osteoclast (OC) differentiation but also to inhibit osteoblast differentiation.

Objectives Here we investigate the impact of activin A on joint destruction in rheumatoid arthritis.

Methods Synovial tissue samples from rheumatoid arthritis (RA) and osteoarthritis (OA) patients were analysed by immunohistochemical staining. For *in vitro* experiments, bone marrow-derived macrophages (BMM) were isolated from femurs and tibias of WT mice and differentiated into osteoclasts in the presence of macrophage colony-stimulating factor (M-CSF) and RANKL with or without activin A. OC differentiation was characterised by TRAP staining. Resorption activity was determined by quantification of osteoclast-mediated pit formation on a calcium phosphate-coated plate. Proliferation of BMMs was evaluated using the CyQUANT Cell Proliferation Assay Kit. Furthermore, osteoclast-specific gene expression as well as the activation of SMAD2, MAPK and NF- κ B signalling were analysed by immunoblotting. The interaction of phospho-SMAD2 with NFATc1 was evaluated by co-immunoprecipitation using Dynabeads.

Results We demonstrate that activin A is highly abundant in the synovium of RA but not of OA patients. *In vitro*, activin A strongly enhanced the RANKL-mediated differentiation of BMMs into mature OCs, reflected by a significantly increased OC number, OC size and number of nuclei per OC compared to the conventional treatment with RANKL alone. Moreover, concomitant administration of activin A led to a significant increase of the total resorption area as well as resorption area per pit, indicating an increased activity of individual OCs. Effects of activin A on BMMs were not caused by increased proliferation since no effect on M-CSF-driven proliferation was observed. Furthermore, activin A alone was not able to induce the expression of OC differentiation markers, but the RANKL-induced expression was enhanced by activin A. After stimulation with activin A, BMMs showed an activation of SMAD2, but not of MAPK p38, ERK, JNK or NF- κ B. Finally, co-stimulation of RANKL and activin A resulted in an increased interaction of activated SMAD2 with NFATc1.

Conclusions The data strongly suggest that increased expression of activin A in the arthritic joint is most likely associated with enhanced osteoclast formation, promoting joint destruction in rheumatoid arthritis.

Disclosure of interest None declared

P074

IL-10 REGULATES SKIN THICKNESS AND SCALING IN IMIQUIMOD-INDUCED PSORIASIS-LIKE SKIN INFLAMMATION IN MICE

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10.1136/annrheumdis-2018-EWRR2018.91

Introduction Psoriasis is an autoimmune skin disease affecting around 0.6% to 3% of the whole population with detrimental physical and societal impacts. Previously, we established a psoriasis-like skin inflammation model in mice using topical application of imiquimod (IMQ). This model successfully recaptures all critical features of clinical psoriasis such as keratinocyte hyperproliferation, Munro's microabscesses, and shares a similar infiltration profile of various immune cells. Previous data suggest up-regulation of IL-10, but its role in this psoriasisiform model is not clear.

Objectives To investigate the role of IL-10 in the IMQ-induced psoriasis-like skin inflammation.

Methods Psoriasis-like skin inflammation was induced by topical application of imiquimod (Aldara) for 5 or 10 days. Mice were injected intraperitoneally with anti-IL-10 or an isotype control antibody or subcutaneously with dexamethasone. Back skin of mice was scored for up to 10 days using a modified Psoriasis Area and Severity Index (PASI) score system adapted from clinical PASI score. Inflammation and skin thickness were scored histologically. Gene expression and immune cells in the skin were analysed using RT-PCR and flow cytometry, respectively.

Results At day 10, both skin thickness and scaling score were significantly higher after neutralising IL-10 compared to isotype control, or either group compared to dexamethasone-treated animals. At days 5 and 10, H and E staining confirmed that epidermal thickness was more prominent in anti-IL-10 treated mice compared to isotype control or dexamethasone-treated mice, with more profound differences at day 10. Ki-67 staining for proliferating keratinocytes showed more proliferation at the epidermal basal layer after neutralising IL-10. In addition, significant more infiltration of neutrophils was found in skin at day 10. At day 5, IL-23/IL-17 pathway cytokines were more significantly upregulated in anti-IL-10 group than the isotype control group, while at day 10, a significant upregulation of IL-19, IL-24 expression were found in anti-IL-10 group compared to isotype control.

Conclusions IL-10 regulates skin thickness and scaling during psoriasis-like skin inflammation. Furthermore, our data suggested that IL-10 might influence psoriatic symptoms through dampening of IL-23/IL-17 axis in early phase (day 5) and reducing IL-19 and IL-24 expression at late stage (day 10). The negative feedback signal of IL-10 partially explains the observed decrease of inflammation in imiquimod-induced skin inflammation after day 5.

Disclosure of interest X. Xu: None declared, E. Prens: None declared, E. Florencia: None declared, L. Boon: None declared, P. Asmawidjaja: None declared, A.-M. Otten-Mus: None declared, E. Lubberts Grant/research support from: Novartis

P075

CHANGES OF METABOLIC BIOMARKER LEVELS UPON ANTI-TNF THERAPY IN RHEUMATOID ARTHRITIS

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10.1136/annrheumdis-2018-EWRR2018.92

Introduction Rheumatoid arthritis (RA) has been associated with cardiovascular disease and metabolic syndrome. Numerous pro-inflammatory cytokines (e.g. TNF- α , IL-1, IL-6) are released, which cytokines cause increased reactive oxygen species (ROS) production and thereby contribute to the increased lipid peroxidation and reduction of many antioxidants. These processes not only lead to the deterioration of joints and other tissues but may also contribute to comorbidities, such as atherosclerosis.

Objectives The aim of this study was to assess the effects of anti-TNF therapy on different metabolic markers, such as PON1 (paraoxonase 1), arylesterase, chemerin and

adiponectin. We also investigated whether these biomarkers correlated with various demographic, clinical and laboratory markers.

Methods We treated 37 RA patients with either etanercept (ETN) or certolizumab pegol (CZP) in a 12 month follow-up study. Assessments were performed at baseline, and 3, 6 and 12 months after treatment initiation. Serum chemerin and adiponectin concentrations were measured by commercially available ELISA kits (R and D System, MN and USA). PON1 and arylesterase activities were measured by spectrophotometry. In addition, age, disease duration, disease activity (DAS28), CRP, anti-CCP, IgM rheumatoid factor and plasma lipid levels were also assessed. Arterial flow-mediated vasodilation (FMD), carotid intima-media thickness (cIMT) and arterial pulse-wave velocity (PWV) were assessed by ultrasound.

Results Anti-TNF treatment resulted in a significant decrease in the levels of chemerin ($p<0.001$) and adiponectin ($p<0.007$) after 12 months. There were no significant changes in the levels of other metabolic biomarkers. We found the following correlations between the baseline values: the PON1 levels correlated with the age of patients ($R=0.466$, $p=0.004$). The adiponectin correlated with the disease activity ($R=0.385$, $p=0.030$), HDL-C ($R=0.417$, $p=0.012$) and the triglyceride levels ($R=0.481$, $p=0.003$). The total cholesterol correlated with the PWV ($R=0.449$, $p=0.021$) and the levels of the LDL-C ($R=0.911$, $p<0.001$). The baseline triglyceride correlated with the IgM rheumatoid factor ($R=0.343$, $p=0.021$); and the levels of LDL-C correlated with the PWV values ($R=0.444$, $p=0.023$).

Conclusions Metabolic factors, such as certain adipokines, PON1 and arylesterase may play a role in oxidative stress and atherosclerosis associated with RA. Anti-TNF treatment may affect adipokine levels.

Acknowledgements The work is supported by the GINOP-2.3.2-15-2016- 00015/I-KOM TEAMING project.

Disclosure of interest None declared

P076

A BIOASSAY TO MEASURE TGF β ACTIVITY REVEALS DECREASED TGF β ACTIVITY IN SYSTEMIC SCLEROSIS SERUM

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10.1136/annrheumdis-2018-EWRR2018.93

Introduction Systemic sclerosis (SSc) is a severe disease characterised by auto-immunity, vasculopathy and excessive fibrosis of connective tissues. The pathophysiology of SSc is still poorly understood, but its symptoms imply a role for dysregulated transforming growth factor β (TGF β) signalling because this cytokine is known to regulate vascular and connective tissue biology. TGF β circulates in blood in an inactive latent form bound to latency associated peptide and latent TGF β binding proteins. This latent TGF β has to be activated before it can become bioactive. With the use of a bioassay TGF β 's bioactivity can be measured in complex mixtures like serum. This is not possible with an ELISA because this technique does not take (cellular) activation processes into account.

Objectives To determine the bioactivity of TGF β in SSc serum compared to that of healthy control serum.

Methods Serum was collected of 10 SSc patients and 10 age and sex matched healthy controls. Primary human fibroblasts

of 3 donors were transduced with CAGA₁₂-luc which produces luciferase in response to TGF β /Smad3 or BRE-luc which produces luciferase in response to BMP/Smad1/5. These cells were treated with 10% serum for 16 hour and luciferase activity was measured. To activate all TGF β , sera were treated with 4M HCl for 1 hour at RT, after which pH was normalised with 4M NaOH. Controls were treated with HCl and NaOH simultaneously. To verify that TGF β signalling was measured in this reporter assay, sera were treated with anti-TGF β 1/2/3 for 1 hour at RT before use.

Results Control sera significantly induced reporter activity by 4.5-fold. However, SSc sera only induced a 2.5-fold increase in luciferase activity, indicating significantly lower bioactivity of TGF β ($p<0.0001$). This difference was not due to a difference in total TGF β levels; after activation of all TGF β both HC and SSc sera induced a similar 6-fold increase in signal strength. These data show that in HC sera approximately 75% of all TGF β is bioactive compared to only 42% in SSc. Addition of anti-TGF β 1/2/3 inhibited all CAGA₁₂-luc reporter activity ($p<0.0001$) of both HC and SSc serum, and of both acidified and not acidified sera ($p<0.0001$), showing that our bioassay is indeed TGF β -dependent. To investigate if reduced bioactivity is a more general phenomenon we measured BMP activity. BMP proteins are structurally closely related to TGF β and also circulate in inactive form. Both HC and SSc sera induced a similar 8-fold increase in BRE-luc activity, and this activity was increased to a 16-fold induction after acidification for both groups. BMPs in SSc sera are thus not less bioactive. This illustrates the uniqueness of our observation on TGF β bioactivity.

Conclusions TGF β in SSc serum is less bioactive than in control serum whereas BMPs are not less bioactive.

Disclosure of interest None declared

P077

EFFECTS OF IL-17 AND THE HEPATOCYTE-MONONUCLEAR CELL INTERACTIONS IN THE HEPATIC INFLAMMATORY RESPONSE

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10.1136/annrheumdis-2018-EWRR2018.94

Introduction Interleukin-17A (IL-17), tumour necrosis factor- α (TNF α) and IL-6 are pro-inflammatory cytokines involved in many autoimmune and inflammatory diseases including rheumatoid arthritis. Because most acute-phase proteins are produced by the liver, the hepatic response plays an important role in systemic inflammation. By producing chemokines such as CCL-20 that attract Th17 cells, hepatocytes can recruit mononuclear cells in liver, which leads to chronic hepatitis.

Objectives To determine the effect of the IL-17/TNF α combination and the contribution of hepatocyte-peripheral blood mononuclear cell (PBMC) interactions in the hepatic inflammatory response.

Methods Human HepaRG cell line and primary human hepatocytes (PHH) were cultured with or without IL-6, anti-IL-6 receptor (anti-IL-6R) antibody, IL-17 and/or TNF α . For co-cultures, PBMC from healthy donors activated or not with phytohemagglutinin (PHA) were added on hepatocytes at a 5:1 ratio. Transwell system was used to study the contribution of the direct cell-cell contact. Cytokine expression and production were quantified by qRT-PCR and ELISA respectively and