Results: In primary synovial fibroblasts, IL37 overexpression significantly reduced IL1 β - and OAS-CM-induced expression of MMP1, MMP3, IL1B and IL6 by more than 50%. In naïve knee joints, IL-37 overexpression did not induce synovial thickening, cartilage damage or osteophyte formation and no overt immune response was detected after 28 days. Strikingly, in CIOA, at day 7, IL37 overexpression significantly reduced synovial thickening and the presence of the metalloproteinase-generated aggrecan-degradation neopeptide VDIPEN in cartilage. At day 28 of CIOA, cartilage degradation was significantly reduced by IL37 treatment at the lateral and medial femur by respectively 46% and 41%, and osteophyte size at the medial femur was reduced by 70%. At day 42 of CIOA, IL37 overexpression could no longer be detected. Osteophyte size formation at the medial femur was still significantly reduced by 46% at this time point, but only a trend ($p = 0.06$) towards reduced cartilage damage was observed on both femur and tibia.

Conclusion: Our results show that IL37 inhibits the production of catabolic mediators in synovial fibroblasts, similar to our previously obtained results in cartilage. Furthermore, IL37 clearly lowered synovial thickening and ameliorated cartilage damage and osteophyte formation in murine experimental OA. These results indicate that IL37 acts on multiple tissues and OA-related processes, which supports IL37 as a potential therapeutic agent for OA.

Disclosure of Interests: : None declared

DOI: 10.1136/annrheumdis-2019-eular.7483

FRI0527

HIGH LDL LEVELS LESSEN BONE DESTRUCTION DURING ANTIGEN-INDUCED ARTHRITIS BY INHIBITING OSTEOCLAST FORMATION AND FUNCTION

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Background: Rheumatoid arthritis (RA) is a chronic inflammatory disease with severe joint inflammation and bone destruction resulting from increased numbers and activity of osteoclasts as important hallmarks. RA is often associated with the metabolic syndrome, characterized by elevated levels of low-density lipoprotein (LDL), which in inflammatory environments is oxidized into oxLDL. However, the effects of high (ox)LDL levels on osteoclasts remains elusive.

Objectives: Here, we investigated the effects of high LDL and oxLDL levels on osteoclast differentiation and bone destruction.

Methods: Antigen-induced arthritis (AIA) was induced in *Apoe*^{-/-} mice that spontaneously develop high LDL levels. Bone erosion was assessed with histology and numbers of osteoclasts were determined with staining for tartrate-resistant acid phosphatase (TRAP). Numbers of CD11b⁺/Ly6C^{high} and CD11b⁺/Ly6C^{high} osteoclast precursors were determined by flow cytometry. *In vitro* osteoclast differentiation from bone marrow cells was induced with macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor kappa-B (RANK) for 7 days. Pre-osteoclasts from day 3 and mature osteoclasts from day 6 of differentiation were exposed to LDL/oxLDL. Gene expression was determined with qPCR.

Results: Whereas basal levels of bone resorption were comparable between WT and *Apoe*^{-/-} mice, induction of AIA resulted in significantly lower bone resorption in *Apoe*^{-/-} mice as compared to WT mice, which was associated with lower number of TRAP⁺ osteoclasts along the bone surface. However, the absence of *Apoe* did not result in altered numbers of osteoclast precursors in the bone marrow of naïve mice, whereas even increased numbers were observed in *Apoe*^{-/-} mice during AIA. Moreover, *in vitro* osteoclastogenesis showed comparable numbers and mRNA expression of osteoclast markers, such as c-Fms, RANK, NFATc1, DC-STAMP, TRAP, CTR, CIC-7, CA-II, Cat K and MMP-9. Together, this showed that the absence of *Apoe* by itself does not affect osteoclastogenesis. Therefore, we determined whether high levels of LDL cholesterol, a characteristic of *Apoe*^{-/-} mice, or oxLDL as the result of LDL oxidation by inflammation led to decreased osteoclast differentiation. Addition of oxLDL, but not LDL, to pre-osteoclasts from day 3 and mature osteoclasts from day 6 of osteoclastogenesis strongly reduced the number of TRAP⁺ osteoclasts and their resorptive capacity. This was accompanied by a decreased expression of various osteoclast markers. To determine the mechanism of how oxLDL stimulation might reduce the osteoclast differentiation, we determined the expression of RANK and various members of the co-stimulatory pathway that promotes osteoclast formation. Interestingly, whereas RANK expression was not affected upon oxLDL exposure, we observed decreased expression of osteoclast-associated receptor (*Oscar*) and the DNAX adaptor protein-12 encoding gene *Tyrbp*, which

regulate the immunoreceptor tyrosine-based activation motif (ITAM) mediated co-stimulation signaling pathway.

Conclusion: *Apoe*^{-/-} mice have decreased bone resorption during experimental RA, probably via oxLDL-mediated interference in the co-stimulatory pathway during osteoclastogenesis.

Disclosure of Interests: : None declared

DOI: 10.1136/annrheumdis-2019-eular.3479

FRI0528

HIGH INTENSIVE THERAPEUTIC LOWERING OF SYSTEMIC CHOLESTEROL DOES NOT AMELIORATE OA DEVELOPMENT IN KNEE JOINTS OF HUMANIZED DYSLIPIDEMIC MICE

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Background: High systemic cholesterol levels have been associated with osteoarthritis (OA) development and lowering cholesterol using statins has been suggested as a potential treatment for OA¹. Recently, novel therapeutic high-intensive cholesterol-lowering treatment has been shown to regress Western-type diet (WTD)-induced atherosclerosis in dyslipidemic APOE*3Leiden.CETP mice, a translational model with a human-like lipoprotein metabolism².

Objectives: Here, we investigated the potency of these therapeutic cholesterol-lowering strategies to attenuate OA development in dyslipidemic APOE*3Leiden.CETP mice.

Methods: Female mice (n=13-16 per group) were fed a cholesterol-supplemented WTD for 38 weeks. After 13 weeks, mice were matched into a baseline group (sacrificed at t=13w) and 5 groups that received WTD alone or with treatment for 25 weeks: atorvastatin alone (A), in combination with PCSK9 inhibitor alirocumab (AA) or ANGPTL3 inhibitor evinacumab (AE) or triple treatment (AAE). Plasma lipids were monitored to assess metabolic dysfunction. Knee joints were analysed for cartilage degradation, synovial inflammation and osteophyte formation. Aggrecanase activity and S100A8 expression were determined using immunohistochemistry.

Results: Systemic administration of cholesterol-lowering interventions resulted in a significant reduction of body weight (A: -16%; AA: -8%; AE: -12%; AAE: -18% at end point) without changes in food intake. WTD-feeding manifested itself in dyslipidemia, with high cholesterol levels that were significantly reduced after administration of therapeutic cholesterol-lowering interventions (A: -46%; AA: -74%; AE: -74%; -AAE: 86% at end point). Using detailed OARSI scoring, dyslipidemic mice showed a mild but significant increase in cartilage degeneration at end point (7.8 ± 1.9) compared to baseline controls (3.8 ± 1.8 , 2.1-fold increase, $p=0.001$), and a strong 52-fold increase in NITEGE staining, representing proteolytic activity by aggrecanases³. However, therapeutic cholesterol lowering did not ameliorate further progression of cartilage destruction. This was supported by comparable NITEGE staining in all treatment groups at end point. Dyslipidemic APOE*3Leiden.CETP mice developed minor synovial inflammation which was not affected by cholesterol-lowering interventions. Alarmin S100A8, a marker for macrophage activation, showed minor expression in the synovial lining of all treatment groups, supporting the absence of joint inflammation in this model⁴. Finally, we determined whether high plasma cholesterol levels promoted osteophyte formation. The total number of osteophytes and osteophyte maturation stage was comparable between all groups despite reduction of systemic cholesterol levels.

Conclusion: Therapeutic cholesterol-lowering interventions did not slow the progression of cartilage degradation and osteophyte formation in dyslipidemic APOE*3Leiden.CETP mice with minor joint inflammation. These findings suggest that therapeutic cholesterol-lowering interventions in the absence of local inflammation within the knee joint cannot prevent progression of OA.

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Disclosure of Interests: None declared
DOI: 10.1136/annrheumdis-2019-eular.4804

FRI0529

SPREAD OF SENESCENCE AND JOINT INFLAMMATION VIA CONNEXIN43-POSITIVE EXOSOMES RELEASED BY OSTEOARTHRITIC CHONDROCYTES

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Background: Chondrocytes (CHs) in articular cartilage undergo phenotypic changes and senescence, restricting cartilage regeneration and favoring osteoarthritis (OA) progression. CHs and synovial cells from OA patients show a chronic increase in the channel protein connexin43 (Cx43), which regulates signal transduction. Extracellular vesicles (EVs), including exosomes, have been shown to play important roles in many biological functions and harbour Cx channels that allow the formation of gap junctions (GJs) between the exosome and the target cell, but the role of these EVs and exosomal-Cx43 in OA progression has not been studied yet.

Objectives: The objective of this study was to investigate the role of EVs released by OA chondrocytes (OACs) in cellular plasticity, inflammation and senescence of surrounding joint tissues.

Methods: CHs, bone and synovial cells were isolated from healthy and OA donors. EVs were obtained by ultracentrifugation and their protein content was analysed by LC-MS/MS. Protein levels were evaluated by western blot, immunofluorescence and flow cytometry. RNA expression was evaluated by RT-qPCR. Senescence and GJ intercellular communication was studied by flow cytometry and scrape loading assay, respectively.

Results: OACs showed increased levels of Cx43 within their EVs in comparison to the EVs isolated from healthy donors. Overexpression of Cx43 in CHs increased senescence and exosomal Cx43 levels. Interestingly, the treatment of CHs, bone cells and synoviocytes (target cells) with Cx43-EVs released by OACs, led to a significant increase in both Cx43 mRNA and protein levels in the recipient cells. The increase of Cx43 in target cells acted as a positive regulator of the reversion to a less differentiated state via EMT by activation of Twist-1, associated with increased levels of the mesenchymal markers, as CD105/CD166. The phenotypic changes detected in OACs lead to a decrease in Col2A1 and aggrecan expression in CHs, and increased the levels of cellular senescence and the senescence associated secretory phenotype (SASP) in the target cells in target cells via p53/p16 and NF-κB. These results were corroborated by analysing the protein cargo of these Cx43 positive EVs by LC-MS/MS, finding enrichment in proteins related with catabolic, senescence and wound-healing pathways.

Conclusion: Our results indicate that Cx43-positive exosomes released by OACs may be involved in the spread of cellular senescence and inflammation involved in wound healing failure. Further understanding of the role of exosomal Cx43 in OA will help to halt the disease spread and progression.

Disclosure of Interests: : None declared
DOI: 10.1136/annrheumdis-2019-eular.4692

FRI0530

ADIPOSE DERIVED STEM CELLS TRANSPLANTATION AMELIORATES OSTEOARTHRITIS THROUGH AUTOPHAGY INDUCING VIA PI3K/AKT/MTOR SIGNALING

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Background: Osteoarthritis is characterized by joint inflammation and cartilage degradation. Adipose derived stem cells (ADSCs), as a source of

adult mesenchymal stem cells, display similar multiple-lineage differentiating potentials to bone marrow MSCs, only are much more abundant, much easier to isolate and expand, have been suggested for suppressing inflammatory responses and repairing cartilage damage. Autophagy are known to be take part in the pathogenesis of OA. Aging related changes of chondrocytes were related to increased mammalian target of rapamycin (mTOR) signaling and defective autophagy (1). We aim to determine the effect of ADSC on autophagy and its underlying mechanism.

Objectives: To investigate the effect of ADSCs on autophagy in a rat osteoarthritis model and IL-1-induced chondrocytes, and to ascertain whether it regulates autophagy via PI3K/AKT/mTOR signaling.

Methods: ADSCs and chondrocytes were isolated from SD rats. Flow cytometry was performed for ADSC phenotypic characterization. ADSCs and chondrocytes coculture were established in the presence of IL-1β. A rat anterior cruciate ligament transection (ACLT) OA model were established. ADSCs or irrelevant cell lines of similar number were injected in the osteoarthritis-affected joints. Autophagic activation was determined by Western blotting for LC3-II, p62, MDC (monodansylcadaverine) staining and GFP-LC3 fluorescence microscopy. Autophagy inhibition was mediated by siRNA knockdown of ATG5. Relevant proteins in the PI3K/AKT/mTOR signaling pathway were detected by western blotting. IL-1β, IL-6, TNFα, IFN-γ, Col2, MMP-1, 3 and -13 were measured. Histology of the target joints were evaluated.

Results: Application of ADSCs resulted in downregulation of MMP13 and upregulation of Col2 in OA model cartilage and IL-1β induced chondrocytes. ADSCs decreased pro-inflammatory cytokines IL-1β, IL-6, and TNF-α in rat OA and IL-1β-induced chondrocytes. Autophagy was inhibited in OA model and IL-1β induced chondrocytes. ADSCs increased cell viability and autophagy-related proteins levels in vitro and in vivo. In IL-1β induced chondrocytes with ATG5 knockdown by siRNA, the effect of ADSCs on autophagy activation and its function as suppression of IL-1β induced pro-inflammatory cytokines was undermined. Furthermore, ADSCs remarkably decreased the expressions of phosphorylated (p)-PI3K, p-AKT and p-mTOR in IL-1β induced chondrocytes.

Conclusion: ADSCs inhibited the progression of cartilage degeneration in a rat OA model and provide an effective approach to decrease the pro-inflammatory cytokines secretion. ADSCs' anti-inflammatory effect was associated with cell autophagy and these roles of ADSCs may be associated with PI3K/AKT/mTOR signaling pathway.

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Disclosure of Interests: None declared
DOI: 10.1136/annrheumdis-2019-eular.718

FRI0531

THE ARTICULAR PROTECTION EFFECT OF ISORHAMNETIN IN THE RATS OF MONOSODIUM IODOACETATE-INDUCED OSTEOARTHRITIS

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Background: Osteoarthritis (OA) is a degenerative joint disease with damage to the articular cartilage. Active production of inflammatory cytokine/chemokine and matrix metalloproteinases may be found during the progression of OA. Isorhamnetin had the effects of anti-inflammatory, antioxidant, anti-ischemia, anti-atherosclerotic hepatoprotective and anticancer activities.

Objectives: Our study was focused on the effects of isorhamnetin treatment in OA.

Methods: We used monosodium iodoacetate (MIA)-induced OA rats to evaluate the effects of isorhamnetin related anti-inflammatory process. The rats in all groups were sacrificed on four weeks post-MIA injection. The measurements of knee joint swelling, histological analysis, serum inflammatory biomarkers and Western blot were evaluated.

Results: We found that isorhamnetin may reduce MIA-induced knee swelling by significantly reduction of articular cartilage damage in rats. The severity of OA lesion was graded using the modified Mankin scoring system, and we found that the overall modified Mankin's scores were significantly decreased after isorhamnetin treatment compared with a MIA-treated group. The production of IL-1β, IL-6, and TNF-α decreased in isorhamnetin treatment group compared with MIA alone group.