In addition, he had/had consultancy relationship within the last 3 years with A. Menarini, Amgen, Abbvie, GSK, Mepha, MSD, Pfizer and UCB in the field of arthritides and related disorders, Caroline Ospelt: None declared, Kerstin Klein: None declared

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THU0039 THE RELATION BETWEEN THE INFLAMMATORY STATUS OF HUMAN END STAGE OSTEOARTHRITIC SYNOVIUM AND LEVELS OF LOW DENSITY LIPOPROTEIN

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Background: High systemic levels of low density lipoprotein (LDL) is a shared metabolic risk factor for osteoarthritis (OA) and cardiovascular disease (CVD), which may point to common biochemical pathways. One of the key events in atherosclerosis is formation and uptake of oxidized LDL (oxLDL) as a consequence of macrophage-mediated inflammation. Studies performed in our lab have previously shown that in mice, high LDL levels enhance synovial activation and ectopic bone formation during development of collagenase-induced OA. Furthermore, injection of oxLDL in naïve macrophage depleted joints led to infiltration of myeloid progenitor cells. Although these results strongly indicate towards (ox)LDL-mediated events that enhance OA severity, these processes have not yet been shown to occur in human osteoarthritic synovium.

Objectives: In the present study, we investigated whether the inflammatory status of OA synovium is associated to systemic and local levels of (ox)LDL.

Methods: Blood was collected from patients planned to undergo total knee replacement and LDL serum levels were determined (n=16). After surgery, synovial explants from the same patients (2-8 per patient) were collected and incubated at 37°C for 24 hours in presence of LDL (50 µg ApoB/mL), oxLDL (50 µg ApoB/mL) or control medium. Levels of IL-1β, TNF-α, IL-10 and MCP-1 secreted in the conditioned medium were measured using Luminex. Levels of S100A8/A9 were measured using ELISA. Concentrations of secreted factors were normalized for tissue sample weight. Synovial explants were further processed for histological analysis. Immunohistochemical staining of ApoB was performed to assess presence and uptake of (ox)LDL in the synovium. Hematoxylin & eosin-stained sections were used to arbitrarily score cellular infiltration and synovial hyperplasia.

Results: Around 50% of the patients in our cohort showed synovial activation exemplified by high secretion levels of IL-1β, TNF-α and IL-10. Cellular infiltration and synovial hyperplasia was observed in the majority of synovial tissue samples, their grades did however not correlate with cytokine secretion. Secretion levels of IL-1β positively correlated with serum levels of LDL and total cholesterol and did not correlate with BMI, suggesting a link that runs via systemic metabolic processes rather than increased weight-mediated joint loading. Considering that around 50% of OA patients suffers from synovitis characterized by increased macrophage presence and activity, high LDL in these patients may trigger oxLDL formation and uptake in the synovium. Staining of LDL-associated protein ApoB in histological sections of synovia showed distinct speck-like staining within macrophages and endothelial cells especially, suggesting oxLDL accumulation in these cells. Unexpectedly, presence of high concentrations of LDL or oxLDL during incubation of synovial explants did not significantly affect secretion of IL-1β, TNF-α, IL-10 and MCP-1, whereas presence of LDL but not oxLDL negatively affected secretion of S100A8/A9.

Conclusion: IL-1β secretion by end stage OA synovium is associated to systemic levels of LDL. Intervention with high concentrations of LDL or oxLDL for 24-hours after synovium extraction did however not alter cytokine secretion, suggesting that LDL and synovitis are linked through more long-term local interactions, or alternatively via systemic inflammatory factors.

Disclosure of Interests:

None declared

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THU0040

USING A NOVEL BEAD-BASED IMMUNOASSAY FOR SIMULTANEOUS DETECTION OF AUTOANTIBODIES AGAINST SERUM AMYLOID A1 AND ALPHA1 ACID GLYCOPROTEIN

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Background: Naturally occurring autoantibodies (Abs) against acute phase proteins (APPs), such as anti-serum amyloid A1 (SAA1) Abs have already been identified in sera of healthy individuals, as well as in patients with autoimmune diseases (1). Currently however, no method exists for simultaneous detection of multiple Abs against APPs.

Objectives: To develop a bead-based duplex immunoassay for simultaneous detection of IgG anti-SAA1 and anti-alpha 1 acid glycoprotein (AGP) Abs and quantify their levels in sera of healthy blood donors (HBD), and patients with systemic autoimmune diseases, as well as in intravenous immunoglobulin preparation (IVIg).

Methods: Fluorescently labeled MagPlex microspheres (Luminex Corp) were used to covalently bind SAA1 and AGP. Sera samples (diluted 1:20) were added to a 96-well microtiter plate and incubated with SAA1- and AGP-coupled microspheres for 2h. Subsequently, PE-conjugated anti-human IgG Abs were added to each well, incubated for 30 min, resuspended in PBS-1%BSA and analysed using the MagPix system (BioMedica, GmbH).

Abstract THU0040 – Figure 1. Levels of antibodies against SAA1 (A) and AGP (B) in HBD as compared to patients with systemic autoimmune diseases. Shown is median and IQR. 5th and 95th percentile of MFI values in HBD are indicated with dotted lines; *p<0.05; **p<0.01. SAA1, serum amyloid A1; AGP, alpha 1 acid glycoprotein; MFI, median fluorescence intensity; HBD, healthy blood donors; GCA, patients with giant cell arteritis; IgAV, immunoglobulin A vasculitis; SLE, systemic lupus erythematosus; SSc, systemic sclerosis; APS, antiphospholipid syndrome; ERA, early rheumatoid arthritis.

Abstract THU0040 – Figure 2. Presence of antibodies against SAA1 and AGP in IVIg. IVIg, intravenous immunoglobulin G; SAA1, serum amyloid A1; AGP, alpha 1 acid glycoprotein; MFI, median fluorescence intensity.

Sera samples were collected from HBD (n=55), patients with giant cell arteritis (GCA; n=30), immunoglobulin A vasculitis (IgAV; n=30), systemic lupus erythematosus (SLE; n=20), systemic sclerosis (SSc; n=27), antiphospholipid syndrome (APS; n=19) and early rheumatoid arthritis (ERA; n=20). Ab levels were determined also in IVIg (Octapharma AG).

Results: We observed the presence of both, anti-SAA1, and anti-AGP Abs in sera of HBD, as well as in patients with GCA, IgAV, SLE, SSc, APS and ERA.

GCA patients had significantly higher anti-SAA1 levels (median (IQR) MFI was 2163 (1113- 3048)) as compared to SSc (816 (480-1462); p<0.01) and ERA (886 (722-1596); p<0.05) patients. Levels of anti-AGP Abs were also significantly higher in sera of GCA patients (495 (298-872)) as compared to ERA (213 (130-338); p<0.01) and APS (264 (154-399); p<0.05) patients. No difference in anti-SAA1 or anti-AGP levels was observed between HBD and the tested groups of patients (Figure 1).

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.

Disclosure of Interests: None declared

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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-