into the Risk-RA clinical research program, and followed-up by our multidisciplinary rheumatology team. A total of 64 patients with complete US records were included between years 2015–2016. Our patient demographics were 84% (n 54/64) females, mean age 49 (range 22–82) years, median relative ACPA (times cut-off) 26 (range 1–174) titer, median visual analogue scale (VAS) pain 30, median VAS patient global 28 and median C-reactive protein was 1 (0–20) at inclusion. Hand (Wrists, MCP's, PIP's, DIP's) and feet joints were US-evaluated for synovial hypertrophy, hyperemia and bone erosions. The presence of wrist (compartments 1–6) and finger (flexor and extensor) tenosynovitis were assessed. Data from inclusion→follow-up visits until September 2017 were analysed. SPSS software version 25 was used (Univariate, Chi-square, T-test and Mann Whitney U-test) for comparisons.

Results At inclusion, none of the 64 patients had any signs of active joint inflammation. However, ultrasound changes for tenosynovitis were seen in 7 out of 64 patients, 3 of who also presented with mild hypertrophy without Doppler activity and one patient with mild Doppler hyperemia (without hypertrophy), and none with bone erosions at inclusion. Among all tendons evaluated bilaterally, tenosynovitis of the Extensor Carpi Ulnaris (ECU) wrist tendons (4 of 7 patients) and the 2nd finger flexor-tendons (3 of 7 patients) were most commonly affected.

Of the 57 patients without US-tendon changes, one had mild Doppler hyperemia (without hypertrophy). Patients with US-tendon changes were 86% (n 6/7) females, had mean age 56 years, median VAS pain 42, median VAS global health 20, mean 2.7 mg/L CRP, median relative ACPA titer 70 in comparison to patients without US-tendon changes, 75% females, mean age of 48 years, median VAS pain 24, median VAS global health 28, mean 2.7 mg/L CRP and median ACPA titer of 23. The numerical difference in pain and relative ACPA titer were non-significant ($p>0.05$).

After follow up for mean 18 months (range 1–18), 7 out of 7 (100%) with US-tendon changes at inclusion and 18 out of 57 (32%) without US-tendon changes developed arthritis. Patients with US-tendon changes compared to those without tenosynovitis at inclusion, developed arthritis within 12 and 11 mean months follow-up, respectively.

Conclusions Our study shows that tenosynovitis is a specific marker for arthritis development in ACPA-positive patients with musculoskeletal symptoms. The role of inflammatory spreading from tendons (synovial sheath) to synovial tissue within joints need to be further investigated.

Disclosure of interest None declared

P020

TOLL-LIKE RECEPTOR 9 INFLUENCES INFLAMMATORY ARTHRITIS AND OSTEOCLASTOGENESIS

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Introduction Release and insufficient removal of endogenous nucleic acids may be involved in triggering autoimmune reactions important in the initiation of systemic autoimmune

diseases including rheumatoid arthritis (RA). Nucleic acid sensing molecules, such as the endosomal Toll-like receptors (TLRs) 7 and 9, have been linked to pathogenic autoimmune processes, but their role in RA is less clear.

Objectives To gain more insight into the role of TLR9 in autoimmune arthritis, TLR9 inhibition was investigated in rats with pristane-induced arthritis (PIA). To further investigate TLR9 involvement, streptococcal cell wall (SCW) arthritis was induced in TLR9^{-/-} mice.

Methods Arthritis was induced in mice with SCW lysates and in rats with the mineral oil pristane. Rats were treated with a TLR9 antagonist, starting before disease induction. Arthritis was scored using established scoring systems, inflammation and bone erosion was quantified by histological analysis of the paws. Levels of α -1-acid-glycoprotein (AGP), rheumatoid factor (RF) and IL-6 in sera were analysed. The role of TLR9 in osteoclast differentiation was investigated *in vitro*.

Results In PIA, which is T cell-dependent, the TLR9 antagonist reduced arthritis severity by ~50%. This was accompanied by a reduction of AGP, IL-6 and RF in the sera of these animals. In addition, TLR9 inhibition led to reduced inflammation, bone erosion and cartilage degradation in the paws. Moreover, the T cell-dependent chronic phase of SCW arthritis was significantly suppressed in TLR9^{-/-} mice. Remarkably, TLR7 and TLR9 mRNA levels strongly differed in the course of *in vitro* osteoclastogenesis. Whereas TLR7 expression did not change throughout osteoclastogenesis, expression of TLR9 was higher in precursor cells than in mature osteoclasts and stimulation with a TLR9 agonist (CpG) completely inhibited osteoclastogenesis.

Conclusions The results suggest a crucial role for TLR9 in the T cell-dependent phases of PIA and SCW arthritis and thus an important involvement of the DNA (CpG) recognising TLR9 in the induction of arthritogenic autoimmune reactions. In addition, TLR9 also seems to play a role in the initiation of osteoclast differentiation which needs to be further elucidated in future experiments.

Disclosure of interest None declared

P021

MEMBRANE TNF EXPRESSION ON MONOCYTES AND DIFFERENTIATION OF MONOCYTES INTO M2-M1 MACROPHAGES: 2 NEW BIOMARKERS OF RHEUMATOID ARTHRITIS

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Introduction Three monocyte subsets have been described based on their CD14 and CD16 expression profiles, the sub-population CD14⁺CD16⁺ being expanded in rheumatoid arthritis (RA) patients. Macrophages contribute *in situ* to the RA pathogenesis. They can display various states of activation or « polarisation ». Two distinct states of polarisation for macrophages have been recognised: the 'classically activated macrophage phenotype' (M1) and the 'alternatively activated macrophage phenotype' (M2). To sum-up, M1 are considered to be pro-inflammatory and M2 to be regulatory and anti-inflammatory.

Objectives Here, we have assessed monocytes subsets and their capacity of differentiation into M2 or M1 macrophages in RA patients and controls.

Methods PBMCs were isolated and we first determined monocytes subpopulations and looked at expression of membrane TNF (mTNF). After negative selection of monocytes, Macrophages were Derived from Monocytes(MDM) by 7 days of culture in the presence of M-CSF (M2 differentiation) or GM-CSF (M1 differentiation). Expression of total macrophages markers (CD11b and CD71) and the M2 macrophage polarisation markers (CD163 and CD206) were evaluated.

Results We have confirmed that the CD14⁺CD16⁺ monocytes subset was expanded in RA patients. For the first time, we have demonstrated that mTNF expression was significantly increased only in monocytes in RA patients (CD14⁺: Mean 5.6% HD versus 10.6% RA, CD14⁺CD16⁺: Mean 3.3% HD versus 11.1% RA, CD16⁺: Mean 2.4% HD versus 6.3% RA). Moreover, mTNF expression on monocytes correlated with the activity of the disease assessed by DAS28 CRP (Spearman r 0.548 and $p=0.0021^*$). We have observed a significant decrease of macrophages induction by M-CSF in RA patients as shown by a decreased expression of CD11b-CD71 (Mean 87.2% HD versus 57.1% RA, P value ***). Among MDM, we have found a specific decreased level of M2 markers (CD206 Mean 78.1% HD versus 27% RA and CD163 56.2% HD versus 37.2% RA) suggesting an impaired maturation to M2 stage in RA patients.

Conclusions Monocytes from RA patients have an increased expression of mTNF linked to activity of the diseases. RA patients have an impaired maturation of monocytes to M2 macrophages. This might suggest that RA monocytes have a propensity for preferential maturation towards a pro-inflammatory M1 phenotype thus contributing to synovial inflammation. Deeper characterisation of M1/M2 and effect of the different types of anti-TNF on this differentiation process are on-going and will be presented.

Disclosure of interest None declared

P022 CHECKPOINT INHIBITORS ACTIVATE STORE-OPERATED CA²⁺ ENTRY AND ERK1/2 SIGNALLING AND PROMOTE TH17 DIFFERENTIATION

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Introduction Anti-tumour therapy with immune checkpoint inhibitors for programmed-death 1 receptor (PD1), such as nivolumab is often accompanied by immune-related adverse events (irAE). The cellular and molecular mechanisms underlying this phenomenon are not defined yet. Interaction of PD-1 with its ligand (PD-L1) mediates potent inhibitory signals to hinder proliferation and effector function of T cells. Interruption of PD1:PD-L1 interaction by nivolumab during anti-tumour therapy might therefore amplify T cell receptor (TCR) signalling and facilitate the development of a pro-inflammatory autoimmune response.

Objectives To investigate the impact of PD1 inhibition on intracellular signalling mechanisms down-stream of the TCR such as calcium (Ca²⁺) influx and activation of mitogen-activated protein kinases (MAPK) pathway and to assess the effect of PD1 inhibition on T cell effector function by evaluating the cytokine profile of the nivolumab-treated T cells.

Methods CD4 memory T cells were stimulated with anti-CD3 and anti-CD28 in the presence of nivolumab for 24 hour.

Afterwards intracellular Ca²⁺ influx in response to ionomycin was assessed by flow cytometry following loading the cells with Fluo-8. Expression of proteins involved in store-operated Ca²⁺ entry (SOCE), stromal activation molecule (STIM) 1, an activator of Ca²⁺ release-activated Ca²⁺ (CRAC) channels, and Orai1, a component of CRAC channels was determined by TaqMan real-time PCR. Phosphorylation of extracellular signal-regulated kinase 1 and 2 (Erk1/2) in response to anti-CD3/28 was determined by intracellular flow cytometry. The cytokine profile of nivolumab-treated cells was assessed after four-day culture by intracellular flow cytometry.

Results Treatment of CD4 T cells with nivolumab led to a pronounced increase of the ionomycin-mediated Ca²⁺ influx. At the same time expression of SOCE proteins, STIM1 and Orai1, was significantly up regulated in the nivolumab-treated cells. Phosphorylation of Erk1/2 in response to short anti-CD3/CD28 restimulation was almost twice as high in CD4 T cells cultured with as without nivolumab. Finally nivolumab-treated cells contained higher frequencies of IL-17-producing T cells (Th17 cells).

Conclusions Interruption of PD1:PD-L1 interaction by nivolumab activate SOCE and promotes Erk1/2 activation. Both T cell signalling pathways are essential for a proper mounting an immune response. Their deregulation might therefore precede an abnormal T cell response as shown for example by increased Th17 cell frequency and facilitate the onset of autoimmune phenomena such as irAE.

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Disclosure of interest None declared

P023 SMOKING IS ASSOCIATED WITH LOW SERUM LEVELS OF SOLUBLE PD-L1 IN RHEUMATOID ARTHRITIS

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Introduction Smoking is a risk factor for the development of rheumatoid arthritis (RA) and associates with positivity for disease specific anti cyclic citrullinated peptide (aCCP) antibodies. It is not known exactly how smoking promotes autoimmunity but we have previously demonstrated that smoking limits the expression of the T cell co-inhibitory receptor programmed death-1 (PD-1).¹

Objectives To investigate if smoking can interfere with the interaction between PD-1 and its ligand by influencing the serum levels of soluble PD-1 ligand 1 (sPD-L1).

Methods Serum samples were collected from 254 RA patients and 168 healthy controls with known smoking status and analysed with ELISA for levels of sPD-L1 and inflammatory cytokines IL-6 and IL-1b. Fc-receptor mRNA expression analysis of peripheral blood monocytes (PBMC) from a group of 10 healthy controls and 15 RA patients was done by qPCR.

Results In RA patients current smokers had 5 times lower median serum levels of sPD-L1 compared to never smokers ($p=0.027$). This difference persisted in former smokers that quit smoking <25 years ago ($p=0.0086$). Expectedly, sPD-L1