Substantial amounts of anti-SAA1 Abs were observed in serially diluted IVIg, while there were 4-fold less anti-AGP Abs detected (Figure 2), which corresponds also to the ratio found in HBD (Figure 1).

Conclusion: Serum IgG Abs against SAA1 and AGP present in HBD, patients with systemic autoimmune diseases and in IVIg can be simultaneously quantified using a customized bead-based multiplex assay. These natural autoAbs found in IVIg could be endogenous immune-regulators of the acute phase response.

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THU0041 MESENCHYMAL STEM CELLS OF DIFFERENT ORIGINS EXHIBIT UNIQUE RESPONSES TO DIFFERENT INFLAMMATORY STIMULI

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Background: Mesenchymal stromal/stem cell (MSC) - based therapies represent promising avenues for treatment of various inflammatory and autoimmune diseases. Currently, a variety of fetal and adult tissues represent a good source of MSC, however further characterization of their effects, especially in an inflammatory environment, is needed.

Objectives: Firstly, to characterize MSC from different origins, namely bone marrow (BM), adipose tissue (AT) and umbilical cord (UC), after stimulation with TNF α , IL-1 β and serum amyloid A (SAA). Secondly, to determine the effects of MSC secretome on migration, proliferation and apoptosis of lung fibroblasts.

Methods: BM-, AT- or UC-MSC were isolated each from 3 healthy donors. The expression and secretion of analytes were studied in each cell type at basal level and following TNF α - (1 ng/ml), IL-1 β - (1 ng/ml) and SAA- (12 μ g/ml) stimulation using qPCR (SDF, CCL5, VCAM, PDE, PGC, IDO, VEGF, IGF) and Luminex technology (IL-6, IL8, MCP-1, ICAM, CH3L1, MMP1, MMP3, tenascin, uPA, HGF, VEGF), respectively. Normal healthy lung fibroblasts (NHLF) were used in the wound scratch assay and treated for 24h with 20% of MSC-conditioned media. For evaluation of BM-, AT- and UC conditioned media effects on proliferative and apoptotic activity, Ki-67-immunolabeled mitotic cells and caspase-3-immunolabeled cells were counted using fluorescence microscopy.

Results: Treatment of MSC with pro-inflammatory cytokines increased mRNA levels of SDF, CCL5, VCAM1, PGC and IDO, while levels of PDE-5A were down-regulated. The greatest increase in expression was observed in TNF α -stimulated MSCs, namely of two immunomodulatory molecules, CCL5 and IDO in AT-MSC (4396- and 928-fold change increase respectively). Secretome analysis showed highest secretion levels of IL-6, IL-8 and MCP1 after IL-1 β -activation in all 3 tissue sources. AT- and BM-derived conditioned media increased NHLF scratch closure, while UC-MSC decreased it. In non-inflammatory conditions, UC-, BM- and AT-MSCs conditioned media decreased the number of mitotic events in NHLF by an average of 40%. Importantly, only the conditioned medium derived from inflammatory cytokine-treated AT- and BM-MSC significantly decreased the mitotic rate of NHLF ($p < 0.01$). The number of apoptotic cells increased 3- to 8-fold after treatment with all MSC conditioned media, with UC-MSC showing the highest effect ($p < 0.01$).

Conclusion: Our results highlight specific roles of MSC from different tissue origins, as well as their response to environmental inflammatory stimuli and suggest that source selection might be critical for the success of treatment approaches in different diagnoses.

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THU0042 EFFECT OF SPLEEN TYROSIN KINASE (SYK)-INHIBITORS ON RHEUMATOID ARTHRITIS SYNOVIAL FIBROBLASTS

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Background: The spleen tyrosine kinase (syk) is an intracellular protein kinase involved in signal transmission processes of immune cells and non-hematopoietic cells, such as fibroblasts. Various syk-inhibitors are therefore currently being tested in preclinical and clinical studies, e.g. for the treatment of different types of cancer as well as autoimmune diseases. In preclinical studies, the syk inhibitor fostamatinib showed a pronounced anti-inflammatory effect on synovial fibroblasts (SF) from patients with rheumatoid arthritis (RA). These cells play an important role in the pathogenesis of RA by mediating matrix destructive processes. On the other hand, clinical trials have been only moderately successful due to the side effects and the limitation regarding increase in dosage. However, further inhibitors such as RO9021 or TAK-659 have been developed. They are characterized by a more specific kinase profile and therefore side effects could potentially be less severe. Whether these inhibitors also convey an anti-inflammatory effect on RASF has not yet been investigated.

Objectives: This study examines the effect of syk-inhibitors on the inflammatory response and functional behavior of activated RASF.

Methods: RASF were isolated from synovial tissue of patients with known rheumatoid arthritis during joint replacement surgery. RASF were pre-treated with different concentrations of RO9021 and TAK-659 and their vehicle control for 2h and additionally stimulated with IL-1 β (10 ng/ml) for 17h or with oncostatin M (OSM, 100 ng/ml) for 24h. Supernatants were collected and the concentration of IL-6 and MMP3 were determined by ELISA. The measurement of cell viability, cytotoxicity and apoptosis was performed to exclude possible cytotoxic or apoptotic effects of the syk-inhibitors. The RASF proliferation was detected by a BrdU-incorporation assay. The effect of syk-inhibitors on cell migration towards a FCS-gradient was measured by cell culture inserts.

Results: Both TAK-659 and RO9021 showed a significant reduction of the release of the pro-inflammatory cytokine IL-6 and the production of MMP3 in IL-1 β stimulated RASF. However TAK-659 showed a stronger effect on IL-6 reduction (48%, $p < 0.005$) than RO9021 (25%, $p < 0.05$) at 10 μ M. MMP3 decrease was also stronger with TAK-659 (92%, $p < 0.001$) than with RO9021 (59%, $p < 0.001$). This effect was apparently not restricted to IL-1 β because both inhibitors also decreased the OSM-induced IL-6 release of RASF by 85% ($p < 0.01$) with TAK-659 and 30% with RO9021 at 10 μ M. The proliferation of RASF was also reduced by both syk-inhibitors at 5 μ M and higher. The highest effect could be observed with TAK-659 for 80% and with RO9021 by 50% both at 15 μ M. The migration of RASF was also decreased by TAK-659 at 10 μ M ($p < 0.01$, $n = 3$). The effects were not mediated by induction of apoptosis or by cytotoxicity.

Conclusion: The syk-inhibitors TAK-659 and RO9021 at concentrations above 5 μ M reduce the inflammatory response, the migration and proliferation of RASF. If appropriate concentrations can be reached in vivo, these syk-inhibitors could offer a possibility to modulate the aggressive phenotype of RASF.

DISCLOSURE OF INTERESTS:

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THU0043 IL-25 ALLEVIATES RHEUMATOID ARTHRITIS BY INHIBITING TH17 IMMUNE RESPONSE

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Background: Rheumatoid arthritis (RA) is a prevalent common autoimmune disease characterized by chronic inflammation of the joint and synovial hyperplasia and progressive destruction of articular cartilage and bone. Accumulating evidence highlighted that an imbalance between pro-

and anti-inflammatory cytokines maybe a key mechanism for disease progression in RA. IL-25 is reported to play an anti-inflammatory role in autoimmune and inflammatory diseases through the downregulation of Th1 and Th17 cell responses. However, the exact role of IL-25 in the pathogenesis of RA remains to be elucidated.

Objectives: Explore the role of IL-25 in the pathogenesis of RA, and provide the scientific evidence of IL-25 relevant biological agent.

Methods: 1. The study was first to analyze the relationship between IL-25 and DAS28 score, CRP and anti-CCP in RA patients. Test IL-1 β , IL-6, IL-17A, TNF- α and IFN- γ through ELISA, then analyze the relationship with IL-25. CD4⁺ T cells from PBMCs of the 5 RA patients and 5 HCs were isolated under the manual of EasySep™ Human CD4⁺ T Cell Isolation Kit and stimulated with anti-CD3 plus anti-CD28 in the presence or absence of rhIL-25. 2. CIA model was established to explore the mechanism of IL-25. The expression of CII specific IgG1 and IgG2a, as well as IL-1 β , IL-6, IL-17A and TNF- α in CIA serum were examined using ELISA assay. The ROR- γ , T-bet, GATA-3 mRNA levels in knee joints and spleen were assessed by real-time qPCR analysis. In vitro, CD4⁺ T cells from spleen were isolated from CIA mice and cultured with or without recombinant mouse (rm)IL-25 for 24h to control the expression IL-17A. Cells were stimulated with plate-bound anti-CD3 plus anti-CD28 in the presence or absence of recombinant mouse (rm) IL-25 with different dose for 24 h.

Results: 1. The expression of IL-25 were upregulated in the serum and synovial fluid in RA patients. Serum IL-25 levels were positively associated with DAS28 score, ESR and C-reactive protein. However, no significant correlation was observed between serum IL-25 levels, RF and anti-CCP. IL-25 serum levels were positively correlated with the concentrations of IL-1 β , IL-6, IL-17A and TNF- α , respectively. However, no significant correlation was observed between serum IL-25 and IFN- γ level. 2. IL-25 can alleviate inflammatory response of CIA mouse and inhibit IL-17 differentiation. The serum levels of IL-1 β , IL-6, IL-17A and TNF- α in IL-25-treated mice were markedly reduced compared with those in WT mice, except IFN- γ . RT-qPCR also demonstrated that the transcription levels of IL-17A and ROR- γ t were decreased in the synovial tissue of IL-25-treated mice. RmIL-25 treatment significantly inhibited IL-17A production in a dose-dependent manner, and the mRNA level of the key TFs for Th17 cells (ROR- γ t), as well as IL-17A, were also decreased.

Conclusion: IL-25 is upregulated in the serum and synovial fluid of RA patients. High levels of IL-25 was associated with disease severity and inflammation response in RA patients. IL-25 inhibits CD4⁺ T-cell activation and differentiation into Th17 cells, without affecting Th1 cells in RA. Systemic administration of IL-25 attenuates arthritis onset and joint damage in CIA mice. IL-25 of multi-target regulation further be used in the clinical treatment of RA.

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THU0044 PRO- AND ANTI-INFLAMMATORY MEDIATORS OF SYSTEMIC INFLAMMATION AND ARTHRITIS IN THE ELDERLY

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Background: Elderly-onset RA (EORA), which is defined as rheumatoid arthritis (RA) with an onset at >60 years of age, and polymyalgia rheumatica (PMR) are common rheumatic diseases in the elderly. Eicosanoids are biological lipids that serve a specific role as either activators or suppressors of systemic inflammation and have been involved in the development and progression of arthritis.

Objectives: We hypothesized that eicosanoid-related perturbations are related to arthritic symptoms in the elderly, and that by defining this eicosanoid profile, we might be able to define elements of inflammation pathobiology in this population.

Methods: Arthritis in the Elderly (ARTIEL) is a recent collection cohort with patients older than 60 years of age who have newly diagnosed arthritis. Blood samples were collected from these patients at baseline (pre-treatment) and 3 months after treatment, along with physician and patient outcome measures through 12 months. These patients were also

compared with randomly-selected control individuals of the same age and gender. A thorough clinical examination was conducted and patients completed a health assessment questionnaire (HAQ). Disease activity score (DAS)28CRP was also calculated. Serum eicosanoids were determined by mass spectrometry at baseline and after 3 months of treatment and were classified into groups according to their eicosanoid precursors: eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) or arachidonic acid (AA). Data processing and statistical analysis were performed in R.

Results: 64 patients (average: 75.15, standard deviation (SD) 6.80) and 18 controls (average: 75.39, SD, 6.04) were analyzed. Of these, 44 were diagnosed with RA and 20 with PMR. At the start of the study, patients had a mean DAS28CRP of 5.72 (SD, 1.05) and a mean HAQ of 1.64 (SD, 0.73). In addition, 84% of the patients reported scapular pain and 56% reported pelvic pain at baseline. After three months of treatment, patients had a mean DAS28CRP of 2.38 (SD, 1.23) and a mean HAQ of 0.36 (SD, 0.41). Several eicosanoids, especially anti-inflammatory species derived from EPA and DHA, were significantly downregulated in both RA and PMR patients at the start of the study as compared to normal controls (Figure 1). Three months after treatment, the levels of anti-inflammatory eicosanoid species derived from EPA and DHA went back to normal only in patients who responded to therapy.

Conclusion: These results suggest that certain eicosanoids may be key effectors in arthritis in the elderly and that the imbalance between pro and anti-inflammatory eicosanoids before and after treatment might be related to clinical and therapeutic outcomes in this population.

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Abstract THU0044 – Figure 1. Anti-inflammatory species derived from EPA and DHA are significantly downregulated in patients at baseline. Pro-inflammatory (in red) and anti-inflammatory eicosanoids (in blue) detected in our patients are represented by pathways and origin PUFA. Significant eicosanoids ($p < 0.05$) are circled in blue if downregulated in patients compared to controls at baseline.

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