(LRA n=20) refractory RA patients failing to respond to one or more biologics. Healthy control group (HC n=20) and additional comparable group of 20 early RA patients treated with methotrexate (MTX).

Results Our previous data evaluating IL-6 pathway (JAK-STAT and also, PI3K/Akt and MAPK/ERK) in T-, B- and monocyte cells showed that p-STAT3 is predominantly affected in CD4 +T cells.¹ Constitutively, p-STAT3 levels in CD4 +T cells were higher in later RA group (MFI:316±33.3) compared to ERA (MFI:296±40.96; p=0.057) and healthy individuals (285±21.6; p=0.01). Upon stimulation of the pathway using cis and trans IL-6 activation, there was little induction in the later RA patient cohort. Whereas early RA group showed a capacity for further activation of p-STAT3. Further analysis is currently being undertaken to understand the kinetics of this variability including response to treatment and biopsies of synovial tissue for phosphoprotein verification.

Conclusions Our results are in line with previous findings,^{2,3} there was a difference in p-STAT3 levels at baseline between early and later RA, and differential response to stimulus with IL-6. Investigation of early vs later RA biologic response profiles will enable us to better understand the multiple cytokine networks, their interaction, and how disease duration and therapy alters this.

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Disclosure of interest None declared

P044

TRANSGLUTAMINASE-2 IN OSTEOARTHRITIS: MMP-13 PRODUCTION THROUGH ENHANCED FOXO3A NUCLEAR TRANSLOCATION

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Introduction Transglutaminase 2 (TG2) also known as tissue transglutaminase, is a calcium-dependent enzyme that has a variety of intracellular and extracellular substrates. It has been well known that TG2 increases in osteoarthritis (OA) tissue and can be used as a biomarker of OA.

Objectives To elucidated the molecular mechanism of TG2 during the cartilage degradation in OA.

Methods The surgical destabilisation of the medial meniscus (DMM) model is used to induce OA in 10-week-old male C57BL/6J mice. Primary chondrocytes were obtained from E15.5 long bones, and ZDON, a cell-permeable, peptide-based, irreversible inhibitor of TG2, was used to inhibit the function of TG2 and calcium ionophore to stimulate TG2.

Results TG2 expression was increased in articular cartilage and growth plate in surgical OA model. When treated with various growth factors, only TGFβ1 increased TG2 expression of primary chondrocyte in a dose-dependent manner. Intra-articular injection of specific TG2 inhibitor, ZDON, ameliorated the severity and MMP-13 expression in surgically-induced OA. ZDON attenuated MMP-3 and MMP-13 expression in TGFβ- and calcium ionophore-treated chondrocytes in a Runx2-independent manner. TG2 activation by calcium ionophore

induced phosphorylation of FoxO3a and ZDON decreased total FoxO3a as well as nuclear FoxO3a level. FoxO3a and TG2 were co-localised in primary chondrocytes and immunoprecipitation analysis revealed a direct interaction of FoxO3a and TG2, suggesting enhanced nuclear translocation of FoxO3a by TG2.

Conclusions Our data provide an evidence of TG2 as an enhancer of FoxO3a-nuclear translocation which was responsible for the TG2-dependent MMP-13 expression.

Disclosure of interest None declared

P045

ABSTRACT WITHDRAWN

P046

ABSTRACT WITHDRAWN

P047

ANTI-COLLAGEN TYPE II ANTIBODIES ARE ASSOCIATED WITH EARLY INFLAMMATION IN MALAYSIAN RHEUMATOID ARTHRITIS PATIENTS WITH THREE DIFFERENT ETHNICITIES

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Objectives We have previously shown that Caucasian rheumatoid arthritis (RA) patients with anti-collagen type II antibodies (anti-CII) have an acute onset phenotype with elevated levels of C-reactive protein (CRP) and erythrocyte sedimentation rate, as well as higher disease activity score and number of swollen joints [Manivel et al. *Ann Rheum Dis* 2017 September;76(9):1529–1536]. Our aim was to replicate this in a multiethnic Malaysian RA cohort.

Methods Anti-CII, anti-CCP2, IgM RF and IgG RF were measured in 1,105 Malaysian early RA patients and 1565 healthy controls of Malay, Chinese or Indian ethnicity in the Malaysian Epidemiological Investigation of RA (MyEIRA) case control study, and related to baseline CRP and to HLA-DRB1* alleles.

Results 106/1,105 (9.6%) of the RA patients had elevated anti-CII. Anti-CII levels were higher in RA patients than in controls (p<0.0001), generally higher in Malay than in Indian or Chinese subjects, and also higher in Malaysian than in Swedish healthy controls. All measured autoantibodies associated with elevated baseline CRP. The occurrence of anti-CII did not associate with the occurrence of anti-CCP2, IgG RF and IgM RF, and when patients with only one antibody were compared to antibody negative patients, only anti-CII associated with elevated CRP (p=0.0310). HLA-DRB1*12:01 positive patients has higher levels of anti-CII (p=0.01) whereas Malaysian Malay patients with HLA-DRB1*12:02 had lower anti-CII levels (p<0.002) as compared to individuals lacking the corresponding genotype.

Conclusions Elevated anti-CII levels at the time of RA onset associate with an early inflammatory RA phenotype not only in Caucasian, but also in an Asian RA population. This supports our hypothesis that the association between early elevations of anti-CII and the acute onset RA phenotype is a finding of global validity.

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Disclosure of interest None declared

P048

ABSTRACT WITHDRAWN

P049

CHARACTERISATION OF THE ANTIBODY RESPONSE TO A CITRULLINATED PEPTIDE DERIVED FROM PORPHYROMONAS GINGIVALIS PAD IN RA

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Introduction Anti-citrullinated protein antibodies (ACPA) – a hallmark of rheumatoid arthritis – antedate joint inflammation. Based on the epidemiological association between RA and periodontal disease (PD),¹ it has been suggested that break of tolerance to citrullinated proteins may occur in the gum mucosa. This hypothesis is primarily based on the unique feature of *Porphyromonas gingivalis* (Pg) – a keystone pathogen in PD – to express a bacterial version of the peptidyl arginine deiminase enzyme (denoted PPAD), capable of autocitrullination.²

Objectives In the present study, we have investigated the antibody response against CPP3, a citrullinated peptide derived from PPAD, in order to address the hypothesis that Pg may drive ACPA-production.

Methods

This study included 2,859 RA cases and 4864 controls from the Epidemiological Investigation of RA (EIRA) cohort; 65 PD patients and 59 periodontally healthy individuals; and 218 monoclonal antibodies, derived from RA B cells. Reactivity with citrullinated Pg and human antigens was assayed by ELISA and/or multiplex. Associations with genetic risk factors and smoking were determined by logistic regression. Monoclonal antibody mutations were analysed by IgBLAST comparison. **Results** Anti-CPP3 IgG was detected in 11% of RA, 10% of PD and <2% of controls, with higher levels in RA compared to PD. These antibodies clustered outside the classical ACPA response, associated with smoking, but not with major genetic risk factors. Two CPP3-reactive monoclonal antibodies were identified; one which cross-reacted with citrullinated human vimentin and had extensive mutations, indicating antigen-driven clonal selection and affinity maturation.

Conclusions Based on these data, we propose that Pg infection triggers an antibody response to CPP3, which cross-reacts with citrullinated human proteins by mechanisms of molecular mimicry. Future studies should address whether anti-CPP3 IgG could serve as a biomarker to identify individuals with PD at increased risk for RA.

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Disclosure of interest None declared

P050

HIGH CHOLESTEROL LEVELS BY APOE DEFICIENCY REDUCE BONE DESTRUCTION IN ANTIGEN-INDUCED ARTHRITIS VIA REDUCTION OF THE NUMBER OF OSTEOCLASTS

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Introduction Rheumatoid arthritis (RA) is a chronic inflammatory disease characterised by immune complex (IC) deposition in the synovium, leading to increased bone destruction. In RA, joint destruction has been associated with high levels of low density lipoproteins (LDL) and enhanced LDL oxidation (oxLDL). Apolipoprotein E (ApoE) is an important regulator of LDL transportation and its absence strongly elevates circulating LDL levels, which may lead to increased oxLDL levels during inflammation.

Objectives In this study, we investigated the effects of high LDL levels on bone destruction during antigen-induced arthritis (AIA) and how increased LDL/oxLDL levels affect osteoclast formation.

Methods AIA was induced by injection of methylated BSA (mBSA) into the right knee joint of *ApoE*^{-/-} and wild type (WT) control mice previously immunised with mBSA and complete Freund's adjuvant (CFA). WT and *ApoE*^{-/-} Hoxb8 myeloid precursor cells were differentiated into osteoclasts using 20 ng/mL RANKL and 30 ng/mL M-CSF, then stimulated for 24 hour with 10 µg/mL LDL/oxLDL. Oil Red O staining was performed to assess lipid uptake by osteoclasts. mRNA levels of c-Fos, RANK, NFATc1, DC-STAMP, TRAP, CTR, CIC-7 and Cat K were measured by qPCR. Bone erosion was quantified by histological analysis using an arbitrary scale from 0 to 3 and TRAP⁺ cells were determined using immunohistochemistry.

Results *ApoE*^{-/-} mice showed significantly higher LDL serum levels than WT controls. At day 21 after AIA induction, bone erosion was significantly decreased in *ApoE*^{-/-} mice (25% reduction from 1.5±0.2 to 1.1±0.1). In line with that, the number of osteoclasts within the knee joints was 36% lower in *ApoE*^{-/-} mice, as determined by image analysis of TRAP staining. To study the role of ApoE and high LDL levels on osteoclastogenesis in more detail, we differentiated WT and *ApoE*^{-/-} myeloid precursor cells (Hoxb8) into osteoclasts and found similar mRNA levels of osteoclast markers. Whereas LDL stimulation did not affect osteoclast formation, oxLDL strongly impaired cell fusion keeping them in a mononuclear state. mRNA levels of DC-STAMP were significantly down-regulated in both WT and *ApoE*^{-/-} osteoclasts (1.4 and 2.3 fold decrease, respectively) as well as TRAP activity (49% and 58% reduction in WT and *ApoE*^{-/-} osteoclasts), underlining a major role of oxLDL in inhibiting osteoclastogenesis.