Conclusions: The in vitro results confirmed that 2ccPA had suppressing effect of cartilage degrading enzymes expression on SFs and chondrocytes, supports the hypothesis that 2ccPA might have played direct role to suppress inflammation and also protect articular cartilage in arthritic condition.
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AB0066 HUMAN MULTIPOTENTIAL STROMAL CELLS EXPRESS LOW SURFACE LEVELS OF PRO-INFLAMMATORY CYTOKINE RECEPTORS IN BONE HEALING DEFECTS

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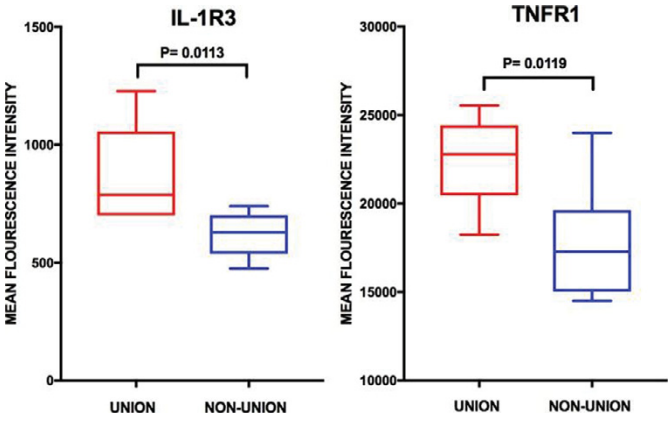
Background: Osteoimmunology is an evolving field where the multipotential stromal cells (MSCs) can be considered as an important player linking immune response with bone generation. A group of pro-inflammatory cytokines including IFN-γ, TNF-α, IL-17 and IL-1, has been proven to have a licensing effect on MSCs promoting the immunomodulatory activities of MSCs (1). Importantly, these cytokines can regulate the osteogenic differentiation capability of MSCs and in particular, IL-1 and IL-17 can enhance the MSC osteogenesis as shown in previous in vitro studies (2,3). However, little is known about the role of these cytokine-MSC interactions in the bone-related diseases in humans.
Objectives: The main focus of this study was to assess if the immune-dependent licensing process of MSCs could be involved in defective bone regeneration.
Methods: We used samples of bone marrow aspirates (n=15) from two groups of traumatic bone fracture patients; normal union and non-union. Bone marrow MSCs were analyzed for the surface expression of the receptors of the pro-inflammatory licensing cytokines using flowcytometry-optimized panels. Additionally, a comparison of the cytokine effect on the proliferation of cultured MSCs was compared between normal union and non-union groups using the cell proliferation XTT test.
Results: Interestingly, there were significant lower expression levels of IL-1 receptors 1 and 3 (IL-1R1 and IL-1R3) on non-union MSCs compared to normal-union MSCs (p=0.0478 and p=0.0113 respectively). Furthermore, the surface levels of TNF-αR1 (CD120a) were significantly lesser on non-union MSCs (p=0.0119). There was a clear trend of reduced expression of IL-17 receptors (CD217) on the surface of non-union MSCs, but it was not statistically significant compared to normal-union (p=0.0726). The XTT data showed a significant less proliferation index for IL-1-treated non-union MSCs compared to normal-union MSCs (p=0.0446). Also, a consistent trend of lower proliferation index of non-union MSCs was detected when these cells were treated by IFN-γ, TNF-α or IL-17.



Conclusions: Together, the lower levels of the pro-inflammatory cytokine receptors indicated a possible mechanism for a defective response of non-union MSCs to the inflammatory signals (particularly IL-1). Further understating of the impact of immune-MSC interactions on human bone healing and regeneration will help to develop new therapies for musculoskeletal diseases involving osteolytic lesions.
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AB0067 INFLUENCE OF ADIPOKINES ON DIFFERENTIATION OF SPONGIOSA-DERIVED MESENCHYMAL STROMAL CELLS FROM OSTEOPOROTIC AND OSTEOARTHRITIS PATIENTS

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Background: Osteoporosis (OP) and osteoarthritis (OA) are two common age-related disorders leading to chronic pain and disability in elderly people. Age-related bone loss and articular cartilage damage are associated with increased bone marrow adiposity due to a possible shift of osteogenic differentiation towards adipogenic differentiation of bone marrow mesenchymal stem cells (MSC). The differentiation of MSC into adipocytes or osteoblasts is an important determinant of bone structural integrity. Adipose tissue is an metabolically active tissue. Therefore adipocyte-derived factors -adipokines- might influence differentiation of bone marrow-derived MSC.
Objectives: The role of fat-bone interactions in the pathogenesis of OP is poorly understood. Therefore, we analyzed the presence of distinct adipokines (visfatin, resistin and leptin) in the bone marrow cavity and their effects on MSC differentiation.
Methods: Spongiosa from femoral heads was collected (hip replacement surgery of OA patients or after osteoporotic femoral neck fracture). MSC were cultured in adipogenic and osteogenic media with/without adipokines. For the transfer and differentiation of MSC on cancellous bone, bone fragments were purified and sterilized. mRNA expression of adipokines, bone marker genes, TIMPs and MMPs of stimulated MSC and bone samples were evaluated by realtime PCR. Matrix mineralization was assayed using Alizarin red S staining. Proinflammatory factors were measured by ELISA.
Results: Visfatin and leptin levels were increased in OP bone vs. non-osteoporotic bone (n=14). In contrast to leptin and resistin, visfatin induced the secretion of proinflammatory factors (IL-6, IL-8, MCP-1) during both, osteogenic and adipogenic differentiation. Visfatin significantly increased the matrix mineralization and downregulated collagen type 1-expression (e.g. d21: -4.6-fold) in osteogenic differentiated cells. Visfatin also reduced the expression of MMP2, MMP13, RunX2, TIMP1 and TIMP2 (e.g. d21: -2.4-fold/-3.18-fold/-5.85-fold/-3.2-fold/-4.3 fold respectively) during osteogenic differentiation, but not leptin and resistin. In contrast to osteogenesis, visfatin significantly induced MMP13 expression (e.g. d21: 104-fold) during adipogenic differentiation under standard cell culture conditions. However, visfatin-induced MMP13-expression was markedly reduced during differentiation on purified autologous cancellous bone.
Conclusions: Visfatin and leptin levels were elevated in osteoporotic bone tissue. Therefore, the visfatin-mediated increase of matrix mineralization and reduction of collagen type 1 expression might lead to enhanced bone fragility and contribute to the pathogenesis of OP. Visfatin induced release of proinflammatory cytokines and dysregulated expression of MMPs and TIMPs during osteogenic and adipogenic MSC-differentiation might influence bone turnover specifically at the adipose tissue/bone interface.
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AB0068 HYPOXIA AND RHEUMATOID PHENOTYPE DECREASE THE CAPACITY OF SYNOVIAL FIBROBLASTS TO SUPPRESS T HELPER CELL PROLIFERATION THROUGH IDO1-MEDIATED TRYPTOPHAN CATABOLISM

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Background: The pathogenesis of rheumatoid arthritis (RA) is linked to functional changes in synovial fibroblasts (SF) and local infiltration of T lymphocytes. Increased synovial inflammation is also associated with a hypoxic joint microenvironment. Oxygen levels in the joints of RA patients are significantly decreased compared to those of osteoarthritis (OA) patients with values of about 22.5mmHg corresponding to ambient oxygen tensions of 3.2%. So far, little is known about the effects of hypoxia on the interaction between fibroblasts and T lymphocytes and its implications on the pathophysiology of RA.
Objectives: The aim of this study was to compare the influence of SF from RA versus OA patients on T helper (Th) cell responses both under normoxic and hypoxic conditions.
Methods: SF were isolated from synovectomy tissues of OA or RA patients, Th cells were isolated from peripheral blood of RA patients or healthy donors. Cell cultures were performed under normoxic or hypoxic (3% O_2) conditions. Th cell proliferation was determined by PKH26 labelling and flow cytometry. Cytokine secretion was quantified by ELISA. Indoleamine 2,3-dioxygenase 1

(IDO1) expression was analysed by Western Blot and expression of enzymes of the kynurenine pathway by real-time PCR. Tryptophan/ kynurenine levels in culture supernatants were quantified by HPLC.
Results: SF strongly inhibited the proliferation of co-cultured Th cells. Tryptophan was completely depleted within a few days in co-cultures of SF and Th cells, resulting in eukaryotic initiation factor (eIF)2α phosphorylation, TCRζ-chain down-regulation and proliferation arrest. Blocking of IDO1 completely restored Th cell proliferation, indicating that SF suppressed the proliferation of Th cells through IDO1-mediated tryptophan catabolism. Interestingly, RASF showed a significantly lower IDO1 expression, tryptophan metabolism and a weaker Th cell suppressive capacity compared to OASF. Under hypoxic conditions, the secretion of IFNg, the expression of IDO1, the tryptophan metabolism and the Th cell suppressive capacity of both OASF and RASF were significantly reduced.
Conclusions: SF suppressed Th cell growth through IDO1-mediated tryptophan catabolism. This mechanism may play an important role in preventing inappropriate Th cell responses under normal conditions. The reduced tryptophan metabolism under hypoxia together with the inferior efficiency of RASF to restrict T cell proliferation likely supports the development of synovitis in RA.
Disclosure of Interest: None declared
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AB0069 STRONG AGE-DEPENDENT EFFECTS OF DOPAMINE ON JOINT INVASION IN ARTHRITIS

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Background: Preventing synovial fibroblasts (SF) from migrating into the adjacent cartilage is a desirable therapeutic target in rheumatoid arthritis (RA) in order to avoid joint destruction and disability. In our previous studies we could show that RASF as well as SF from osteoarthritis (OA) patients express all dopamine receptor (DR) subtypes and dopamine stimulation alters pro-inflammatory cytokines (*Capellino S et al, A&R 2014*).
Objectives: Therefore, we aimed to elucidate a potential dopamine-mediated impact on joint invasion and destruction in arthritis.
Methods: SF from RA and OA patients were obtained from patients undergoing knee joint replacement surgery (mean age: 74.3±11.3yrs at OA and 73.7±10.3 yrs at RA patients) to investigate dopamine receptor (DR)-distribution within the RA synovium and in the invasion zone, immunohistochemistry was performed for all five DR-subtypes. Migration and motility assays were performed under D1-like (D1DR and D5DR) and D2-like (D2DR, D3DR and D4DR) receptor stimulation. Dopamine effects on MMP3 and proMMP1 were evaluated using ELISA.
Results: D1DR, D4DR and D5DR were found to be stronger expressed close to the invasion zone and more cells were expressing the respective DR. Migration of RASF and OASF was significantly correlated with patients' age at surgery: younger patients ($\leq$75years) showed an increase in migration up to 78% whereas older patients ($\geq$75years) showed a reduced migration of up to 50% (OA n=8; RA n=7). There was no difference between RA and OA patients and between D1-like and D2-like receptor stimulation. The same effect could be observed in the motility assay (OA n=5; RA n=6). MMP3 levels are altered under DR activation (OA n=6; RA n=6).
Conclusions: The high DR expression close to the invasion zone suggests a direct role of dopamine on RASF aggressiveness and may contribute to cartilage invasion. This was confirmed in the in vitro assays, and supports the idea of a therapeutic potential of the dopamine pathway in RA.
Disclosure of Interest: None declared
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AB0070 CHONDROPROTECTIVE EFFECTS LINKED TO REDUCTION OF ADIPOGENESIS AND REGULATION OF GAP JUNCTION INTERCELLULAR COMMUNICATION: REGENERATIVE POTENTIAL THERAPEUTIC APPROACH FOR OSTEOARTHRITIS

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Background: Human bone marrow mesenchymal stem cells (hMSCs) exhibit an age-dependent reduction in osteogenesis and an increased propensity toward adipocyte differentiation. This switch has been associated with different bone disorders characterized by reduced bone formation and increased bone marrow fat accumulation. Connexin43 (Cx43) is an integral membrane protein that forms gap junction channels (GJs) and it is implicated in multiple cellular functions including cellular differentiation and control of bone remodelling and cartilage structure and function.
Objectives: In this study we investigated the effect of oleuropein and other molecules isolated from the *Olea europaea* in cellular differentiation to test if these molecules act as adipogenic suppressors in order to promote bone ad cartilage regeneration through a Cx43-dependent mechanism.
Methods: hMSCs were obtained from bone marrow donors. Human chondrocytes were isolated from cartilage of healthy donors and patients with osteoarthritis (OA). Differentiation assays were carried out in the presence of different concentrations of oleuropein and olive extract (OE). Cellular differentiation was evaluated using histological stains. Scrape loading assays, western-blot, real-time qPCR and immunohistochemistry (IHC) assays were used to study the cellular communication through gap junction (GJs) and the levels and changes in subcellular localization of Cx43 and extracellular matrix (ECM) proteins such as Collagen type II (Col2).
Results: hMSCs treated with olive derived molecules showed a two-fold decrease in adipogenic differentiation, while osteogenesis and chondrogenesis were significantly increased. IHC, RT-qPCR and Western-Bot analysis of hMSCs supplemented with oleuropein/OE showed increased levels of Cx43. On the other hand, OA chondrocytes showed higher levels of Cx43 in comparison with normal chondrocytes; oleuropein and OE treatments significantly decreased Cx43 levels and dye transference through GJ channels. The treatment of OA chondrocytes micromasses with oleuropein and OE increased the levels of proteoglycans and Col2 in the extracellular matrix. These changes in the micromasses were accompanied by a decrease in Cx43 levels improving protein subcellular localization.
Conclusions: Together, our results suggest that the molecules used in this assays, via Cx43 and GJ intercellular communication increase the propensity towards osteogenesis and chondrogenesis, reducing adipocyte differentiation. Our assays indicate that these molecules may represent a potential therapeutic approach for cartilage and bone age-related disorders such as OA in order to promote cartilage regeneration.
Disclosure of Interest: None declared
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AB0071 ANALYSIS OF SYNOVIAL EXPRESSION OF PARVOVIRUS B19 ASSESSED IMMUNOHISTOCHEMICALLY AND CORRELATED TO SYNOVITIS SCORE

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Background: Apart from systemic symptoms of viral infection parvovirus B19 (B19) could lead to acute and chronic arthropathy. It has been found in synovial tissue of rheumatoid arthritis (RA) patients, sometimes, being associated with some forms of undifferentiated arthritis. Possibly, it could promote inflammation in various forms of arthritis.
Objectives: To determine the expression of B19 antigens in different compartments of synovial membrane; correlate these findings to the estimated synovitis score.
Methods: 7 RA and 54 osteoarthritis (OA) patients were enrolled in this study. nPCR was used to detect the presence of B19 genomic sequence in 61 samples of synovial tissue. PCR B19 positive tissue samples were immunohistochemically treated with the anti-B19 antibodies detecting B19 capsid proteins' VP1/VP2 expression estimated thereafter semiquantitatively. The intimal and subintimal layers of synovium as well as vasculature were estimated. Synovial inflammation was evaluated using synovitis score.
Results: 3 RA and 3 OA patients were PCR B19 tissue positive. B19 antigens' expression was observed in synovial lining, immune infiltrates and vascular endothelium. Correlation between B19 expression observed in synovial cells and inflammatory infiltrates' lymphocytes and macrophages was r=0.555 ($p<0.0001$) and r=0.793 ($p<0.0001$), respectively. Likewise, correlation between vascular endothelial B19 expression and synovial lymphocytic infiltrates was demonstrated r=0.616 ($p<0.0001$). Determined synovitis score varied from low up to intermediate revealing median value 2. Simultaneously, there was no correlation found between the synovitis score and B19 antigens' expression.
Conclusions: B19 capsid proteins' VP1/VP2 expression is detectable in different structural constituents of synovial membrane. Under conditions studied, the tissue expression of B19 antigens does not correlate with the inflammatory indices scored.
Disclosure of Interest: None declared
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