different anti-inflammatory treatment, stronger in RA than OA patients, with resulting poor control of inflammation in OA.

REFERENCES

1. Kontny E, et al. *Ann Rheum Dis* 2012;**71**(2):262–267.
2. Kontny E, et al. *Rheumatology* 2013;**52**:2158–2167.

Acknowledgements This work was funded by the National Science Centre (grant number 2012/N/NZ5/00074).

Disclosure of interest None declared

P065

INSULIN-LIKE GROWTH FACTOR 1 RECEPTOR REGULATES THE PHENOTYPE AND FUNCTION OF CD21+ B CELLS

M Erlandsson*, C Wasen, G Gravina, MI Bokarewa. *Rheumatology and Inflammation Research, Gothenburg University, Gothenburg, Sweden*

10.1136/annrheumdis-2018-EWRR2018.84

Introduction Ligand to the inducible T cell costimulator (ICOSL) on B cells is essential for the ICOS-dependent follicular recruitment of activated T cells. In patients with Rheumatoid arthritis (RA) the IGF1-IGF1R axis is altered. Inhibition of IGF1R alleviated arthritis by reducing IL-6-dependent formation of Th17 cells. Here we study the role of IGF1R on CD21 +cells in experimental arthritis.

Methods Female Balb/c mice were immunised with methylated BSA or with CII. Consequences of the IGF-1R inhibition for arthritis were studied in mBSA and CII-immunised mice treated with NT157 compound promoting degradation of insulin receptor substrates (mBSA) or using shRNA producing construct (mBSA +CII). At termination three sub-populations of CD21 +cells were analysed: follicular dendritic cells (FDc, CD21 +CD19-CXCR5-); marginal zone B cells (MZBc, CD21 +CD19+CXCR5-); follicular B cells (FBc, CD21 +CD19+CXCR5+). Supernatants of LPS-stimulated splenocytes and serum were analysed for production of antigen specific and autoantibodies.

Results In spleen of mBSA-immunised mice, ICOSL expression on CD21 +cells correlated to IGF1R ($r=0.70$). Inhibition of IGF1R induced a 20% reduction in ICOSL expression in all CD21 +subsets followed by an increase in MZBc ($p=0.003$), while FDc and FBc were unchanged. ICOSL +MZBc were mostly IgMhi, while ICOSL +FBc were mostly IgDhi. Inhibition of IGF1R had no effect on the expression of ICOS +on CD4 T cells and the subset of CXCR5 +follicular T cells. Reduction of the ICOSL +CD21 +B cells, reduced production of mBSA-specific IgM and increased production of autoreactive RF-IgM levels.

Conclusions The study shows that IGF1R controls expression of ICOSL on CD21 +cells. ICOSL on MZBc is required to balance between antigen-specific response and autoantibody production.

Disclosure of interest None declared

P066

ABSTRACT WITHDRAWN

P067

IL-17 RESULTING FROM CELL INTERACTIONS DURING CHRONIC INFLAMMATION: COMPARISON BETWEEN JOINT-DERIVED- AND SKIN-DERIVED-MESENCHYMAL CELLS

M Noack*, P Miossec. *Immunogenomic and Inflammation Unit, Hospital Edouard Herriot, University Claude Bernard Lyon 1, Lyon, France*

10.1136/annrheumdis-2018-EWRR2018.85

Introduction IL-17, mainly produced by Th17 cells, is a major pro-inflammatory cytokine involved in several chronic inflammatory diseases. Furthermore, during chronic inflammation, immune cells, notably Th17 cells, migrate to the inflammatory site, synovium or skin for example, and interact with the local mesenchymal cells (synoviocytes or skin fibroblasts).

Objectives The aim is to study and compare the effect of cellular interactions between immune cells and mesenchymal cells (MC) from different origins on pro-inflammatory cytokine production, with a focus on IL-17, and to identify the involved mechanisms.

Methods A co-culture system with MC (synoviocytes or skin fibroblasts) and PBMC was developed. MC were cultured overnight and PBMC were seeded at a 5:1 ratio for 48 hour, in the presence or not of TCR activation with PHA. Transwell system was used to study cell-cell contact. Monocytes were removed to study their involvement. An antibody against podoplanin was pre-incubated with PBMC before co-culture. Supernatants were collected at 48 hour and IL-6, IL-1 β and IL-17 production measured by ELISA. Extracellular (CD3, CD4) and intracellular (IL-17) staining of PBMC was analysed by flow cytometry.

Results In control conditions, IL-6 and IL-1 β production was increased more than 20 fold and 10 fold respectively, in PBMC-MC co-culture compared to PBMC alone ($p\leq 0.05$). No additional effect was observed with PBMC activation. Flow cytometry showed no significant difference in the percentage of Th17 cells in activated-PBMC alone or co-cultured with MC ($p=0.4$). Conversely, IL-17 production was highly increased at least 10 fold only in co-cultures with activated-PBMC ($p\leq 0.02$). Transwell experiments confirm that cell-cell contact was critical for IL-17 secretion. The removal of monocytes highly decreased the IL-1 β production by 80%–90% ($p\leq 0.05$) with both MC, while the IL-17 secretion was decreased with skin fibroblasts by 60% but not with synoviocytes. The inhibition of podoplanin, interaction molecule involved in the modulation of IL-8 secretion during synoviocyte-platelet interactions, was tested. The addition of an anti-podoplanin antibody decreased significantly IL-17 secretion by 60%, similarly with skin fibroblasts and synoviocytes.

Conclusions Cellular interactions between mesenchymal cells and immune cells play a major role in the pro-inflammatory cytokine production, leading to a heightened IL-17 secretion. The podoplanin molecule seems play a crucial role in this mechanism. Nevertheless, the inhibition of IL-17 remains only

partial that means podoplanin is one of the involved mechanisms but others have to be identified.

Disclosure of interest None declared

P068 INCREASE OF AEROBIC GLYCOLYSIS MEDIATED BY ACTIVATED T HELPER CELLS DRIVES SYNOVIAL FIBROBLASTS TOWARDS AN INFLAMMATORY PHENOTYPE

¹P Kvacskay*, ¹M Souto-Carneiro, ²R de Albuquerque Carvalho, ¹J-H Schnotz, ¹S Krienke, ³KD Klika, ¹T Tretter, ¹H-M Lorenz, ¹L-O Tykocinski. ¹Internal Medicine V – Division of Rheumatology, University Hospital Heidelberg, Heidelberg, Germany; ²NMR Centre, Department of Biochemistry, University of Coimbra, Coimbra, Portugal; ³Molecular Structure Analysis, German Cancer Research Centre (DKFZ), Heidelberg, Germany

10.1136/annrheumdis-2018-EWRR2018.86

Introduction There is growing evidence for a dysregulated glucose metabolism of synovial fibroblasts (SF) in rheumatoid arthritis (RA) being a prerequisite for their aggressive phenotype. As yet, little is known about the influence of immune cells on the metabolism of SF although local infiltration of leucocytes constitutes a hallmark in the pathogenesis of RA.

Objectives In this study, we investigated the effect of T helper (T_H) cells on the glucose metabolism and cytokine production of non-inflammatory and inflammatory SF *in vitro*.

Methods RASF as well as SF from patients with osteoarthritis (OA) were cultured in the presence of a stable glucose isotope (U-¹³C) and stimulated with culture supernatants (SN) of activated T_H cells. Lactate production was measured by proton nuclear magnetic resonance spectroscopy (H-NMR). Secretion of interleukin (IL)–6, IL-8 and matrix metalloprotease (MMP)–3 was quantified by ELISA. The expression of hexokinase (HK)–II, pyruvate kinase (PK)–M2 and pyruvate dehydrogenase (PDH) was analysed by PCR, western blot and immunofluorescence. Janus kinases (JAKs) were blocked by Baricitinib or Tofacitinib, glycolysis was inhibited by 3-Bromopyruvate (3 BP) or Fx11.

Results In the absence of stimulation, RASF showed a significantly higher lactate production and IL-6 and MMP-3 secretion compared to OASF. Stimulation by T_H cell SN strikingly changed the metabolic profile of both RASF and OASF by inducing a shift towards aerobic glycolysis with strongly increased lactate production. In parallel, a significant increase in IL-6 and MMP-3 secretion was induced. Interestingly, Baricitinib and Tofacitinib as well as glycolytic inhibitors significantly reduced both the production of lactate and the secretion of inflammatory cytokines. Finally, perpetual stimulation of non-inflammatory OASF by T_H cell SN triggered an inflammatory phenotype characterised by significantly higher amounts of lactate, IL-6 and MMP-3 compared to non- or single stimulated SF.

Conclusions Chronic stimulation of non-inflammatory SF by activated T_H cells provoked an aggressive phenotype with strongly increased glycolytic activity and upregulated secretion of inflammatory cytokines. This was blocked by both JAK- and glycolytic inhibitors. These observations suggest that the T_H cell-mediated metabolic switch towards aerobic glycolysis in SF is an important step in the pathogenesis of RA. Targeting this mechanism could provide a new strategy in the therapy of RA.

Disclosure of interest None declared

P069 S100A9 MEDIATES ACUTE NOCICEPTIVE PAIN IN EXPERIMENTAL SYNOVITIS

A Blom, M van den Bosch, E Geven, E Blaney Davidson, P van der Kraan, P van Lent*. *Experimental Rheumatology, Radboud University Medical Centre, Nijmegen, Netherlands*

10.1136/annrheumdis-2018-EWRR2018.87

Introduction Synovitis-associated pain is an important aspect of arthritis pathology. Several inflammatory mediators released by the synovium have been implicated in the regulation of pain, including S100A8 and S100A9 which may regulate pain either via direct stimulation of TLR4 on the nerve endings in the synovium or via stimulation at the site of the dorsal root ganglia (DRG), thereby enabling an increased phagocyte infiltration, which may cause sensitisation.

Objectives To elucidate the role of S100A9 in the pain response after induction of an acute synovitis using streptococcal cell walls (SCW) as a trigger, comparing S100A9^{-/-} mice and their WT controls.

Methods Acute synovitis was induced by a single i.a. injection of SCW in the knee joint of C57Bl6 (WT) mice and S100A9^{-/-} mice, control mice received an i.a. saline injection. Serum S100A8/A9 levels were investigated by ELISA. Joint swelling and cell influx was assessed by ^{99m}Tc accumulation and histology. Pain response were investigated using an Incapacitance Tester (weight bearing), Catwalk (gait analysis) and von Frey's filaments (mechanical allodynia). Gene expression of inflammatory mediators and neuron activation markers in DRG were determined by q-PCR. Monocyte influx and protein expression was monitored by immunohistochemistry (IHC).

Results A single i.a. injection of SCW resulted in increased synovial expression of S100A8 and S100A9 and subsequent increased serum S100A8/A9 levels at day 1, which returned to basal levels at day 7. Joint swelling and cell influx were similar in WT and S100A9^{-/-} mice at day 1 day excluding a role for S100A8/9 in the level of synovitis. WT mice showed a marked and significant decrease in percentage of weight bearing on the SCW injected hindpaw (28%) compared to saline injection (47%, p<0.001) at day 1, whereas S100A9^{-/-} mice did not. In addition, the stand-phase of the unaffected paws were significantly increased in WT mice 1 day p.i., while in S100A9^{-/-} mice these parameters were not altered. Both mouse strains showed a similar reduction of paw withdrawal threshold, excluding a role for S100A8/9 in allodynia. Analysis of DRG showed no increased phagocyte infiltration after SCW injection and no change in gene expression of MCP-1, KC, IL-1β or TNF was measured. In addition, no change in F4/80 staining was seen in both WT and S100A9^{-/-} mice. However, expression of neuron activation markers NAV1.7, ATF3 and GAP43 were significantly increased at 1 day after SCW injection in WT mice as compared to saline injected mice (p=0.022, 0.004 and 0.030, respectively) while no regulation was found in S100A9^{-/-} mice, which is in line with the reduced pain response observed earlier in S100A9^{-/-} mice. The difference in NAV1.7 expression in the DRG was further confirmed at protein level with IHC.

Conclusions These findings show that S100A9 is an important mediator of inflammatory nociceptive pain response in the knee, rather than being involved in peripheral sensitisation. During the acute phase of inflammation S100A8/A9 is likely regulated via direct activation of TLR4 on nerve endings in the synovium and not via monocyte infiltration in the DRG.

Disclosure of interest None declared