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 μM) 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-1β, IL-6, PGE_2 and NO release, as well as the concurrent enhancement of IL-10 levels. Besides, RvD1 decreases bone resorption through the inhibition of plots 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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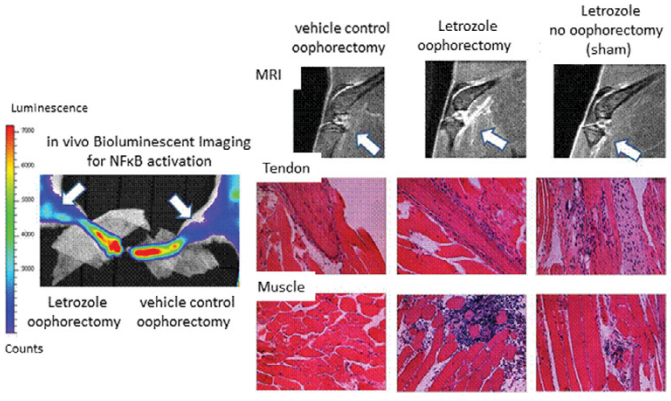
Background: Aromatase Inhibitors (AIs) block physiological estrogen production in peripheral tissues and significantly improve overall survival rates of post-menopausal, hormone receptor-positive breast cancer patients by reducing tumor recurrences. However, half of patients taking these drugs develop 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 (NFκB-RE-luc)-Xen mice, which have a firefly luciferase cDNA reporter gene under the regulation of 3 κB 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 NFκB 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 NFκB 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 lymphoplasmocytoid infiltrate, obliterative phlebitis and storiform fibrosis often associated with eosinophilia and increased levels of IgG4. Cytotoxic CD4 T cells producing IL-1b [D1], TGFb1 and IFN-gare 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-g production. The parallel increase of sIL-1R1 and sIL-1R2 suggests an efficient dampening of inflammatory IL-1bsignaling 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 patents 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 predonisolone and methotraxate between ADA and TCZ groups. In ADA group, baseline DAS28 for the 15 patients was 4.8±0.3 (2.5–7.2).

On the other hands, baseline DAS28 for the 20 patients was 4.8±0.3 (2.5–6.8) in TCZ group. There were no differences between ADA and TCZ groups. RA patients with an insufficient response to ADA or TCZ showed highly significant improvement of DAS28 after 12 weeks (2.9±0.3 and 2.2±0.4, respectively), and 24 weeks (2.5±0.4 to 2.2±0.2, respectively). ADAM-10 highly correlates with CDAI, and fractalkine/CX3CL1. Serum ADAM-10 levels were no remarkable change after treatment with ADA despite decrease of disease activity of RA. On the other hand, serum ADAM-10 levels in patients who were treated with TCZ were significantly diminished following successful treatment and clinical improvement (baseline 408±88 pg/ml and 54 weeks 138±51 pg/ml, $p<0.05$). Univariate logistic regression analysis, baseline of DAS28 (ESR), baseline of CDAI, and ADAM-10 were selected as significant variables for improvement of DAS28 (ESR) at 24 weeks. Multiple regression analysis showed that ADAM-10 was only identified as independent predictive variable for improvement of DAS28 (ESR) at 24 weeks. ADAM-10 baseline in TCZ responder was significantly higher than TCZ nonresponders at 24 weeks (620±134 pg/ml and 109±25 pg/ml, respectively, $p<0.05$).

Conclusions: This study indicates that ADAM-10 is correlated with RA disease activity, and is higher in TCZ responders. These results suggest that ADAM-10 may be a predictor of treatment effectiveness for RA with TCZ.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.4594

THU0065 CYTOKINES AND LIPOCALIN-2 IN PREGNANT WOMEN WITH RHEUMATOID ARTHRITIS AND SYSTEMIC LUPUS ERYTHEMATOSUS

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Background: Rheumatoid arthritis (RA), and especially seronegative RA, is often ameliorated by pregnancy, while systemic lupus erythematosus (SLE) is prone to flare and associated with pregnancy complications. Cytokines and chemokines are of great importance for immune processes during pregnancy. The inflammatory marker Lipocalin-2 (LCN2) has become increasingly relevant as a potential clinical biomarker of rheumatic diseases (1). LCN2 is produced in the maternal-fetal interface during normal pregnancies and correlates with the presence and severity of preeclampsia (2).

Objectives: To obtain a better understanding of immune regulation and the disparate immune responses in pregnant women with RA and SLE. In this pilot study, we analyzed levels of multiple cytokines, chemokines and LCN2 in women with seropositive RA, seronegative RA, SLE and healthy controls during pregnancy and postpartum.

Methods: The Norwegian National Advisory Unit on Pregnancy and Rheumatic Diseases collect serum samples in a biobank from women with inflammatory rheumatic diseases before pregnancy, during pregnancy week 10–12, week 23–25, week 30–32 and 6 weeks, 6 months and 12 months postpartum. Control serum samples were collected from healthy pregnant women at matching time-points. We analyzed serum cytokine and chemokine levels using a multiplex assay. A sandwich ELISA was used to measure LCN2. In this pilot study we included pregnant women with SLE (n=4), seropositive RA (n=4), seronegative RA (n=2) and healthy pregnant controls (n=4). The total cohort consists so far of 18 pregnant women with SLE and 23 pregnant women with RA.

Results: We observed lower LCN2 levels during pregnancy in SLE patients, compared to controls and RA patients. LCN2 levels in seropositive RA patients and controls were found to be comparable during pregnancy, whereas pregnant women with seronegative RA showed higher LCN2 levels. Levels of IFNγ, IL-6 and IP-10 were higher in SLE than in RA patients during the course of pregnancy. IL-17 was slightly higher only in seropositive RA patients compared to controls. TNFα was slightly higher in both SLE and RA patients compared to controls, levels of anti-inflammatory IL-10 were very low or undetectable in all groups.

2. Trimester	Controls	SLE	Seropos RA	Seroneg RA
LCN2 (ng/ml)	285,5 (±74,7)	126,5 (±44,6)	232,6 (±67,4)	330,2 (±3,2)
IFNγ (pg/ml)	15,0 (±22,4)	40,4 (±65,1)	6,9 (±5,2)	26,1 (±9,9)
IL-6 (pg/ml)	3,4 (±1,9)	5,9 (±2,0)	3,8 (±1,5)	5,3 (±1,0)
IP-10 (pg/ml)	60,3 (±14,5)	138,3 (±92,1)	61,7 (±8,9)	64,9 (±7,3)
IL-17 (pg/ml)	1,3 (±1,3)	2,3 (±2,2)	4,8 (±9,0)	0,6 (±0,2)
TNFα (pg/ml)	2,2 (±2,5)	4,2 (±5,4)	3,3 (±3,5)	3,2 (±0,7)

Conclusions: We found interesting differences in cytokine, chemokine and LCN2 levels during pregnancy in women with SLE, seropositive RA and seronegative RA. The results need confirmation in the total cohort and will be further explored for a better understanding of the disparate immune modulation of RA and SLE during pregnancy.

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Acknowledgements: This study is supported by grants from St. Olavs Hospital and Norsk Revmatikerforbund.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.1916

THU0066 OSTEOCLAST DIFFERENTIATION GENE EXPRESSION PROFILING REVEALS CCL4 MEDIATES RANKL-INDUCED OSTEOCLAST MIGRATION

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Background: The migration of osteoclast from circulation and bone marrow into bone surface has suggested as a novel therapeutic point for bone erosion in RA.

Objectives: We explored the mechanisms involved in osteoclast migration.

Methods: Gene expression profiling was identified by microarray analysis and validated by Real-time PCR during differentiation of bone marrow-derived macrophages (BMMs) into osteoclast (OCs). RANKL induced osteoclast precursor cell line RAW264.7 migration and invasion in the presence and absence of anti-CCL4 antibody was measured in vitro. Intracellular signaling pathway was assessed by Western blotting. Osteoclast formation was identified by TRAP staining.

Results: A panel of 11 chemokines signal was significant increase in osteoclastic differentiation of BMMs by Microarray. High expression of CCL4 was validated in primary BMMs and RAW264.7 cell line during differentiated into OCs. RANKL induced osteoclast precursor cell migration and invasion was decreased upon addition of anti-CCL4 antibody. OCs formation and OCs related genes expression were not affected by CCL4 inhibition. Neutralization of CCL4 promoted the PI13K phosphorylation at 45 to 60min after RANKL stimulation in RAW264.7.

Conclusions: CCL4 regulates RANKL-induced OCs migration, suggesting that CCL4 inhibition could be bone protective in RA

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Acknowledgements: Support for this work was obtained from the National Natural Science Foundation of China (NSFC): 30701129 (WT), 30901332 (FW), 81172845 (WT), 81273294 (MZ), National Natural Science Foundation of Jiangsu province: BK2011851 (WT), BK2012875 (MZ), the special project of clinical medicine from Jiangsu province: BL2013034 (MZ), A Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD), and scholarship from Asia Pacific League of Associations for Rheumatology (APLAR) and International League of Associations for Rheumatology (ILAR) (WT).

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.4255

THU0067 NLRP3 INFLAMMASOME ACTIVITY IN MONOCYTES IS REGULATED BY 12/15-LIPOXYGENASE

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Background: Activation of the NLRP3 inflammasome is a major inflammatory pathway in monocytes in response to various exogenous and endogenous stimuli. However, negative regulation of inflammasome activity is not well understood. Glucocorticoids (GC) are drugs of choice for the treatment of many inflammatory diseases. Recently, we could show that treatment of monocytes with GC leads to re-programming towards a specific population involved in resolution of inflammation. Gene analysis has shown up-regulated expression of 12/15-lipoxygenase (12/15-LOX) in GC-and LPS/GC-treated monocytes. 12/15-LOX reacts with polyunsaturated-fatty-acids to generate anti-inflammatory lipid-mediators, which contribute to resolution of inflammation.

Objectives: The aim of our study was to determine the contribution of 12/15-LOX on the inflammatory response on murine monocytes.

Methods: Bone marrow-derived monocytes were isolated from wild-type (wt) C57BL/6 and 12/15-LOX$^{-/-}$ mice and stimulated with GC and/or LPS as well as various inhibitors or stimulants. Gene expression was analyzed using qRT-PCR. Protein expression was examined by Western-Blot, Flow-Cytometry and ELISA. T-cell response was analyzed by co-culture of stimulated monocytes with allogenic T-cells.

Results: 12/15-LOX$^{-/-}$ monocytes showed slightly higher secretion of IL-1β as compared to wt cells after LPS stimulation. The differences between wt and 12/15-LOX$^{-/-}$ were much more pronounced when monocytes were additionally exposed to ATP. LPS treatment markedly enhanced expression of pro-IL-1β in 12/15-LOX$^{-/-}$ monocytes. No differences could be observed between wt and 12/15-LOX$^{-/-}$ monocytes in secretion of other proinflammatory mediators as well as the expression of inflammasome components. However, expression of cleaved caspase-11 was up-regulated in 12/15-LOX$^{-/-}$ monocytes exposed to LPS. Additionally, inhibition of caspase-11, caspase-1 and 5-LOX significantly reduced the high secretion of IL-1β in 12/15 LOX$^{-/-}$ monocytes. Interestingly, 12/15-LOX$^{-/-}$ rather than wt monocytes stimulated with LPS led to enhanced T-cell proliferation.