in starved SF indicated an important role for PI3K-mediated signals in the ACPA-induced increase of SF mobility.
Conclusions: We demonstrated that additional stimuli (such as stress-induced citrullination and cytokine priming) were needed for SF to react upon ACPA stimulation. This is an indirect proof supporting the idea that a synovial insult that will normally resolve unobserved, might be essential for the transition towards chronic synovial changes in the presence of ACPA.
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FRI0014 ANTIOXIDANT ROLE OF MICROVESICLES FROM ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS IN HUMAN OSTEOARTHRITIC CHONDROCYTES

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Background: Oxidative stress results in the disruption of normal physiologic signaling leading to inflammatory changes, cartilage degradation and osteoarthritis (OA) progression (1). Recent advances have revealed the role of cell-derived microvesicles (MV) as a new mechanism of cell-to-cell communication with potential therapeutic applications. We have shown previously the antiinflammatory effects of human adipose tissue-derived mesenchymal stem cells (AD-MSC) conditioned medium in OA chondrocytes (2).
Objectives: We have isolated the MV fraction from the secretome of AD-MSC to investigate its activity on oxidative stress and inflammation in OA chondrocytes stimulated with interleukin (IL)-1β. Furthermore, we have characterized the MV protein content by proteomic analysis.
Methods: AD-MSC were isolated from fat of patients who undergone abdominoplasty (without obesity). MV were isolated from AD-MSC conditioned medium by differential centrifugation with size filtration. MV size and concentration were determined by resistive pulse sensing. Proteomic analysis was performed by LC-MS/MS, with ProteinPilot and PeakView software and the bioinformatic tools UNIPROT and PANTHER. OA chondrocytes were isolated from knee specimens of advanced OA patients, stimulated with IL-1β (10 ng/mL) and treated with MV ($3.6x10^7$ particles/mL) for 24h. Accumulation of 4-hydroxy-2-nonenal (HNE)-modified proteins and cytokines were measured by ELISA, NO production and MMP activity by fluorometry. Expression of specific proteins was evaluated by confocal microscopy or immunostaining. The data were analysed by ANOVA followed by Dunnett's test.
Results: MV reduced the accumulation of HNE-modified proteins, a biomarker of oxidative stress–induced lipid peroxidation, in OA chondrocytes stimulated with IL-1 β. The production of NO, IL-6 and TNFα, as well as MMP activity were also significantly reduced by MV treatment, whereas IL-10 and collagen II were enhanced. Proteomic analysis of MV showed high levels (5.89-fold upregulation) of peroxiredoxin 6 (Prdx6), a member of the peroxiredoxin family of antioxidant proteins which is downregulated in OA cartilage (3). MV treatment increased the expression of Prdx6 in OA chondrocytes suggesting a protective role against oxidative stress in these cells.
Conclusions: MV from AD-MSC downregulate the production of oxidative stress and inflammatory mediators in OA chondrocytes. Prdx6, an antioxidant enzyme, may contribute to the protective effects of MV. Our data support the interest of these MV to develop new therapeutic approaches.
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FRI0015 MODELLING THE INITIAL PHASE OF FRACTURE HEALING IN VITRO – 3D BONE-LIKE MODELS OF ENDOCHONDRAL OSSIFICATION

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Background: Immunosuppressed patients with ongoing inflammation experience more often difficulties in the process of fracture healing. Hereby amongst others immunomodulated activation of osteoclasts leads to augmented osteolysis. Most notably numerous cytokines and invading lymphocytes provide an inflammatory environment within the fracture gap, utmost during the first phase of fracture healing. Nowadays researchers, whilst investigating fracture healing, use small rodent models, facing the problem of translation towards the human system. Hence, there is a lack of valid *in vitro* models to examine the first phase of fracture healing. To test new therapeutic strategies, we develop a valid 3D-model using human cells to mimic the first phase of fracture healing *in vitro* which is characterized by the formation of a fracture hematoma, a hypoxic microenvironment as well as inflammation that initiate the healing cascade and the process of endochondral ossification.
Objectives: To develop 3D bone-like models displaying endochondral ossification.
Methods: As a first step, we focus on establishing 3D bone-like, matrix-free models consisting of human mesenchymal stromal cells (hMSC). MSC were isolated from bone-marrow samples of patients undergoing total hip replacement and characterized with regard to their typical surface markers as well as their differentiation potential towards osteogenic, adipogenic and chondrogenic lineage. 3D bone-like models were generated from micro-mass culture of hMSC (Research Center of Medical Technology and Biotechnology, Bad Langensalza). After initial maturation, 3D bone-like models were cultured under hypoxic conditions (37°C, 1% O2) in osteogenic medium for up to 3 months. *In vitro* μCT analyses were performed at day 0, after one and two months focusing on the total volume (TV) and bone volume (BV). Additionally osteogenic-relevant genes/factors (Runx2, SPP1, Dlx5, ALP, RANKL, SPI1) as well as exclusion markers (SOX9, PPARg2) were investigated after 3 months of cultivation using qRT-PCR. Finally, we implemented histological/immunohistochemical methods (van Kossa, ALP, Col1 staining).
Results: The 3D bone-like models achieved diameters between 0.5 and 0.7 and a thickness of 0.3 cm. *In vitro* μCt analysis revealed a high amount of mineralized tissue at day 0 and substantial increase in the bone volume (BV/TV) after cultivation. At day 0 *in vitro* μ Ct analysis implied mineralization mostly at the margin but penetrated the tissue within further cultivation. These results are also supported by positive van Kossa staining. Additionally, qRT-PCR results yielded higher expressions of *RUNX2, SPP1, SPI1, RANKL* and *DLx5*. Furthermore, immunohistochemistry showed high ALP-activity and CoII-expression.
Conclusions: Preliminary results of our study focusing at developing a 3D bone-like model displayed a promising trend towards modelling endochondral ossification *in vitro* by increased mineralization (*in vitro* μCt analysis and van Kossa staining), and upregulation of osteogenic-relevant *RUNX2* and *SPP1* expression as well as ALP-activity and CoII-expression. High expression of *SPI1* and *RANKL* could refer to osteoclast-like activities, which will be in the focus of further investigations. Finally, the complete 3D model will leave us the opportunity for studying the first phase of fracture healing under *in vitro* conditions.
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FRI0016 INVOLVEMENT OF RUNX-2 AND β-CATENIN SIGNALING IN THE PRODUCTION OF ADAMTS-7 AND ADAMTS-12 IN OSTEOARTHRITIC SYNOVIAL FIBROBLASTS

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Background: Proteinases released from synovial membrane of osteoarthritis (OA) patients contribute to cartilage damage. We recently reported in synovial fibroblasts (SF) the expression of ADAMTS (a disintegrin and metalloproteinase domain with thrombospondin motifs)-7 and -12, involved in the destruction of the cartilage oligomeric matrix protein (COMP) (1). Signaling pathways regulating these ADAMTS are poorly understood. As Runx2 and β-catenin are two transcription factors involved in chondrocytes metabolism and OA pathology (2–5), we studied whether these factors are implicated in the production of ADAMTS-7 and 12 in SF. Moreover, we analyzed the induction by two inflammatory mediators present in OA joints: interleukin-1β (IL-1β), and fibronectin fragments (Fn-fs), previously described in ADAMTS expression (1).
Objectives: To elucidate the signaling pathways involved in the production of ADAMTS-7 and -12 in healthy donors (HD) - and OA-SF.
Methods: ADAMTS-7 and -12 were detected in HD- and OA-SF protein extracts by Western blot. Blockade experiments were performed after stimulation with IL-1β or 45-kDa Fn-fs. We used inhibitors for two mitogen-activated protein kinases (MAPKs), ERK and p38, implicated in the activation of Runx2, PD98059 and SB203580, respectively. We also used an inhibitor of Wnt/β-catenin signaling, DDK-1. Levels of ADAMTS-7 and -12 were analyzed by quantitative RT-PCR and ELISA in SF culture supernatants.
Results: Intracellular presence of ADAMTS-7 and -12 was confirmed in HD- and OA-SF, with higher levels of ADAMTS-7 in OA. After IL-1β or Fn-Fs stimulation, DDK-1 decreased ADAMTS-7 transcript in HD and OA-SF that was translated to a protein reduction in OA. Besides, ERK inhibitor decreased ADAMTS-12 mRNA and protein exclusively in OA-SF.
Conclusions: We reported that ADAMTS-7 protein expression is higher in OA-SF compared to HD confirming previous data at mRNA level (1). As DKK decreased ADAMTS-7, Wnt-β-catenin signaling seems to be implicated in its expression. By contrast, the expression of ADAMTS-12 is regulated by ERK, pointing to a possible implication of ERK-Runx2 axis, exclusively in OA-SF.

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FRI0017 A NOVEL CONCEPT OF M1 AND M2 MONOCYTES IN RHEUMATOID ARTHRITIS: PRO-INFLAMMATORY MONOCYTE POLARIZATION IMBALANCE, ANTI-CITRULLINATED PROTEIN ANTIBODY AND OSTEOCLASTOGENESIS

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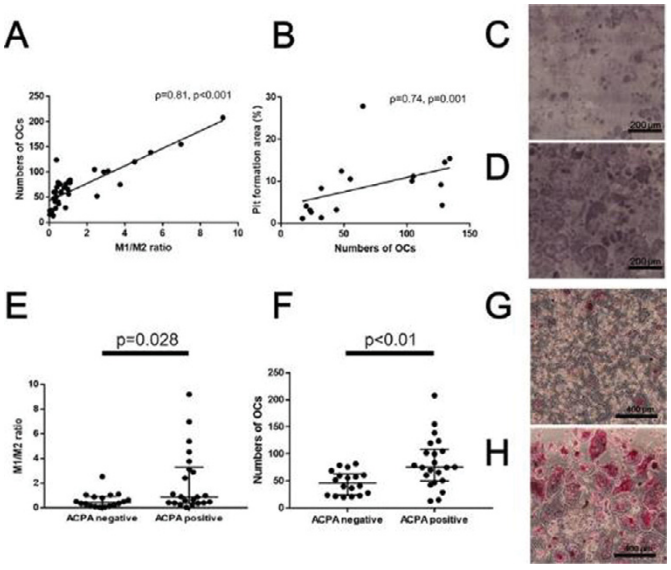
Background: Monocytes can differentiate into either proinflammatory, microbicidal M1 macrophage or anti-inflammatory M2 macrophage subtypes. In addition to macrophages, regarding monocyte subsets, M1 monocytes and M2 monocytes mirroring the M1/M2 macrophage polarization concept were suggested.
Little is known regarding the relationships between osteoclastogenesis and M1/M2 monocyte subsets.
Objectives: We investigated the relationships among M1 monocytes, M2 monocytes, osteoclast differentiation ability and clinical characteristics in patients with rheumatoid arthritis (RA).
Methods: Peripheral blood mononuclear cells (PBMCs) were isolated from RA patients and healthy donors, and we then investigated the number of M1 monocytes or M2 monocytes by fluorescence-activated cell sorting. We defined positive CD14, CD68 and CCR2 monocytes as M1 monocytes, and in separate tubes, we defined positive CD14, CX3CR1 and CD163 or CD206 monocytes as M2 monocytes.
We also obtained and cultured CD14-positive cells from PBMCs from RA patients and healthy donors to investigate osteoclast differentiation in vitro. We defined osteoclasts as tartrate-resistant acid phosphatase (TRAP) staining-positive cells with >3 nuclei. We counted the osteoclasts in the whole wells of a 96-well dish. Pit formation assays were performed to evaluate function of osteoclasts.
Results: Forty RA patients and 20 healthy donors were included. Twenty-two patients (55%) were ACPA-positive.
The median M1/M2 ratio was 0.59 (0.31–1.11, IQR). There were no significant differences between the RA patients and healthy donors.
There was a positive correlation between the M1/M2 ratio and the differentiated osteoclast number in vitro in RA patients (ρ=0.81, p<0.01) (A). The numbers of osteoclasts in vitro were significantly correlated with the area percentage of the pit formation area (ρ=0.74, p=0.001) (B).We demonstrated that the RA patients who had lower M1/M2 ratio and fewer osteoclasts (C) had smaller resorbed areas compared to the RA patients who had higher M1/2 ratio and greater numbers of osteoclasts (D).
The ACPA-positive patients had significantly higher M1/M2 ratio in vivo (p=0.028) (E) and significantly greater numbers of osteoclasts in vitro (p<0.01) (F) than the ACPA-negative patients. We show an ACPA-negative patient's osteoclasts in vitro (G) and those of an ACPA-positive patient (H).



Multivariable regression analysis revealed that the M1/M2 ratio was the sole significant contribution factor to in vitro osteoclastogenesis (β-coefficient 16.3, p<0.0001).
RA patients with M1/M2 ratio >1 (having relatively more M1 monocytes) had higher erythrocyte sedimentation rates (p=0.011) and C-reactive protein (p=0.032) than RA patients with M1/M2 ratio ≤1.
M1-dominant monocytes in vitro produced higher concentrations of IL-6 upon stimulation with lipopolysaccharide than M2 monocytes (p=0.032).
Conclusions: The M1/M2 ratio is strongly correlated with the in vitro differentiation of osteoclasts in patients with RA. The RA patients with positive ACPA had higher M1/M2 ratio and higher numbers of osteoclasts.
M1 and M2 monocyte subsets may become a new target of treatments for RA.
Disclosure of Interest: None declared
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FRI0018 TARGETED INHIBITION OF JANUS KINASES ABATES IFN-GAMMA-INDUCED INVASIVE BEHAVIOR OF FIBROBLAST-LIKE SYNOVIOCYTES

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Background: Emerging evidence suggests that fibroblast-like synoviocytes (FLS) are key effector cells in rheumatoid arthritis (RA) and research into the mechanisms defining FLS activity in RA indicate that cytokines secreted by leukocytes play a crucial role. Nevertheless, the contribution of IFNγ, which is increased in rheumatoid synovitis, to the inflammatory synovial tissue reaction is not known.
Objectives: To explore the function of the T-cell cytokine IFNγ for mesenchymal tissue remodeling in RA, and to determine whether IFNγ-signaling controls the invasive potential of FLS.
Methods: To assess architectural responses, FLS were cultured in three-dimensional micromasses. FLS motility was analyzed in migration-, spreading- and invasion assays. Signaling events relevant to cellular motility were defined by western blots. Baricitinb and siRNA pools were used to suppress Janus Kinase (JAK) functions.
Results: Histological analyses of micromasses revealed unique effects of IFNγ on FLS shape and tissue organization. This was consistent with accelerated migration, pronounced actin and focal adhesion (FA) re-organization upon IFNγ stimulation. Since actin and FA dynamics and, thus, cell motility are integrated by the focal adhesion kinase (FAK), we next analyzed its activity. Indeed, IFNγ stimulation induced the phosphorylation of FAK-Y925, a phosphosite implicated in FAK-mediated cell migration. siRNA knockdown of JAK2, but not JAK1, abrogated FAK activation by IFNγ. Correspondingly, IFNγ-inudced FAK activation and invasion of FLS was abrogated by the JAK-inhibitor baricitinib.
Conclusions: Our study contributes insight into the synovial response to IFNγ and reveals JAK2 as a potential therapeutic target for FLS-mediated joint destruction in arthritis, especially in RA.
Disclosure of Interest: None declared
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FRI0019 HUMAN MESENCHYMAL STROMAL CELLS REDUCE TNFα SECRETION OF ACTIVATED PBMC VIA CTLA-4

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Background: The inhibitory costimulatory molecule Cytotoxic T-Lymphocyte Antigen-4 (CTLA-4) plays a crucial role in conveying immune tolerance in both inflammatory disorders and regenerative processes such as bone healing. During regenerative processes, mesenchymal stromal cells (MSC) provide the building bricks for reestablishing structural integrity but do also control inflammation by their immunomodulatory activities under restrictive microenvironmental conditions such as hypoxia. Here, we hypothesize these cells to support the control of inflammation via CTLA-4 in order to facilitate tissue regeneration such as bone fracture healing.
Objectives: Therefore, we analyzed expression of CTLA-4 by human MSC and their ability to convey immune suppression.
Methods: MSC were isolated from bone marrow of patients undergoing total hip replacement and characterized (i) by surface marker staining using flow cytometry and (ii) via assessing their osteogenic and adipogenic differentiation potential. MSC were cultured under normoxic (~18% O_2) and hypoxic (<1.5% O_2) con-