and TTK_hsa_circ_0077158- miR-212-3p/miR-132-3p/miR-129-5p-TTK might be potential RNA regulatory pathways to regulate the disease progression of early RA.

Conclusion: This work identified three haematologic/immune system-specific expressed genes, namely, GZMA, PRC1, and TTK, as potential biomarkers for the early diagnosis and treatment of RA and provided insight into the mechanisms of disease development in RA at the transcriptome level. In addition, we proposed that NEAT1-miR-212-3p/miR-132-3p/miR-129-5p-TTK, XIST-miR-25-3p/miR-129-5p-GZMA, and TTK_hsa_circ_0077158-miR-212-3p/miR-132-3p/miR-129-5p-TTK are potential RNA regulatory pathways that control disease progression in early RA.

REFERENCES:

- [1] Smolen JS, Aletaha D, McInnes IB: **Rheumatoid arthritis**. *Lancet* 2016, **388**:2023-2038.
- [2] Aletaha D, Smolen JS: **Diagnosis and Management of Rheumatoid Arthritis: A Review**. *Jama* 2018, **320**:1360-1372.
- [3] Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, 3rd, Birnbaum NS, Burmester GR, Bykerk VP, Cohen MD, et al: **2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative**. *Arthritis Rheum* 2010, **62**:2569-2581.

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2021-eular.135

AB0043 EFFECT OF ANTI-INFLAMMATORY MEDICATION ON INTERACTION OF SYNOVIAL FIBROBLASTS WITH MACROPHAGES IN RHEUMATOID ARTHRITIS

M. Schmeller¹, M. Diller¹, R. Hasseli¹, A. Knothe¹, S. Rehart², I. Tarner¹, W. Hermann¹, U. Lange¹, U. Müller-Ladner¹, E. Neumann¹. ¹Justus Liebig University Giessen, Campus Kerckhoff, Department of Rheumatology and Clinical Immunology, Bad Nauheim, Germany; ²Agaplesion Markus Hospital, Department of Orthopaedics and Trauma Surgery, Frankfurt, Germany

Background: One of the key mechanisms in the pathogenesis of rheumatoid arthritis (RA) is the interaction of macrophages and synovial fibroblasts during joint inflammation. Increased synergistic proinflammatory activity of both cell types leads to the release of high levels of proinflammatory cytokines, especially of interleukin-6 (IL-6), and of matrix degrading enzymes. If this mechanism is uncontrolled, progressive destruction of articular cartilage and bone will take place.

In active disease, immediate anti-inflammatory treatment with glucocorticoids is usually replaced by disease-modifying anti-rheumatic drugs (DMARDs), especially by methotrexate (MTX) and biologics such as TNF- α - or IL-6-inhibitors. This led to great improvements in prognosis and outcome for RA patients. However, about 40% of patients experience no remission or suffer from side effects of medication. To optimize established substances and to develop new treatment strategies, it is necessary to understand the mechanisms underlying the limited therapeutic effects.

Objectives: Evaluation of the effect of prednisolone, MTX, adalimumab, tocilizumab on IL-6 secretion by RA synovial fibroblasts (RASf) and macrophages.

Methods: RA synovium was used for RASf isolation. Peripheral blood mononuclear cells (PBMCs) were isolated from blood of healthy donors and RA patients by using Ficoll medium followed by density gradient centrifugation. Mononuclear cells were seeded on six well plates (6x10⁶/well) and incubated for one week. Then they were stimulated with Interferon- γ (20 ng/ml) and LPS (50 ng/ml) for 48h to initiate differentiation into proinflammatory M1 macrophages. The M1 macrophages were co-cultured with RASf (100.000/well) and different treatments added (prednisolone: 10, 25, 50, 75, 100 nM, 1 μ M; adalimumab: 100, 500 μ g/ml; tocilizumab: 1, 5 μ g/ml; MTX: 0.5, 1, 5, 10, 100 nM, 1 μ M). After 24h culture supernatants were collected and IL-6- and TNF- α -ELISAs were performed.

Results: IL-6 concentrations of untreated controls were comparable, regardless whether M1 macrophages from healthy donors or RA-patients were used for co-culture. Prednisolone reduced co-culture-induced IL-6 up to 56% (p<0.001)

in co-culture of RASf and M1 macrophages of healthy donors and up to 60% (p<0.001) in co-culture of RASf and RA M1 macrophages. Adalimumab reduced IL-6 up to 28% (p<0.05) in M1 of healthy donors and up to 45% (p<0.01) in RA M1 macrophage co-cultures. A minor reduction by 10-20% of IL-6 was observed with tocilizumab and no significant effect could be achieved after treatment with MTX.

Conclusion: Prednisolone and adalimumab clearly decrease but do not eliminate proinflammatory synergistic activity of RASf and M1 macrophages. These results confirm the clinical observation, that there is a large number of RA-patients that independent of anti-inflammatory treatment still suffer from low-level joint inflammation.

The synergistic proinflammatory activity of M1 macrophages and RASf seems to be a complex and multifactorial mechanism that is difficult to eliminate by a single treatment substance. Since it is one of the key mechanisms in RA pathogenesis, there is a critical need to investigate how therapy effects could be optimized. This study confirmed RASfs as one of the leading effector cells of increased synergistic proinflammatory activity, thus underlining their promising role as a treatment target in rheumatoid arthritis.

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2021-eular.247

AB0044 ENDOTHELIAL CELL AND RHEUMATOID ARTHRITIS SYNOVIAL FIBROBLAST MIGRATION AND ADHESION ARE ALTERED BY ACTIVIN/FOLLISTATIN

H. M. Scholz¹, I. Aykara¹, K. Frommer¹, S. Rehart², U. Müller-Ladner¹, E. Neumann¹. ¹Kerckhoff-Klinik GmbH, Rheumatologie und Klinische Immunologie, Bad Nauheim, Germany; ²Agaplesion Markus Krankenhaus, Klinik für Orthopädie und Unfallchirurgie, Frankfurt am Main, Germany

Background: Activin A and follistatin belong to an anti-inflammatory auto-regulatory cycle. Patients with rheumatoid arthritis (RA) have increased activin A levels in the synovial fluid and tissue. During inflammation, activin A is released systemically, then inducing its antagonist follistatin. This negative feedback is active in different cell types but not RA synovial fibroblasts (SF). Fibroblasts interact with endothelial cells in inflamed tissues inducing angiogenesis.

Objectives: Evaluation of the role of activin A and follistatin on RASf and endothelial cell interactions.

Methods: RA synovium was used for RASf isolation, HUVEC were commercially obtained. RASf and HUVEC were stimulated in mono- and coculture. Direct: RASf together with HUVEC; indirect: inserts with HUVEC separated by a membrane from RASf. Stimuli: activin A 15ng/ml, follistatin 500ng/ml, IL-1 β 1ng/ml. Proliferation was analyzed by BrdU assay. RASf were Calcein-AM stained. Cells were transferred to 24-well plates after 18h stimulation. After adhesion for 1h, non-adherent cells were removed by shaking 3x for 5min. Afterwards, fluorescent cells were quantified. For the flow-adhesion assay, HUVEC were cultured on rattail collagen coated capillary slides. HUVEC and RASf were stimulated for 4h with TNF α , TNF α +activin A or TNF α +follistatin. After stimulation, 2x10⁶ RASf were resuspended in 20ml medium and sent through the capillaries. Two 1min videos were recorded (18.4ml/h, 30.5ml/h). Settings: TNF α -stimulated RASf on HUVEC stimulated with TNF α or activin A+TNF α or follistatin+TNF α . For migration assays, 2% FCS medium with 1x10⁵ cells were placed in inserts (8 μ m membrane) into wells with 10% FCS (control: 2%FCS vs. 2%FCS) and stimulated with, IL-1, IL-1+activinA and IL-1+follistatin. After 16h, migrated cells were quantified. For scratch-assay 4x10⁵ cells were cultured overnight, then cells were scratched and stimulated, afterwards 3 pictures per scratch were taken at start, after 10h and every 1.5h. Cells migrating into the scratch area were quantified.

Results: IL-1 induced activin A in RASf and HUVEC in all settings. IL-1-induced activin A release was reduced by follistatin in HUVEC monoculture and both cocultures compared to IL-1 alone but not in RASf monoculture. IL-1-induced IL-6 release was reduced by activin A in HUVEC and indirect coculture but not in RASf monoculture and direct coculture. Follistatin did not alter IL-6 responses. IL-1 induced VEGF in RASf but not in HUVEC and was not altered by activin A. Short-term adhesion showed no significant influence of activin A or follistatin (n=4). Flow adhesion assay showed reduced adherence/rolling of RASf on HUVEC stimulated with TNF α and activin A (n=4). Migration assays showed that IL-1 decreased migration but without significant difference between the induced effects mediated by IL-1+activinA and IL-1+follistatin (n=4). Scratch assay showed increased migration in direct coculture with greater difference between stimulated and unstimulated cells in RASf monoculture and indirect coculture (n=4). Proliferation was not altered by activin A or follistatin.

Conclusion: In direct and indirect coculture of HUVEC with RASf the effect on HUVEC was dominant leading to reduced IL-1-induced activin A release. However, the IL-1-induced IL-6 release in RASf or HUVECs was decreased by activin A in HUVEC monoculture and indirect coculture but not during cell-contact between both cell types. The direct interaction of RASf with HUVEC seems to prevent the reducing activin A effect on IL-6 release in HUVECs. Activin A seems to not to have an impact on short-term cell adhesion but first observations show, that activin A alters selectin-mediated adhesion under flow conditions. The migration assay shows that IL-1-induced effects on cell migration were enhanced by activin

A and follistatin. Migration assay shows that IL-1-induced decrease on migration more prominent in indirect coculture and RASF monoculture than in direct coculture although in gap migration in the scratch assay was highest in direct coculture.

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2021-eular.271

AB0045

THE RELATIONSHIP BETWEEN CD14 AND VIMENTIN-POSITIVE SYNOVIAL DENDRITIC-SHAPED CELLS AND SYNOVITIS IN RHEUMATOID ARTHRITIS

R. Kurose^{1,2}, T. Satoh³, K. Murakami⁴, A. Kurose⁵, Y. I. Satoh⁶, Y. Ishibashi², K. Ishida⁷, K. Ogasawara⁷, A. Morikawa⁸, T. Sawai⁹. ¹Hirosaki Memorial Hospital, Orthopedic Surgery, Hirosaki, Japan; ²Hirosaki University, Orthopedic Surgery, Hirosaki, Japan; ³Iwate Medical University, Pathology, Morioka, Japan; ⁴Iwate Medical University, Orthopaedic Surgery, Morioka, Japan; ⁵Hirosaki University, Pathology, Hirosaki, Japan; ⁶Iwate Medical University, Medical Education, Morioka, Japan; ⁷Iwate Medical University, Technical Support Center, Morioka, Japan; ⁸Hitachi High-Technologies Corporation, Electron Microscope Application Group, Hitachinaka, Japan; ⁹Tohoku University, Pathology, Sendai, Japan

Background: Inflammation in rheumatoid arthritis (RA) is caused by multiple cell types, including infiltrating inflammatory cells, such as lymphocytes, neutrophils, macrophages, and spindle-shaped fibroblasts. Especially, we are focusing on fibroblast-like synoviocytes (FLSs). In our previous study, we have reported that FLSs were positive for multiple markers including CD14, CD68 and HLA-DR, and were dendritic-shaped cells constituting nursing phenomenon between lymph or plasma cells. In addition, in our recent study, we found that CD14+FLSs were positive for vimentin (VIM), which is a marker for mesenchymal cells. There are still many issues to be discussed regarding CD14+VIM+ cells.

Objectives: To investigate the relationship between CD14+VIM+ cells and the degree of synovitis in rheumatoid arthritis.

Methods: Synovial tissues collected from RA patients who underwent joint surgeries were prepared for this study. First, the proportions of CD14+ cells in RA synovial tissue and control were analyzed using flow cytometry and the concentrations of inflammatory cytokines released by CD14+ cells in RA synovial tissue and control were examined by ELISA. Next, the proportions of CD14+VIM+ cells in RA synovial tissue and control were examined immunohistologically and then we analyzed the results using image analysis software. Also, we statistically analyzed the relationship between the proportion of CD14+VIM+ cells, the degree of synovitis, and clinical data.

Results: Results of flow cytometry showed that CD14+ cells were frequently observed in RA synovial tissue than control. Cultured CD14+ cells released more inflammatory cytokines than cultured CD14- cells. Also, results of immunohistological staining showed that many CD14+VIM+ cells were observed in RA synovial tissue than in control. The proportion of CD14+VIM+ cells was correlated with Krenn synovitis score. High proportion cases significantly showed high level of CRP and MMP-3.

Conclusion: CD14+VIM+ cells might be involved in the mechanism of chronic immunological inflammation in RA and the proportion of these cells might influence the clinical data.

REFERENCES:

- [1] Ochi T, Yoshikawa H, Toyosaki-Maeda T, Lipsky PE. Mesenchymal stromal cells. Nurse-like cells reside in the synovial tissue and bone marrow in rheumatoid arthritis. *Arthritis Research & Therapy* 2007; 9(1): 201.
- [2] Ochi T, Sawai T, Murakami K, Kamataki A, Uzuki M, Tomita T, et al. Nurse-like cells in rheumatoid arthritis: Formation of survival niches cooperating between the cell types. *Mod Rheum* 2018; 29: 1-5.
- [3] Krenn V, Morawietz L, Burmester GR, Kinne RW, Muller-Ladner U, Muller B, Haupl T. Synovitis score: discrimination between chronic low-grade and high-grade synovitis. *Histopathology* 2006; 49: 358-64.

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2021-eular.428

AB0046

THE EXPRESSION AND CLINICAL SIGNIFICANCE OF TAM RECEPTOR TYROSINE KINASE ON MONOCYTE SUBSETS IN RHEUMATOID ARTHRITIS

J. Xue¹, L. Xu¹, F. Hu¹, Y. Su¹. ¹Peking University People's Hospital, Department of Rheumatology and Immunology, Beijing, China

Background: The TAM receptor tyrosine kinases (Tyro3/Axl/Mer TK) are a subfamily of receptor tyrosine kinases, the role of which in autoimmune diseases such as systemic lupus erythematosus has been well explored, while their functions in rheumatoid arthritis (RA) remain largely unknown [1].

Objectives: The study aimed to investigate the expression and clinical significance of Tyro3, Axl and MerTK on monocyte subsets in rheumatoid arthritis (RA).

Methods: The expression of Tyro3, Axl and MerTK on CD14+ monocytes, nonclassical monocytes (NCM, CD14⁺CD16⁺), intermediate monocytes (IM, CD14⁺CD16⁺), and classical monocytes (CM, CD14⁺CD16⁻) were evaluated in peripheral blood of RA by flow cytometry and qPCR. And the correlation between

the expression of Tyro3TK and MerTK on NCM, IM, and CM with RA patient clinical feature were further analyzed.

Results: The results revealed that the expression of Tyro3TK on CD14+ monocytes was significantly upregulated in RA patients ($F = 9.18$, $P < 0.0001$), while there was no significant difference of the expression MerTK on HC, OA, and RA, and the expression of AxlTK was minimal (Fig 1). The expression of Tyro3TK on CM was significantly upregulated in RA patients as compared with HC and osteoarthritis (OA) patients ($P < 0.05$, Fig 2-3), and positively correlated with the disease manifestations, such as swollen joint count (SJC), tender joint count (TJC) and the disease activity score (Fig 4).

Figure 1. The expression of Tyro3, Axl and MerTK on CD14+ monocytes is increased in RA. (a) Gating strategy for flow cytometry of Tyro3, Axl and MerTK on CD14+ monocytes. **(b)** The expression of Tyro3, Axl and MerTK on CD14+ monocytes were compared between HC (n = 40), OA (n = 27), and RA patients (n = 40). * $P < 0.05$, ** $P < 0.01$, ns, not significant.

Figure 2. The expression of Tyro3TK on IM and CM were increased in RA. (a) Gating strategy for flow cytometry of TAMTK on monocyte subsets. The expression of **(b)** Tyro3TK and **(c)** MerTK on NCM, IM, and CM were compared between HC (n = 40), OA (n = 27), and RA patients (n = 40). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns, not significant.