Conclusions: The study showed that exosomes in the serum of RA patients can prohibit the apoptosis of FLSs and enhance the secretion of inflammatory cytokines to promote bone destruction. Exosomes play an important role in the pathogenesis of RA. Exosomes can be used as a potential predictor for early bone destruction.

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Acknowledgements: This work was supported by grants from Top Six Types of Talents Financial Assistance of Jiangsu Province Grant (BRA2016527), The Natural Science Foundation of China under Grant (81671616), The Natural Science Foundation of China under Grant (81471603).

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.5065

THU0060

ALTERATIONS OF SPLICING IN LEUKOCYTES FROM RHEUMATOID ARTHRITIS PATIENTS AND ITS INFLUENCE ON THE AUTOIMMUNE, INFLAMMATORY AND ATHEROTHROMBOTIC PROFILE OF THE DISEASE. POTENTIAL ROLE OF U4ATAC

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Objectives: The aim of this study was the identification, in leukocytes of Rheumatoid Arthritis (RA) patients, of the alterations present in the spliceosome and the machinery responsible for the splicing, as well as their influence on the activity of the disease and its atherothrombotic profile.

Methods: We evaluated, using a microfluidic qPCR array (Fluidigm), a set of 45 elements of the splicing machinery: the complete major and minor spliceosome components and a series of splicing factors with potential pathological role. Monocytes, neutrophils and lymphocytes of purified from 74 RA patients and 29 healthy donors (HD) were assessed. In parallel, extensive clinical/serological evaluation, and correlation and association analyses were carried out.

Results: A significant alteration in several components of the spliceosome was observed in all the three leukocyte subtypes from RA patients compared to HD. Various spliceosome components were specifically altered in different leukocyte subtypes; it should be noted that a general downregulation was observed. Likewise, it was striking that 7 elements, including two small nuclear RNA (snRNA) of the major spliceosome (U1 and U5), the snRNA of the minor spliceosome, U4atac, and the splicing factors RBM3, RBM17, SAM68 and SRSF10 showed the same alteration pattern: all significantly reduced in the 3 leukocyte subtypes of patients with RA, except for U4atac, which was consistently over expressed and virtually absent in HD leukocytes. Although this process needs further analysis, it is likely that the overexpression of U4atac could interfere in the normal functioning of the major spliceosome, by binding to U5, thus altering the splicing of most introns (>99%), favouring non-canonical splicing, and generating aberrant proteins involved in the development of this pathology. Correlation and association studies showed a significant association between the expression levels of the 7 splicing factors cited and several clinical/serological parameters, including the activity of the disease, the positivity for anti-CCP and RF antibodies, and the expression of different inflammatory mediators. Likewise, reduced values of other splicing factors, differentially deregulated in the three leukocyte subtypes, were associated with radiological involvement, as well as with the presence of atheroma plaques, hyperlipidemia and arterial hypertension.

Conclusions: We have identified specific alterations in the splicing machinery of leukocytes from RA patients, associated with the activity of the disease, as well as with its inflammatory and atherothrombotic profile. Altogether, the generalised reduction of multiple elements of the splicing machinery and the consistent elevation of U4atac will deem necessary to examine the possible role of this snRNA in the alteration of the spliceosome in the near future, as well as its specific implication in the regulation of the expression of key proteins in the pathology of RA.

Acknowledgements: Junta de Andalucía (CTS-7940) and ISCIII (PI15/01333 and RIER RD16/0012/0015) co-funded with FEDER funds

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.6469

THU0061

RESTRICTED AXL SYNOVIAL EXPRESSION AND INCREASED CLEAVAGE OF AXL ECTODOMAIN CORRELATE WITH HIGHLY INFLAMED SYNOVITIS AND MORE SEVERE RHEUMATOID ARTHRITIS

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Background: Tyrosine kinase Axl, member of the TAM family, is expressed by antigen presenting cells and behaves as a negative regulator of the inflammatory cascade. Inflammatory stimuli up-regulate Axl expression in bone-marrow-derived macrophages¹. Soluble (s) Axl, generated by ADAM10, is a potent decoy for the TAM-ligand Gas6 and can impair TAM axis activation in lupus². In rheumatoid arthritis (RA) dendritic cells, Axl is epigenetically down-regulated³. Emerging evidence has emphasised the significant role of Axl/sAxl in the pathogenesis and progression of autoimmune diseases, but little is known about TAM expression and regulation within the rheumatoid synovium.

Objectives: We aimed to quantify Axl/ADAM10 in synovial tissue (ST) and sAxl/Gas6 in synovial fluid (SF) and to correlate Axl/sAxl expression with synovial inflammation and disease severity in RA patients.

Methods: ST/SF were sampled from early treatment-naïve RA patients undergoing ultrasound (US)-guided synovial biopsy of the most inflamed accessible joint. RA was diagnosed according to ACR/EULAR2010 criteria. The Krenn's synovitis score of inflammation was determined by H and E. Immunohistochemistry (IHC) staining of CD3/CD20/CD138/CD68 allowed to define the synovial immune infiltrate. Axl/ADAM10/CD68 were assessed in ST by IHC/immunofluorescence (30 patients). sAxl/Gas6 were quantified in 17 SF by ELISA. Synovial gene expression data were obtained by next-generation sequencing (80 patients).

Results: Axl was predominantly expressed only by CD68-positive macrophages of the lining and co-localised with ADAM10. AXL synovial mRNA significantly negatively correlated with the Krenn's score and with the degree of infiltration by B/T lymphocytes, plasma cells, and sub-lining macrophages, but not lining macrophages. In SF, sAxl positively correlated with Gas6 and was significantly more abundant in patients with highly inflamed synovitis and more active disease. AXL synovial gene expression showed strong negative correlation with indexes of disease severity, including ESR, CRP, DAS28, and US synovial thickening/power-doppler, and with the mRNA of pro-inflammatory cytokines, e.g. TNF and IL6.

Conclusions: Our data demonstrate that, despite the highly-inflamed environment characterising RA synovia, Axl expression is restricted to the synovial lining, where it can be cleaved and released into the synovial fluid by ADAM10. There, sAxl can bind Gas6 with high affinity, preventing its interaction with transmembrane functional TAM receptors. Raised levels of sAxl and down-regulated AXL mRNA expression correlate with highly inflamed synovitis and more severe disease. Defects in the TAM system could provide an original mechanistic explanation of the persistent inflammation in the RA joints, representing a novel therapeutic target exploitable to regain tissue homeostasis.

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Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.6988

THU0062

WNT5A INVOLVEMENT IN MIGRATION, INVASION AND THE PRO-INFLAMMATORY PHENOTYPE OF RHEUMATOID SYNOVIOCYTES

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Background: Fibroblast-like synoviocytes (FLS) are pivotal in the inflammation and joint damage of rheumatoid arthritis (RA). These cells acquire an aggressive phenotype; they migrate and invade articular structures perpetuating synovial inflammation. Also, they contribute to cartilage and bone damage by secretion of cytokines, metalloproteinases and cathepsins. The mechanisms modulating migration and invasion of FLS are not yet completely known. Recently, the role of the non-canonical pathway of Wnt5a has been highlighted in these processes, as well as, its contribution to osteoclastogenesis. Moreover, Wnt5a could be involved in other pathogenic aspects of RA, as suggested by its involvement in tissue

inflammation through regulation of cytokine, chemokine and metalloprotease expression. Thus, the study of the non-canonical pathway of Wnt5a in the aggressive phenotype of FLS and the inflammatory response may provide new therapeutic targets for the RA.

Objectives: To analyse the involvement of Wnt5a in the proliferation, migration, invasion and inflammatory responses of FLS from RA patients.

Methods: FLS were obtained from eight RA patients. Expression of Wnt5a was suppressed by siRNA transfection (Dharmacon). Cellular proliferation was determined using the CellTiter-Glo Luminescent Cell Viability Assay (Promega). Migration was analysed by the wound-healing assay using Ibidi inserts. The occupation area was determined by Image J. Invasion was tested by quantifying Giemsa stained cells on the bottom side of Matrigel coated trans-membrane (Millipore). The expression of inflammatory mediators and metalloproteases was analysed by real-time PCR after stimulation with recombinant Wnt5a protein (rWnt5a), TNF and combined treatment.

Results: We analysed the effect of the absence of Wnt5a and rWnt5a on the proliferation, migration and invasion of RA FLS. FLS lacking Wnt5a showed a significant migration decrease, 27.9% in basal condition and 16.9% after TNF stimulation, when compared with FLS transfected with siRNA control. Accordingly, migration of RA FLS treated with rWnt5a was increased by 48.5% when compared with cells treated with the vehicle. No change in migration was observed in TNF-stimulated FLS. Invasive capacity was reduced, reaching 71.4% of that observed in FLS transfected with siRNA control in basal conditions. In addition, invasion in FLS treated with rWnt5a was 35.7% higher than in controls. We also analysed the effect of rWnt5a on the inflammatory response of RA FLS. We found a significant increase of IL6, IL8, CCL2, CXCL5, MMP9 and MMP13 basal expression and the TNF-induced expression of IL6, IL8, CXCL5 and MMP3. Nevertheless, the lack of Wnt5a or the addition of rWnt5a did not modify the spontaneous or the TNF-induced proliferation.

Conclusions: These results indicate that Wnt5a contributes to the aggressive phenotype of FLS by potentiating their migration and invasion, and by stimulating the inflammatory response.

Acknowledgements: Supported by grant of the ISCIII/P14/01660/RETICS Program, RD16/0012/0014/cofinanced FEDER.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.4538

THU0063 CD11c+ DENDRITIC CELLS IN INFLAMMATORY ARTHRITIS

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Background: Dendritic cells (DCs) are important antigen presenting cells (APCs) and therefore they play an important role in bridging the innate and the adaptive immune response. DCs can be divided in different subsets with specific functions. As powerful APCs, DCs are thought to play an important role in the induction of autoimmune diseases such as rheumatoid arthritis. However, the active role of DCs in joint inflammation is not known yet.

Objectives: We analysed histological sections of K/BxN serum transfer arthritis as well as hTNFtg arthritis for the presence of CD11c+cells by immunohistochemistry. We also performed synovial biopsies and analysed the cellular composition of the inflammatory infiltrate with respect to DCs. We used CD11c-diphtheria toxin receptor (DTR) transgenic mice, which express the human diphtheria toxin receptor under the CD11c promoter, allowing for specific depletion of CD11c+cells by administration of diphtheria toxin (DT). K/BxN serum transfer arthritis was induced, and mice were given either DT or PBS or in wt and BARF3 deficient mice. In addition CD11c DTR mice were crossed into hTNFtg animals and also received either DT or PBS. The severity of arthritis was determined clinically and histologically.

Methods: We analysed histological sections of K/BxN serum transfer arthritis as well as hTNFtg arthritis for the presence of CD11c+cells by immunohistochemistry. We also performed synovial biopsies and analysed the cellular composition of the inflammatory infiltrate with respect to DCs. We used CD11c-diphtheria toxin receptor (DTR) transgenic mice, which express the human diphtheria toxin receptor under the CD11c promoter, allowing for specific depletion of CD11c+cells by administration of diphtheria toxin (DT). K/BxN serum transfer arthritis was induced, and mice were given either DT or PBS or in wt and BARF3 deficient mice. In addition CD11c DTR mice were crossed into hTNFtg animals and also received either DT or PBS. The severity of arthritis was determined clinically and histologically.

Results: We show that CD11c+cells are present in significant numbers in the synovia of K/BxN and TNF driven arthritis. Both CD8 +CD11c+and CD11b +CD11c+, can be found in synovial tissue. Upon depletion of CD11c+cells clinical signs of K/BxN serum transfer arthritis were significantly reduced. Histological analysis found reduced synovial inflammation after the depletion of CD11c+cells in K/BxN arthritis. In addition, local bone destruction and the number of

osteoclasts was also significantly reduced. Analysis of K/BxN arthritis in wt mice and BATF3^{-/-} mice, which lack a subset of DCs, namely CD8 +CD11+DCs, revealed no difference in arthritis severity between the two groups. In addition to K/BxN arthritis, we found that also in TNF-driven arthritis depletion of CD11c+cells led to a striking reduction of synovial inflammation and a complete depletion of osteoclasts.

Conclusions: These data show that in addition to initiating an adaptive immune response, CD11c+dendritic cells, are also involved in innate effector mechanisms of inflammatory arthritis. Especially CD11b+CD11c+and monocyte derived inflammatory seem to play a role in inflammatory arthritis, suggesting that they could be an important therapeutic target for patients suffering from inflammatory arthritis.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.7320

THU0064 ANTIBODIES AGAINST CARBAMYLATED PROTEINS FROM PATIENTS WITH RHEUMATOID ARTHRITISACTIVATE ENDOTHELIAL CELLS

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Background: Inflammation contributes to the excess of cardiovascular morbidity in rheumatoid arthritis (RA), by promoting endothelial activation; this brings toward the production of adhesion molecules and the activation of signalling mediators. Antibodies against carbamylated proteins (anti-CarP) detected in RA patients correlate with subclinical atherosclerosis.

Objectives: Aims of the present study were: 1) to determine the effect of anti-CarP antibodies purified from the sera of RA patients, on the production of VCAM1 and ICAM1 as well as the activation of IRAK1 and NF-kB by human endothelial cell line EAhy926. 2) To evaluate endothelial cell apoptosis induced by anti-CarP.

Methods: An indirect ELISA was used to detect the presence of anti-CarP in the sera of RA patients. To purify anti-CarP, carbamylated-FCS used as an antigen was spotted onto a nitrocellulose filter and incubated with patient's sera that recorded the highest titer. Antibodies were eluted with glycine 100 mM, pH 2.5 and neutralised with Tris-HCl 1M, pH 8. Antibodies concentration was measured by using a colorimetric Bradford assay. The immortalised hybridoma cell line EAhy926 was cultured in Dulbecco's Modified Medium containing 10% fetal bovine serum, 1 mM l-glutamine, 100 U/ml penicillin and 10 ml HAT. After cell stimulation with purified anti-CarP at different time points (30 min-48 h) and different concentrations (5–20–50 µg/ml), supernatants were gathered to investigate the production of VCAM-1, ICAM-1 using commercial ELISA kits while activation of IRAK1 and NF-kB was detected by Western Blot analysis using cell lysates. Apoptosis was measured using FITC-conjugated annexin V (AV) and a propidium iodide (PI) apoptosis detection kit at different times (30 min-48 h).

Results: After EAhy926 stimulation with anti-CarP we observed: 1) induction of VCAM-1 but not ICAM production in cell supernatants; 2) activation of IRAK1 and NFkB transcription factor in cell lysates and 3) induction of endothelial cell apoptosis.

Abstract THU0064 – Figure 1. Expression of adhesion molecules (A, B), activation of IRAK1 (C), NFkB (D) signalling and apoptosis (E) in EAhy 926.

Conclusions: These data suggest a potential involvement of anti-CarP antibodies in endothelial cell activation allowing to further speculate on its possible effect in excess cardiovascular risk in RA patients.

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