functions. The "classically activated macrophage phenotype" is considered to be pro-inflammatory and contribute to RA pathogenesis by secreting pro-inflammatory cytokines. The "alternatively activated macrophage phenotype" is considered to be regulatory and anti-inflammatory in tissues. Actually, there is a continuum from pro-inflammatory to anti-inflammatory macrophages, with high plasticity between the different states. We have previously shown that RA patients, and not patients with other inflammatory rheumatic diseases, have an impaired maturation of monocytes in anti-inflammatory-macrophages with increased differentiation in a pro-inflammatory-phenotype. We have found that an increased expression of miR-155 in monocytes/macrophages could be responsible for this defect and thus, could represent a new therapeutic target in RA ([2]).

Objectives: Our aim is to assess if the defect of monocytes polarization in anti-inflammatory-macrophages and the impact of miR-155 in this defect are present in 2 pre-clinical models of RA: the CIA (collagen-induce-arthritis) and STA mice (Serum transfert arhritis), both in which macrophages infiltration of synovium play a key role in pathophysiology. Then, we have test a new therapeutic strategies to correct this defect using PEG-liposomes containing antagomiR-155-5p.

Methods: AntagomiR-155-5p or antagomiR-control were encapsulated in PEG-liposomes of 100 nm in size and -10mV in zeta potential with high antagomiR loading efficiency (above 80%). Mice were injected intravenously with 1,5nmol/100µL PEG-liposomes containing antagomiR-155-5p or control after induction of arthritis.

Results: As in humans, we found that monocytes defect in anti-inflammatory-macrophages was associated with an increase of miR-155-5p in both mouse models. Moreover, we demonstrated the biodistribution of tagged-PEG-liposomes to inflamed joints 1 hour after injection and as well as their subsequent liver's accumulation after 48 hours, indicative of hepatic clearance, in arthritic mice. Subsequently, we demonstrated that treatment with an antagomiR-155-5p encapsulated in PEG-liposomes was able to decrease joint inflammation, to restore bone-marrow monocytes polarization in anti-inflammatory-macrophages, to reduce immune cells infiltration in synovial tissues, to increase the CD206$^+$ and CD163$^+$ tissue infiltrating macrophages and to decrease expression of mRNAs target of miR-155-5p, without any significant functional change in other immune cells including splenic B and T cells.

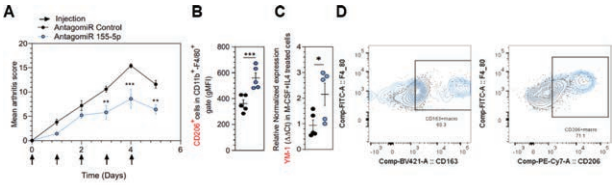

Figure 1: Treatment of STA mice with PEG-liposomes containing antagomiR-control or antagomiR-155-5p. For STA mice, antagomiR-control or 155 entrapped in PEG-liposomes are intravenously injected on days 0, 1, 2, 3, and 4. Mice were evaluated every 2-day, based on a visual scoring scale of the paws with evaluation of the erythema, swelling and ankylosis of each paw (score 1 to 4) **(A)**. The differentiation of sorted bone-marrow monocytes to BMDM-M2 was assessed by flow cytometry with pan-macrophages markers and specific markers of M2 macrophage (CD206) **(B)** and YM-1 relative expression **(C)**. Synovial tissue cells were assessed by flow cytometry analysis using anti-CD45, DUMP channel: CD19-CD3-NK1.1-LY6G, anti-CD11b, anti-F4/80, anti-MHCII, anti-CD64, anti-LY6C, anti-MerTK, anti-CD163, anti-CD206 and anti-CX3CR1. Determination of tissue infiltrating CD206$^+$ or CD163$^+$ macrophages in synovial tissue **(D)**.

Conclusion: The injection of antagomiR-155-5p encapsulated in PEG-liposomes allows delivering small RNA to monocytes/macrophages, lead to reduce joint inflammation in murine models of RA, providing a promising strategy in human disease.

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OP0208 MECHANICAL UNLOADING PREVENTED ARTHRITIS IN THE RAT ADJUVANT-INDUCED ARTHRITIS MODEL

Keywords: Rheumatoid arthritis, Animal Models

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Background: Rheumatoid arthritis (RA) is the most common chronic inflammatory rheumatic disease, characterized by synovitis associated with progressive bone loss and joint swelling. The adjuvant-induced arthritis (AIA) model mimics the pathophysiological features of RA, with a very high prevalence and reproducibility. YAP plays a key role in synovial hyperplasia. It is a transcription factor which can be activated in response to inflammation but also by a mechanical stimulus as tissue stiffening. We already showed a decrease arthritis severity by inhibiting YAP in the AIA model [1]. Then, different mechanical challenges could potentially impact arthritis development by regulating YAP.

Objectives: The objective of this study was to investigate the impact of mechanical loading and unloading on the development of arthritis in the AIA model.

Methods: Arthritis was induced in female Lewis rats by injection of the adjuvant *Mycobacterium butyricum*, defining day (D)0. The AIA model normally develops arthritis at D10, with a peak of inflammation at D17 [2]. Rats were randomized into three groups: an AIA+mechanical loading group (n=11) with free access to an activity wheel from D0 to D17, an AIA+mechanical unloading group (n=11) by tail suspension from D0 to D17, and an AIA-only group (n=11) as positive control. Daily clinical monitoring (arthritic index and ankle circumference) was used to follow the progression and severity of arthritis. At D17, the ankles of the rats were collected to perform RT-qPCR.

Results: Arthritis onset was observed at the same time (D10) in the AIA control and AIA+mechanical loading groups with the same kinetic of arthritis index and ankle circumferences. However, the majority of rats in the AIA+mechanical unloading group did not develop any signs of arthritis. At D17, gene expression of YAP and CYR61 (YAP target gene), IL1B, IL6, RANKL, ACP5 (encoding TRAPc), CTSK (encoding cathepsin K), MMP9, and MMP13 was decreased in the ankle of AIA+mechanical unloading rats compared to other groups. In the AIA+ mechanical loading group, rat stopped their physical activity at D10 which may explain the lack of clinical and molecular differences between the AIA and AIA+mechanical loading groups at D17.

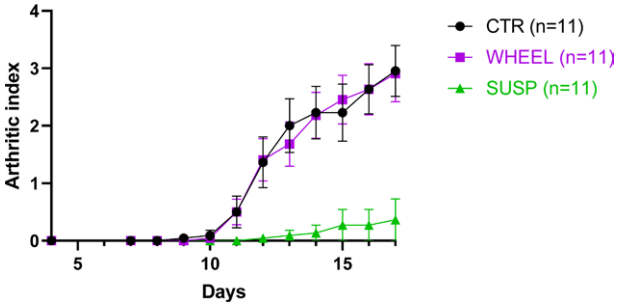

Figure 1. Arthritic index from day(D) 0 to D17 in the AIA control (CTR) group (n=11), the AIA+mechanical loading (WHEEL) group (n=11) and the AIA+mechanical unloading (SUSP) group (n=11).

Conclusion: Mechanical unloading of the hindpaws by the suspension system strongly prevented arthritis by a reduced expression of pro-inflammatory genes, bone resorption, and bone degradation genes. The decrease in inflammation may be partly explained by the decrease in YAP transcriptional activity. Mechanical stress is therefore a key factor in inflammation during AIA. The precise mechanisms linking these two mechanisms are still under investigation.

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Disclosure of Interests: None Declared.

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OP0209 IMPACT OF CHRONIC INFLAMMATION AND COLLAGEN IV FRAGMENT CANSTATIN ON RHEUMATOID ARTHRITIS SYNOVIAL FIBROBLASTS AND ENDOTHELIAL CELL INTERACTIONS IN VITRO AND IN VIVO

Keywords: Cell biology, Synovium, Rheumatoid arthritis

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Background: Increased neovascularization in the inflamed synovium of patients with rheumatoid arthritis (RA) is a pathological feature in the pathogenesis of RA. Key players in RA progression are chronically activated RA synovial fibroblasts (RASF), which secrete proinflammatory factors (e.g. IL-6), proangiogenic

VEGF, IL-11 and matrix metalloproteases (MMP) promoting inflammation, synovial angiogenesis, and invasion of cartilage and bone by degrading the consecutive matrix, resulting in the release of the anti-angiogenic collagen IV fragment canstatin. Canstatin may further activate RASF leading to altered expression of vascular markers such as angiopoietin-2 (ANGPT2) and neovascularization.
Objectives: Analysis of repetitively stimulated RASF- and canstatin-mediated effects on vessel formation in the tube formation assay, in the SCID-mouse model for RA and in synovial tissue of RA patients with respect to ANGPT2 expression and RASF-endothelial cell (EC) interactions.
Methods: 2D tube formation assay was performed using HUVEC seeded on Matrigel. 15% Calcein AM-stained RASF were added. RASF/HUVEC were treated with 0.5µg/ml canstatin. HUVEC were pre-treated with 0.2µg/ml canstatin. RASF were repetitively stimulated three times with 0.05ng/µl IL-1ß every 24h starting at day 2. Tube thickness and the area covered by the formed cellular network were measured. RNA was extracted from tubes and ANGPT2 expression was analysed by qPCR. IL-6 and IL-11 in supernatants were measured by ELISA. Healthy cartilage was subcutaneously co-implanted with RASF into SCID mice. Contralaterally, healthy cartilage without RASF was implanted. Vessel formation was evaluated after 3-45 days. RA and osteoarthritis (OA) synovial tissue was stained for ANGPT2 and CD31.
Results: RASF or HUVEC stimulated once showed a significant IL-6 increase compared to unstimulated controls. After subsequent repetitive stimulation of RASF or HUVEC, a significant decrease in IL-6 (1st stimulation: 5076±1730pg/ml vs. 3rd: 1890±758pg/ml, p<0.0001/ 1st: 488±186pg/ml vs. 3rd: 382±109pg/ml, p=0.041) and IL-11 in RASF (1st: 451±205pg/ml vs. 3rd: 69±51pg/ml, p<0.0001) compared to the first stimulation was observed. Repetitive stimulation of HUVEC+RASF resulted in a significant IL-6 increase for each subsequent stimulation (1st vs. 3rd: p=0.02). RASF significantly reduced tube thickness (22.9µm (SD=6.3) vs. 16.6µm (SD=2.2), p=0.014) and the network area (p<0.0001) and significantly increased ANGPT2 2-fold compared to HUVEC alone. RASF stimulated only once further reduced the network area (p=0.038), while repetitive stimulation significantly attenuated the proinflammatory effect (IL-6, p=0.029). Stimulation of pre-treated HUVEC and unstimulated RASF with canstatin led to disturbed tube formation with reduced tube thickness from 22.9 to 16.9µm (SD=4.4, p=0.011). Co-culture of RASF with pre-treated HUVEC with canstatin further increased the RASF-mediated effect by reducing tube thickness (22.9 to 14.6µm (SD=1.4), p<0.001), with a significant 1.6-fold decrease of ANGPT2. In human RA synovium, ANGPT2 was also found to be significantly upregulated in vessels compared to OA tissue. In SCID mice, RASF-mediated altered vessel formation started at day 3. Helix-like vessels were detectable at early time points of vessel formation in implants with RASF (d3-9), e.g. at day 3, 60% of vessels were helix-like.
Conclusion: RASF induced helix-like vessels in the SCID mouse model suggest an influence on vessel formation. This could be confirmed in vitro as well as on molecular level inducing the vascular marker ANGPT2 in newly formed tubes. ANGPT2-upregulation in vessels was also observed in human RA synovium. Repetitive stimulation of RASF resulted in an inflammatory adjustment to IL-1ß, leading to a significant reduction of IL-6 and improved neovascularization in vitro. The anti-angiogenic canstatin could in part enhance the RASF-mediated effect and was not able to restore RASF-altered tube formation in vitro.
REFERENCES: NIL.
Acknowledgements: NIL.
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Novel insights into disease taxonomy and immunophenotyping

OP0210 BRUTON'S TYROSINE KINASE (BTK) IS A NOVEL INDEPENDENT BIOMARKER OF LYMPHOMA IN PRIMARY SJÖGREN'S SYNDROME: DATA FROM 346 PATIENTS OF THE ASSESS COHORT

Keywords: Sjögren syndrome, Malignancy, Biomarkers

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Background: Primary Sjögren's syndrome (pSS) is a B-cell driven systemic auto-immune disease associated with the highest risk of lymphoma among systemic auto-immune diseases. In the prospective ASSESS cohort (395 patients enrolled, with a 10-year follow-up), transcriptomic analysis recently showed the association between baseline peripheral blood overexpression of Bruton's tyrosine kinase (BTK) and lymphoma.
Objectives: This study aimed to confirm the transcriptomic findings at the mRNA and protein level and to evaluate the relevance of BTK as a lymphoma predictor with regards to other predictors.
Methods: BTK mRNA expression was assessed using qRTPCR in whole blood samples from 63 patients (12 patients with a history of lymphoma, 9 patients with incident lymphoma occurring during the 10-year follow-up, 21 patients without lymphoma and no risk factors for lymphoma and 21 patients without lymphoma and ESSDAI ≥ 5). BTK protein expression in peripheral blood was analyzed by flow cytometry in 7 healthy controls, 5 pSS patients without risk factors for lymphoma, 8 pSS patients with ESSDAI ≥5, and 6 pSS patients with lymphoma. In 346 patients of the ASSESS cohort for whom all data were available, the association between pSS-related lymphoma and BTK expression at enrolment, with 9 validated lymphoma predictors (parotid enlargement, purpura, ESSDAI ≥ 5, lymphocytopenia, CD4/CD8 ratio ≤ 0.8, RF positivity, cryoglobulinemia, monoclonal component, and low C4) and other potential biomarkers (serum FLT3L, BAFF, digital interferon alpha, gammaglobulins, IgG, beta2microglobulin, kappa and lambda free light chains, quantitative levels of anti-SSA/SSB) was analyzed. Multivariate analyses took into account in a first model, BTK and the 9 validated predictors of pSS-related lymphoma, and in a second model variables associated with lymphoma in univariate analysis selected by a stepwise procedure.
Results: In qRTPCR, BTK was significantly upregulated between pSS patients with and without lymphoma. In flow cytometry, BTK protein expression in $CD19^+$ B cells (p=0.03), and in most B-cell subpopulations, was significantly increased in patients with lymphoma compared to healthy controls. A gradual increase was observed in BTK expression of $CD19^+$ B cells, between patients without risk factors for lymphoma, those with ESSDAI ≥5, and those with lymphoma. In multivariate analysis, taking into account 9 validated predictors of lymphoma in pSS, BTK expression at enrolment was significantly associated with lymphoma (adjusted OR [aOR] 1.45; 95% CI [1.19-1.82]; p<0.001), along with CD4/CD8 ratio ≤ 0.8 (aOR 6.03; 95% CI [1.23-26.13]; p=0.02). In a second model including 4 biomarkers selected by a stepwise procedure, BTK expression remained significantly associated with lymphoma (aOR 1.36; 95% CI [1.14-1.64]; p<0. 001), as well as serum BAFF levels (mean [SD]:1028.5 pg/ml [710.3]; per 1000 BAFF units, aOR 2.04; 95% CI [1.3-3.19]; p=0.001), low C4 (aOR 4.16; 95% CI [1.43-13.88]; p=0.01) and CD4/CD8 ratio ≤0.8 (aOR 10.62; 95% CI [2.49-44.15]; p=0.001).
Conclusion: These results confirm that increased expression of BTK in peripheral blood is associated with pSS-related lymphoma. These results also show the potential interest of combining B-cell (BTK and BAFF) and T-cell (CD4/CD8 ratio)-related biomarkers to evaluate the risk of lymphoma in pSS.
Acknowledgements: To the patients for their participation to the ASSESS cohort; To the investigators of the ASSESS cohort; plateforme de transcriptomique de l'Institut Cochin; centre de ressources biologiques de l'Hôpital Bichat; The French Society of Rheumatology.
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OP0211 CHANGE IN URINARY BIOMARKERS AT THREE MONTHS PREDICTS 1-YEAR TREATMENT RESPONSE OF LUPUS NEPHRITIS BETTER THAN PROTEINURIA

Keywords: Biomarkers, Systemic lupus erythematosus, -Omics

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