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.

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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+CD11c+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

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THU0064 ANTIBODIES AGAINST CARBAMYLATED PROTEINS FROM PATIENTS WITH RHEUMATOID ARTHRITIS ACTIVATE 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-κB 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-κB 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 NFκB transcription factor in cell lysates and 3) induction of endothelial cell apoptosis.

Abstract THU0064 – Figure 1. Expression of adhesion molecules (A, B), activation of IRAK (C), NFκB (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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