoptosis [7]. DR3 regulates the gene expressions of various key molecules in RA-FLS and may affect the pathogenesis of RA by regulating gene expression of RA-FLS.

References:

- [1] Takami N. et al., Arthritis Rheuma. 2006;54:779-787.
- [2] Fukuda K. et al., Biomed Rep. 2019;1:1-5.
- [3] Teixeira VH. et al., Arthritis Research & Therapy. 2009;11:R45.
- [4] Hamann J. et al., Pharmacol Rev. 2015;67:338-367.
- [5] Bruni F. et al., PLoS One. 2013;8:e64670.
- [6] Hulusi I. et al., BMC Genomics. 2015;16:138.
- [7] Tanaka M. et al., Mol Cell Biol. 2009;29:402-413.

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2020-eular.1466

AB0091 PD-1 AND GAL3 REGULATE OSTEOCLAST DEVELOPMENT IN RHEUMATOID ARTHRITIS

S. R. Greisen^{1,2}, M. A. Nielsen^{1,2}, M. Hvid³, B. Deleuran^{1,2}. ¹Aarhus University, Dept. of Biomedicine, Aarhus, Denmark; ²Aarhus University Hospital, Dept. of Rheumatology, Aarhus, Denmark; ³Aarhus University, Dept. of Clinical Medicine, Aarhus, Denmark

Background: Bone erosions in rheumatoid arthritis (RA) is a major complication. Despite improved treatment, erosions still occur and progress. Therefore, a continuous investigation of the interplay between bone regulation and immune activity is needed.

Co-inhibitory receptors, like CTLA-4, participate in modulating osteoclast activity¹, and blocking these receptors in cancer treatment results in autoimmune disease². Programmed death 1 (PD-1) is a central co-inhibitory receptor, also present in a soluble (s) form. We have previously shown that sPD-1 is associated with disease activity and reduced bone erosions in RA³. PD-1 and its ligands are glycosylated and the glycosylation affects signaling through the PD-1 pathway^{4,5}. Galectins (GAL) bind to specific glycosylation patterns on glycoproteins by their carbon recognition domain. Gal-3 can bind to multiple immune receptors shaping the immunological response⁶.

Objectives: We aimed to investigate if PD-1 and GAL-3 modulate osteoclastogenesis in RA.

Methods: Plasma and synovial fluid (SF) samples were collected from patients with chronic (c) RA (>8 years of disease, n=14) SF and blood samples were obtained when patients presented with disease flare. Soluble PD-1 and GAL-3 were investigated by ELISA. GAL-3:PD-1 complexes were captured in an optimized ELISA using both GAL-3 and PD-1 antibodies.

Surface plasmon resonance was used to evaluate on the binding properties between GAL-3 and PD-1, on a Biacore 3000. Lactose was used to block the potential binding. Cells from the synovial fluid (SFMC) were differentiated into osteoclasts with M-CSF and RANKL, recombinant human (rh) PD-1 and rhGAL-3 were added and osteoclast formation evaluated by TRAP in the supernatant.

Results: Soluble PD-1 and GAL-3 were present in both plasma and SF from cRA patient, and the ratio (PD-1/GAL-3) was increased in SF. PD-1:GAL-3 complexes were detected in both plasma and SF, with the highest concentration in SF. The binding between PD-1 and GAL-3 was confirmed by surface plasmon resonance analyses, with an estimated K_d of 5uM. Binding could be blocked by addition of lactose, confirming the binding to be glycan dependent. In SFMC osteoclast cultures, rhPD-1 and rhGAL-3 slightly decreased osteoclast formation evaluated by TRAP. However, combining rhPD-1 and rhGAL3 further potentiated the reduction in osteoclast formation by 37%.

Conclusion: We confirm glycan dependent binding between the co-inhibitory receptor PD-1 and GAL-3. Both sPD-1 and Gal-3, and the PD-1:GAL-3 complexes, are upregulated in the inflamed joint at site of erosions. *In vitro* RA culture demonstrates that GAL-3 potentiate the functions of PD-1 and reduces osteoclastogenesis. These findings indicate that the binding between Gal-3 and PD-1 could provide a novel target to control erosions in RA. Future *in vivo* studies on this interaction is needed.

References:

- [1] Axmann, R. et al. CTLA-4 directly inhibits osteoclast formation. *Ann Rheum Dis* **67**, 1603–1609 (2008).
- [2] Friedman, C. F., Proverbs-Singh, T. A. & Postow, M. A. Treatment of the Immune-Related Adverse Effects of Immune Checkpoint Inhibitors. *JAMA Oncol* **2**, 1346–8 (2016).
- [3] Greisen, S. et al. Increased soluble programmed death-1 (sPD-1) is associated with disease activity and radiographic progression in early rheumatoid arthritis. *Scand J Rehabil Med* **43**, 101–108 (2013).
- [4] Verdura, S. et al. Resveratrol targets PD-L1 glycosylation and dimerization to enhance antitumor T-cell immunity. *Aging (Albany NY)* **12**, 8–34 (2020).
- [5] Wang, M. et al. Identification of a monoclonal antibody that targets PD-1 in a manner requiring PD-1 Asn58 glycosylation. *Com Biol* **1**–10 (2019). doi:10.1038/s42003-019-0642-9
- [6] de Oliveira, F. L. et al. Galectin-3 in autoimmunity and autoimmune diseases. *Exp Biol Med (Maywood)* **240**, 1019–1028 (2015).

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2020-eular.5375

AB0092 FIBRIN ADHESION IS A PANNUS-INDEPENDENT MECHANISM OF CARTILAGE DEGENERATION IN RHEUMATOID ARTHRITIS

T. Hügle¹, S. Nasi², D. Ehrichou¹, A. So³, N. Busso³. ¹University Hospital Lausanne (CHUV), Lausanne, Switzerland; ²University Hospital Münster, Institute of Musculoskeletal Medicine, Münster, Germany; ³University Hospital Lausanne (CHUV), Rheumatology, Lausanne, Switzerland

Background: Current concepts of cartilage destruction in inflammatory arthritis include pannus infiltration by inflamed synovial tissue as well as direct detrimental effects of inflammatory cytokines and proteinases. Fibrin maintains chronic inflammatory processes in arthritis but has never been shown to be directly involved in cartilage damage occurring in rheumatoid arthritis (RA).

Objectives: To investigate fibrin-mediated cartilage degradation and the possible underlying mechanisms in arthritis.

Methods: Human cartilage samples were obtained from patients with RA undergoing joint replacement and investigated by H.E. and immunohistochemistry for cartilage damage and fibrin deposition. Cartilage explants from RA patients were incubated *in vitro* with autologous synoviocytes and assessed by immunohistochemistry for cell-adhesion and colocalization with fibrin. Experimental RA was studied in the RA murine model of adjuvant-induced arthritis (AIA), in wildtype (WT) and fibrinogen deficient (Fg^{-/-}) mice. Cartilage damage and chondro-synovial adhesion were analyzed by safranin-O staining and fibrin deposition by immunohistochemistry. Fibrinogen expression (Fga, Fgb, Fgy) was studied in murine primary chondrocytes by qRT-PCR. Cartilage explants were stained with alizarin-red staining and assessed for colocalization of calcific deposits and fibrin. Calcification of murine primary chondrocytes stimulated with secondary calciprotein particles (CPP) and treated with purified human plasma fibrinogen (100 µg/ml) was assessed by alizarin red staining and gene expression for chondrocytic differentiation (Agg, Col2, Col10, Sox9, Runx2), calcification (Alpl, Ank, Anx5, Pc1, Pit1, Pit2), and extracellular matrix remodeling (Adamts4, Adamts5, Mmp3, Mmp13, Comp) by qRT-PCR.

Results: Abundant fibrin deposition on cartilage co-localized and positively correlated with cartilage damage in knee joints of patients with RA. In the AIA model,