Results: S100A11 protein was up-regulated in the muscle from patients with IIMs compared to MG. In PM/DM patients, S100A11 was accumulated in the cytoplasm of regenerating and necrotizing muscle fibers and on the sarcolemma of most fibers. Only some mononuclear infiltrate cells showed S100A11 positivity. In patients with MG, S100A11 was detected on the sarcolemma only. Moreover, S100A11 was increased in plasma of patients with IIMs compared to HC (3.82 [1.47–72.9] vs. 2.84 [1.67–11.18] ng/ml; $p=0.0076$). When divided into groups, patients with DM and CAM showed significant elevation of plasma S100A11 compared to HC (4.10 [2.22–50.81] and 4.52 [1.53–9.11] vs. 2.84 [1.67–11.18] ng/ml; $p=0.004$ and $p=0.02$, respectively). Levels of S100A11 did not differ between PM and HC. In all patients, S100A11 correlated with LD ($r=0.300$, $p=0.001$), CK ($r=0.217$, $p=0.025$) and AST ($r=0.343$, $p<0.001$), with MYOACT ($r=0.279$, $p=0.005$) and with muscle, pulmonary and skin disease activity ($r=0.199$, $p=0.040$; $r=0.224$, $p=0.025$ and $r=0.307$, $p=0.001$). S100A11 levels were elevated in autoantibody-positive group compared to autoantibody-negative group of patients with IIMs (5.22 [1.45–69.29] vs 3.44 [1.58–12.12], $p=0.010$).

Conclusions: S100A11 is up-regulated in the circulation of patients with IIMs and correlates with disease activity. Accumulation of S100A11 in the muscle of patients with myositis may indicate its potential role in the process of cell necrosis and fiber regeneration.

Acknowledgements: Supported by grant 15–34065A of the Agency for Healthcare Research of the Czech Republic and MHCR 023728.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.4758

THU0059 ONCOSTATIN M INDUCES INFLAMMATION AND DIFFERENTIALLY REGULATES TNF ALPHA-INDUCED PRO-INFLAMMATORY MECHANISMS AND NOTCH SIGNALLING IN THE RA JOINT

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Background: Oncostatin M (OSM) is a pleiotropic cytokine, highly expressed in the RA joint that displays both agonistic and antagonistic effects depending on the inflammatory microenvironment. This study examines the effect of OSM on inflammation, the Notch-1 signalling pathway which plays a critical role in vascular development and angiogenesis and finally on TNF α -induced pro-inflammatory mechanisms in Rheumatoid Arthritis.

Objectives: To examine the effect of OSM on cytokine/chemokine production, angiogenesis and the Notch-1 signalling pathway in synovial fibroblasts and endothelial cells and whether OSM potentiates the effects of TNF α -induced pro-inflammatory effects.

Methods: Primary RA synovial fibroblasts (RASFC) isolated from RA synovial biopsies obtained at time of knee arthroscopy and human dermal microvascular endothelial cells (HMVEC) were grown to confluence. RASFC and HMVEC were cultured with OSM (10ng/ml) alone or in combination with increasing concentrations of TNF α (0.01–1ng/ml). IL-6, IL-8, RANTES, GRO α and MCP-1 cytokines/chemokines were quantified in culture supernatants by ELISA. Functionally, angiogenesis and invasion were assessed by matrigel tube formation and Transwell invasions assays respectively, and VEGF in cell lysates quantified by Real-time PCR. Finally, Notch-1, its ligands Delta-like-ligand 4 (DLL-4) and Jagged-1 (Jag-1) and downstream transcriptional repressors – Hey-1 and Hey-2 were quantified by Real-time PCR.

Results: OSM alone significantly induced IL-6 and MCP-1 while inhibiting IL-8 and GRO α in RASFC and HMVEC culture supernatants, compared to basal control. OSM alone induced RANTES expression in HMVEC with little effect observed for RASFC. OSM potentiated the effect of increasing concentrations of TNF α on IL-6 and MCP-1 secretion from RASFC and HMVEC. Conversely OSM significantly inhibited TNF α -induced IL-8 and GRO α secretion from both RASFC and HMVEC. Interestingly, OSM significantly inhibited TNF α -induced RANTES expression in HMVEC yet conversely potentiated this effect in RASFC. At a functional level, OSM induced both RASFC and HMVEC invasion and induced network formation and VEGF expression in HMVEC. OSM significantly induced Notch-1 in RASFC and HMVEC in a time dependent manner, but interestingly differentially regulated the Notch-1 ligands, with induction of Jag-1 only observed in RASFC, and induction of DLL-4 only observed in HMVEC. Finally OSM differentially regulated Notch-1 downstream transcriptional repressors in HMVEC, significantly inducing Hey-2 while simultaneously inhibiting Hey-1.

Conclusions: OSM is a pleiotropic cytokine that displays divergent effects with both pro- and anti-inflammatory mechanisms within the inflamed joint, effects that appear to be dependent on cell type and the inflammatory microenvironment. Targeting OSM or downstream signalling pathways may lead to new potential therapeutic strategies or adjuvant therapies, particularly for those patients who have sub-optimal responses.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.6334

THU0060 T CELL-DERIVED IL-17A AND IL-17F DRIVE BONE FORMATION FROM HUMAN PERIOSTEAL STEM CELLS: IMPLICATIONS FOR ENTHESOPHYTE FORMATION

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Background: Pathological bone formation associated with spondyloarthropathies (SpA) is a major cause of structural tissue damage causing permanent disability. A paucity of *in vitro* models that faithfully replicate human skeletal biology has impeded research into the cellular and molecular triggers for this osteoimmunological phenomenon. Nevertheless, clinical and animal studies have defined IL-17 signalling as a key regulator of SpA disease; however, the role of IL-17 in bone pathology is unclear. IL-17-producing $\gamma\delta$ -T cells have a critical function in periosteal bone formation for fracture repair¹. The periosteum has also been implicated in pathological bone formation during SpA disease progression².

Objectives: To investigate IL-17 signalling in the context of pathological bone formation using a biomimetic human periosteum derived stem cell (hPDSC) model of osteogenic differentiation.

Methods: hPDSCs were obtained through enzymatic digestion of periosteal biopsies from patients undergoing orthopaedic surgery. Expanded cultures were then treated with recombinant human IL-17A, IL-17F, or both over 96h. The expression of gene markers was then evaluated. Alternatively, hPDSCs were stimulated using a biomimetic protocol in combination with IL-17A and IL-17F, or human T-cell supernatants (SNs) (as a surrogate disease-like inflammatory milieu). Antibodies with strong-affinity to IL-17A, IL-17F, or bimekizumab (a humanised monoclonal IgG1 antibody with strong affinity for both IL-17A and IL-17F) were used to define the role of these cytokines in the SNs. Expression of osteogenic markers and matrix mineralisation was assessed to define *in vitro* bone formation.

Results: Under basal conditions IL-17A and IL-17F significantly up-regulated IL-6 expression. Additionally, IL-17A and IL-17F transiently enhanced the expression of the osteogenic transcription factor RUNX-2. When IL-17 cytokines were combined in a biomimetic differentiation protocol, both IL-17A and IL-17F promoted osteogenic differentiation. Importantly, IL-17F enhanced the expression of most osteogenic markers to a greater extent than IL-17A alone following 9 days' exposure. Conversely, IL-17A treatment resulted in elevated *in vitro* mineralisation vs IL-17F. Th17 and $\gamma\delta$ -T cell SNs potentially enhanced hPDSC osteogenic differentiation and mineralisation. Whilst IL-6 expression and *in vitro* bone formation were blocked by neutralisation of IL-17A or IL-17F, dual neutralisation of IL-17A and IL-17F in the inflammatory milieu exhibited the greatest effect on most of the tested parameters.

Conclusions: These data show that both IL-17A and IL-17F enhance *in vitro* osteogenic differentiation and bone formation from hPDSCs. The source of these cytokines has not been established but is likely to involve enthesal resident $\gamma\delta$ -T cells. We propose that following their release, IL-17A and IL-17F drive pathological bone formation resulting in enthesophytes at the enthesis/periosteum interface. Current therapeutics display limited efficacy in blocking enthesophyte formation, hence inhibition of both IL-17A and IL-17F offers an attractive therapeutic strategy to prevent this debilitating feature of SpA.

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Disclosure of Interest: M. Shah Grant/research support from: UCB Pharma, Employee of: UCB Pharma, A. Maroof Employee of: UCB Pharma, R. Al-Hosni: None declared, P. Gikas: None declared, N. Gozzard Employee of: UCB Pharma, S. Shaw Employee of: UCB Pharma, S. Roberts Employee of: UCB Pharma

DOI: 10.1136/annrheumdis-2017-eular.3826

THU0061 REGULATION OF OSTEOCLAST RECRUITMENT AND ACTIVATION BY RESOLVIN D1

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Background: Resolvin-D1 (RvD1) is a derivative of omega-3 fatty acids and a potent anti-inflammatory agent synthesized during the resolution phase of inflammation. In human cartilage, we recently reported that RvD1 strongly inhibited a number of factors involved in inflammation, catabolism, oxidative stress, and apoptosis.

Objectives: The overall objective of this study is to further investigate RvD1 effects on bone metabolism *in vitro*.

Methods: First, murine macrophages RAW267.4 were used to assess osteoclasts (OC) recruitment and bone resorption. RAW267.4 cells were incubated with 50 ng/ml LPS with or without RvD1 (0 - 10 μ M) for 48 hours. Cell viability was verified with the MTS test. OC phenotype markers, namely TRAP and cathepsin-K, were assessed by western blot, enzymatic staining and immunocytochemistry. Levels of TNF- α , IL-1 β , IL-6, IL-10, were measured by ELISA, and PGE₂ levels by EIA. NO release was measured by Griess reaction.

To investigate bone resorption, RAW267.4 cells were seeded in hydroxyapatite plates, then treated with 50 ng/ml LPS with or without RvD1 (0.5 and 1 μ M) for 48 hours. Plot formation was assessed by Von Kossa staining.

Second, human osteoblasts (Ob) were obtained from post-surgery discarded trabecular bone of osteoarthritic (OA) patients who underwent total knee arthroplasty. First passage human OA Ob were treated either with RvD1 (0.1 - 1 μ M) alone, or with 20 nM VitD3 with or without RvD1 (0.1 - 1 μ M), for 48 hours. Cell viability was evaluated with the MTS test. Alkaline phosphatase (PAL) activity and osteocalcin (OCN) release was determined by colorimetric reaction and ELISA, respectively.

Results: In RAW264.7 cells, our results clearly show that RvD1 strongly reduces OC recruitment and activation as indicated by the inhibition of TRAP and cathepsin K expression as well as TNF- α , IL-1 β , IL-6, PGE₂ and NO release, as well as the concurrent enhancement of IL-10 levels. Besides, RvD1 decreases bone resorption through the inhibition of pits formation in hydroxyapatite matrix. In human OA Ob, RvD1 partially decreases VitD3-induced PAL activity, while it maintains OCN expression at control levels.

Conclusions: Our in vitro results clearly show that RvD1 may play an important role in the regulation of bone metabolism. Additionally to our previous data, our findings suggest that RvD1 may offer a novel and original perspective to make a real contribution to musculoskeletal and bone diseases therapy.

Acknowledgements: This study was supported by Canadian Institute of Health Research (CIHR) grant (#IMH 131570) and by the Center of Excellence for Arthroplasty Research.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.2934

THU0062 NOVEL ANIMAL MODEL OF AROMATASE INHIBITOR-INDUCED ARTHRALGIA SUGGESTS AN ESTROGEN-INDEPENDENT INFLAMMATORY MECHANISM

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Background: Aromatase Inhibitors (AIs) block physiological estrogen production in peripheral tissues and significantly improve overall survival rates of post-menopausal, hormone receptor-positive breast cancer patients by reducing tumor recurrences. However, half of patients taking these drugs develop aromatase inhibitor induced arthralgia (AIIA), which is characterized by severe pain and inflammation in various joints. Since AIIA leads to suspension of therapy in 20% of patients, reducing incidence may provide sustained AI treatment and enhanced long-term survival.

Objectives: In order to establish a better understanding of the inflammatory mechanism and to create a platform that can be used to explore interventional strategies, our objective in this study was to design a novel animal model of AIIA.

Methods: Female BALB/C-Tg (NFkB-RE-luc)-Xen mice, which have a firefly luciferase cDNA reporter gene under the regulation of 3 κ B responsive binding sites, were oophorectomized and treated with AI (letrozole) by daily subcutaneous injections. Control groups included oophorectomized mice receiving vehicle control injections and non-oophorectomized mice treated with AI. Bioluminescent imaging of hind limbs was performed after 3 weeks on the in vivo imaging system (IVIS) to measure NFkB activation. At 5 weeks, knee joints and surrounding tissue were imaged on the BioSpec 94/30 micro-MRI. Legs were collected for histopathological analysis and serum cytokine levels were measured at experimental endpoint.

Results: Bioluminescent imaging showed significantly enhanced NFkB activation in the hind limbs compared to oophorectomized controls receiving vehicle treatment. Analysis of knee joints and legs by MRI imaging showed enhanced signal detection in the joint space and surrounding tissue following AI treatment. Surprisingly, enhanced MRI detection was also demonstrated in non-oophorectomized mice that were treated with AI. Histopathological analysis further demonstrated mild inflammation in the synovial tissue and joint damage in mice treated receiving AI both with and without oophorectomy. Moreover, tenosynovitis and inflammatory muscle tissue infiltrates were detected in AI-treated mice and serum cytokine levels of IL-2, IL-4, IL-6, and CXCL1 were significantly elevated.

Conclusions: Collectively, these data establish a novel mouse model of AIIA and suggest that the pathogenesis of AI-induced inflammation is estrogen-independent. Future studies will be directed into the characterization of this inflammatory mechanism to provide insight into potential therapeutic strategies directed at mitigating this adverse inflammatory burden.

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Acknowledgements: Stefanie Spielman Fund for Breast Cancer Research and The Wexner Medical Center.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.5424

THU0063 IL-1 FAMILY CYTOKINES AND RECEPTORS IN IGG4-RELATED DISEASE

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Background: IgG4-related disease (IgG4-RD) is a fibroinflammatory condition that can affect almost any organ, characterized by lymphoplasmacytoid infiltrate, obliterative phlebitis and storiform fibrosis often associated with eosinophilia and increased levels of IgG4. Cytotoxic CD4 T cells producing IL-1 β [D1], TGF β 1 and IFN- γ are detectable in peripheral blood of patients and high IL-18 expression has been found in affected organs.

Objectives: To evaluate the role of IL-1 family cytokines in IgG4-RD, by analyzing cytokines and receptors in sera.

Methods: Nine patients fulfilling the proposed criteria (Umehara, 2012) for the diagnosis of IgG4-RD were recruited. Cytokines of the IL-1 family (IL-1a, IL-1b, IL-33, IL-18), soluble receptors (sIL-1R1, sIL-1R2, sIL-1R3, sIL-1R4) and antagonists (IL-1Ra, IL-18BP) were measured in sera by multiarray ELISA assay. Free IL-18 was calculated using the law of mass action.

Results: Most patients had a multiorgan disease; retroperitoneum, salivary glands, pancreas and lymph nodes were most frequently affected. IL-18 (p=0.007) and free IL-18 (p<0.0001), sIL-1R1 (p=0.0005), sIL-1R2 (p=0.0013), and sIL-1R4 (p=0.0006) were significantly increased in IgG4-RD sera compared with healthy controls.

Conclusions: In IgG4-RD patients, at variance with other autoimmune or autoinflammatory conditions, the increase in IL-18 levels is not counterbalanced by IL-18BP, leading to high levels of free IL-18. The free cytokine may affect T cell subset balance and induce IFN- γ production. The parallel increase of sIL-1R1 and sIL-1R2 suggests an efficient dampening of inflammatory IL-1 signaling at the tissue level, while high levels of sIL-1R4 may be associated with vascular remodeling and fibrosis, as observed in animal models of obesity and in human cardiovascular disorders.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.5816

THU0064 ADAM-10 AS A TOCILIZUMAB TREATMENT PREDICTIVE FACTOR IN RHEUMATOID ARTHRITIS

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Background: A disintegrin and metalloproteinases (ADAMs) are a family of transmembrane and secreted proteins. ADAM-10 has been reported to be the enzyme responsible for the release of a number of chemokines and cytokine receptors. We have shown that ADAM-10 is overexpressed on rheumatoid arthritis (RA) synovial tissue endothelial cells (ECs) and lining cells compared with osteoarthritis and normal tissues. We also demonstrated that ADAM-10 mediates EC migration and tube formed.

Objectives: In order to demonstrate for ADAM-10 in clinical side, we focused on ADAM-10 as predictive factor for treatment with biologics in RA.

Methods: The serum was collected from patients before the initial treatment with biological therapies. Fifteen patients were treated with adalimumab (ADA), and 20 patients were treated with tocilizumab (TCZ). ADAM-10 and fractalkine/CX3CL1 were measured by enzyme-linked immunosorbent assay at 0, 12, 24 and 54 weeks. Clinical disease activity was evaluated by clinical disease activity index (CDAI). Following biological therapies, we defined biologic-responders as patients whose DAS28 scores decreased by more than 1.2 at 24 weeks. ADAM-10 baseline was also compared between responders and nonresponders at 24 weeks.

Results: There were no significant differences were observed in the mean age, gender ratio, dosages of prednisolone and methotrexate between ADA and TCZ groups. In ADA group, baseline DAS28 for the 15 patients was 4.8 $\pm$ 0.3 (2.5-7.2).