Disclosure of Interest: None declared
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FRI0006 PROTEIN CITRULLINATIONS BY PAD ENZYMES PROMOTE DENDRITIC CELL TRANSDIFFERENTIATION INTO OSTEOCLAST AND GENERATE TARGETS FOR RA-SPECIFIC ANTIBODIES

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Background: Immature dendritic cells (DCs) are able to trans-differentiate into osteoclasts (OCs) although the mechanisms regulating this process are little understood. We have recently described an important role for protein citrulliantion and peptidylarginine deiminase (PAD) enzyme activity in the regulation of OC development (1).
Objectives: We studied the molecular bases of DC-OC trans-differentiation and aimed at understanding the role of protein citrullination in this process.
Methods: Monocyte-derived DCs and peripheral blood CD1c+ DCs were cultured in the presence of osteoclastogenic cytokines. Polyclonal ACPAs were isolated from the serum of RA patients and applied in OC cultures. DC and OC differentiation was analyzed *in vitro* using gene expression analyses, flow cytometry-based methods, DC-T cell co-culture experiments, tartrate-resistant acid phosphatase stainings and osteolysis assays. Protein citrullination was monitored with the help of mass spectrometry. PAD activity was measured by ELISA.
Results: Different DC types showed different capacities to develop into OCs. OC-prone DCs were characterized by little immunogenicity and their development was potentiated by the increase of lactic acid, a side product of glycolytic metabolism. The more immunogenic DC types, characterized by prominent ability to migrate towards secondary lymphoid tissues and trigger T cell activation, showed a limited capacity to develop into OCs (2). The differentiation switch towards the OC lineage was associated with increased activity of the Protein Arginine Deiminase (PAD) enzymes and with higher level of protein citrullination in DCs. The PAD inhibitor Cl-Amidine efficiently interfered with OC development form DC precursors. In addition, the deposition of citrullinated proteins on the cell surface made the cells sensitive for anti-citrullinated protein autoantibodies, which could further stimulate DC-OC trans-differentiation through inducing the cytokine IL-8.
Conclusions: Our results indicated that DCs are heterogenic in their ability to form OCs and lineages for immunostimulatory and OC-prone DCs might separate early during DC differentiation. Plasticity towards OC differentiation might be influenced by the metabolic environment and the upregulation of PAD activity in DCs.
References:
[1] Krishnamurthy A et al. Ann Rheum Dis 2016.
[2] Nasi A et al. J Immunol 2013.
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FRI0007 METHYL GALLATE INHIBITS OSTEOCLAST FORMATION AND FUNCTION THROUGH SUPPRESSING THE AKT AND BTK-PLCγ2-CA2+ SIGNALING, AND PREVENTS LPS-INDUCED BONE LOSS

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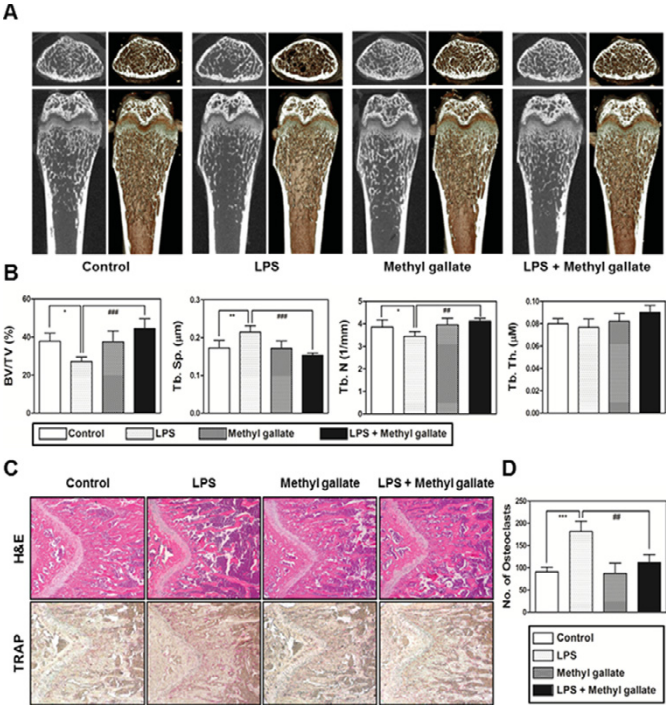
Background: Methyl gallate, a plant-derived phenolic constituent has been known to possess numerous pharmacological features against inflammation, oxidation, and cancer. But so far, there have been no evidences to describe relationship between methyl gallate and bone metabolism.
Objectives: In order to propose a promising candidate for osteoporosis, we performed experiments in this study by using methyl gallate.
Methods: we performed screening of methyl gallate utilizing TRAP staining and revealed intracellular mechanisms responsible for methyl gallate-mediated regulation of osteoclastogenesis through western blotting and quantitative RT-PCR. Also, we assessed the role of methyl gallate on characteristics of mature osteoclasts. we used LPS-induced bone loss mice as a model of osteoporosis and analyzed using micro-CT system and the right femurs were stained with TRAP and H&E.
Results: we observed that methyl gallate significantly suppressed osteoclast formation through Akt and Btk-PLCγ2-Ca^{2+} signaling. The blockade of these pathways was reconfirmed through transduction of CA-Akt retrovirus and evaluation of Ca^{2+} influx intensity stained with Fluo-3/AM. Indeed, methyl gallate down-regulated the formation of actin ring-positive osteoclasts and resorption pit areas. In agreement with in vitro results, we found that the administration of methyl gallate restored osteoporotic phenotype stimulated by acute systemic injection of LPS in vivo through micro-CT and histology.



Conclusions: Consequently, the overall data strongly indicated that methyl gallate could be a useful substance for development of plant-based anti-osteoporotic agent.
Disclosure of Interest: None declared
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FRI0008 EFFECTS OF THE HUMAN IL4-10 FUSION PROTEIN IN THE CANINE GROOVE MODEL OF OSTEOARTHRITIS

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Background: Ideally a disease modifying osteoarthritis drug (DMOAD) combines treatment for pain, tissue damage and inflammation, all in one molecule. Intra-articular application of a DMOAD brings additional value to treatment for two reasons, (i) lower risk of systemic side effects and (ii) higher drug concentration and potentially improved penetration of non-vascularized articular cartilage. Interleukin-4 (IL-4) and Interleukin-10 (IL-10) have been shown to prevent joint degeneration and can work synergistically[1].
Objectives: This study evaluates the DMOAD activity of repetitive intra-articular injections with a human fusion protein of IL-4 and IL-10 (hIL4–10FP) in the canine Groove model of osteoarthritis (OA).
Methods: In 8 dogs joint degeneration was induced according to the Groove model. Six weeks after surgery dogs were treated with ten weekly intra-articular injections of either hIL4–10FP (n=4) or PBS (n=4). Subsequently, dogs were euthanized and cartilage and synovium were harvested. Cartilage damage and synovial inflammation were macroscopically evaluated. Proteoglycan release and content were determined ex vivo by staining of glycosaminoglycans (GAGs) with Alcian Blue. Proteoglycan synthesis was measured by $^{35}SO_4^{2-}$ incorporation and precipitation with cetylpyridium chloride and liquid scintillation analysis of $^{35}SO_4^{2-}$-labeled GAGs. Potential antibody formation against hIL4–10FP was evaluated with ELISA and a cell based assay. Immunohistochemistry of CD79α and CD10 was used to evaluate the presence of B-cells in synovium.
Results: Affected knees of PBS treated dogs showed enhanced macroscopic cartilage damage compared to their controls (0.31 vs 2.44, p=0.068). Also differences in proteoglycan release, content and synthesis indicated a degenerative state in the affected knees.
Unexpectedly, enhanced synovial inflammation was observed in hIL4–10FP treated joints compared to PBS treated joints, demonstrated by enhanced macroscopic (3.5 vs 2.3 out of 5) and histologic (2.5 vs 1.8 out of 6) scores. CD79α and CD10 showed enhanced expression in synovium of the hIL4–10FP group compared to the PBS group, although not statistically significant (fig 1). Additional analyzes showed that hIL4–10FP was immunogenic in dogs after multiple injections. Formation of neutralizing antibodies was shown in a cell based assay where the activity of hIL4–10FP was inhibited in the presence of serum of hIL4–10FP treated dogs (fig 2). Despite the enhanced inflammatory response

in hIL4–10FP group there was no enhanced cartilage degeneration detected compared to the PBS group (fig 3).

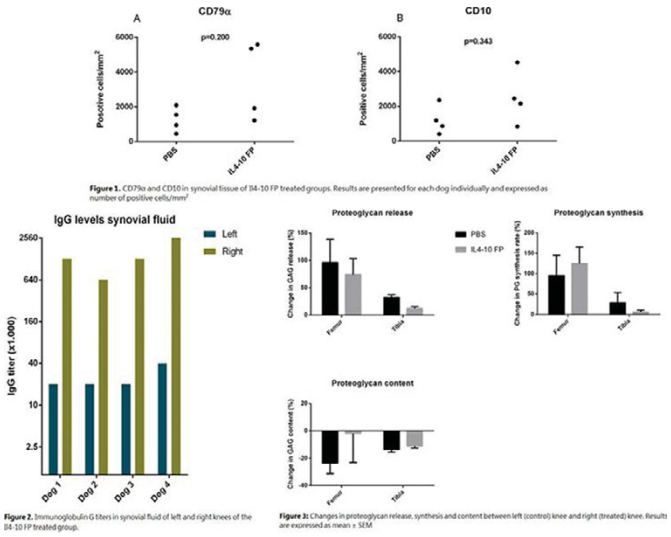

Figure 1. CD79α and CD10 in synovial tissue of Il4-10 FP treated groups. Results are presented for each dog individually and expressed as number of positive cells/mm²

Figure 2. Immunoglobulin G titers in synovial fluid of left and right knees of the Il4-10 FP treated group.

Figure 3: Changes in proteoglycan release, synthesis and content between left (control) knee and right (treated) knee. Results are expressed as mean ± SEM

Conclusions: Repetitive intra-articular injection of human IL4–10FP led to antibody formation in a non-inflammatory canine model of OA. Despite the immune response, proteoglycan turnover parameters were comparable between the two treatment groups, suggesting a beneficial effect of hIL4–10FP. This study also shows that it is not evident to use a human protein in a (canine) animal model, although this is often done. Instead, a species specific protein is warranted. Therefore a canine version of IL4–10FP will be developed to study its DMOAD activity in this model.

References:

[1] van Roon JA, Lafeber FP, Bijlsma JW. Synergistic activity of interleukin-4 and interleukin-10 in suppression of inflammation and joint destruction in rheumatoid arthritis. Arthritis and rheumatism. 2001;44(1):3–12.

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FRI0009 ACCELERATED DEVELOPMENT OF AGING-ASSOCIATED AND INSTABILITY-INDUCED OSTEOARTHRITIS IN 12/15-LIPOXYGENASE DEFICIENT MICE

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Background: 12/15-Lipoxygenase (12/15-LOX) catalyzes the generation of various anti-inflammatory lipid mediators, and has been implicated in several inflammatory and degenerative diseases. However, there is currently no evidence that 12/15-LOX has a role in osteoarthritis (OA).

Objectives: The aim of this study was to investigate the role of 12/15-LOX in the pathogenesis of OA

Methods: The development of aging-associated and destabilization of the medial meniscus (DMM)-induced OA were compared in 12/15-LOX-deficient (12/15-LOX-/-) and wild-type (WT) mice. The extent of cartilage damage was evaluated by histology. The expression of OA markers was evaluated by immunohistochemistry and RT-PCR. Cartilage explants were stimulated with IL-1α in the absence or presence of the 12/15-LOX metabolites, 15-HETE, 13-HODE or LXA4, and the levels of MMP-13, NO and PGE_2 were determined. The effect of LXA4 on the progression of OA was evaluated in WT mice.

Results: The expression of 12/15-LOX in cartilage increased during the progression of DMM-induced OA and with aging in WT mice. Cartilage degeneration was more severe in 12/15-LOX-/- mice compared to WT mice in both models of OA, and this was associated with increased expression of MMP-13, ADAMTS5, iNOS, and mPGES-1. Treatment of cartilage explants with 12/15-LOX metabolites, suppressed IL-1α-induced production of MMP-13, NO and PGE_2, with LXA4 being the most potent. Intra-peritoneal injection of LXA4 reduced the severity of DMM-induced cartilage degradation.

Conclusions: These data demonstrate an important role of 12/15-LOX in OA and suggest that activation of this pathway may provide a novel strategy for prevention and treatment of OA.

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FRI0010 METABOLIC DYSREGULATION ACCELERATES JOINT DEGENERATION UPON MECHANICALLY INDUCED CARTILAGE DAMAGE, DRIVEN BY LOCAL INFLAMMATION; AN IN VIVO RAT STUDY

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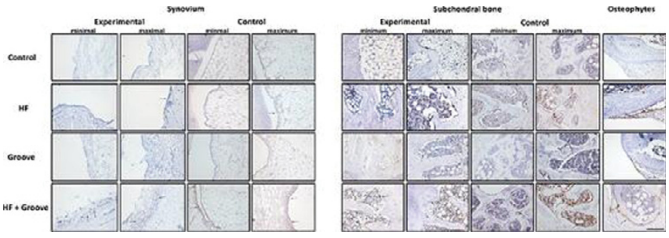
Background: Obesity is a well-known and important risk factor for osteoarthritis (OA). Moreover, obesity is highly associated with the metabolic syndrome (MetS)[1]. Growing evidence indicates that both OA and MetS are low-grade inflammatory conditions with elevation in systemic inflammatory markers. Nonetheless, it is unclear whether MetS low-grade inflammation induces OA, or contributes to the disease.

Objectives: To determine the contribution of metabolic alterations, induced by a High-Fat Diet (HFD), on the onset or progression of OA in a rat model of local cartilage damage.

Methods: Forty Wistar rats (12 weeks old, male), were randomly divided over two groups: twenty rats were fed a HFD (60% of the kcal contained fat:D12492i, Research Diets Inc.) while the other animals received a standard diet. After 12 weeks, local articular cartilage damage was induced on the femoral condyles, in one knee joint according to the groove model in 14 rats of each diet group. Remaining animals served as a control group in each arm. At week 24, serum was collected, subchondral bone was assessed by μCT scan (Quantum FX, PerkinElmar,USA), OA severity was evaluated by rat OARSI histopathology score and macrophage presence with CD68 immunostaining from histological sections was assessed.

Results: HFD feeding resulted in metabolic dysregulation as indicated by significantly increased metabolic parameters (weight, fasting insulin and total cholesterol) compared to the standard fed rats. HFD feeding alone resulted in mild cartilage degeneration (2±1.1 vs 0.58 ±0.7; p=0.06) and synovial membrane inflammation (1.0±0.6 vs 0.3±0.5; p=0.075) both subscores of the rat OARSI histopathology score. However, when HFD feeding is combined with the surgical model of applied local cartilage damage, OA severity is statistically significant increased compared to the local cartilage damage group on a standard diet (6.2±2.1 vs 3.4±1.4; p=0.001). Synovial membrane inflammation (1.3±0.9vs 0.5±0.5; p=0.011) and multiple large osteophyte formation, demonstrated by histology (0.9±1 vs 0.2±0.4; p=0.04) and quantified on μCT (328±349 μm^3 vs 7±14 μm^3; p=0.0001), contributes most to this increased OA severity. Immunohistochemical CD68 expression as observed on both the synovial membrane as well as in the subchondral bone and around the formed osteophytes can explain the increase in selected inflammatory parameters when groove surgery is combined with a HFD (Figure 1).



Conclusions: This study shows that a HFD induces metabolic alterations and increases the inflammatory state of the joint. This by itself does not result in severe OA. However, when adding a HFD to a mild cartilage damage model of OA, joint degeneration is significantly increased. This progression of joint degeneration appears to be driven mainly by inflammatory responses as demonstrated by an increased CD68 expression in both the subchondral bone and synovium membrane with increased osteophytosis. Hence, our findings indicate that systemic metabolic and subsequent inflammatory factors need an additional trigger to contribute to the progression of the OA.

References:

[1] Zhuo Q, Yang W, Chen J and Wang Y, Metabolic syndrome meets osteoarthritis. Nat Rev Rheumatol, 2012. 8(12): p. 729–37.

Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.1639

FRI0011 TARGETING NEUTROPHIL MICROVESICLES TO DAMAGED CARTILAGE USING ANTIBODIES TO POST TRANSLATIONALLY MODIFIED COLLAGEN II

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Background: Microvesicles (MV) are double membrane-bound extracellular vesicles released from the plasma membrane of cells. MV derived from polymorphonuclear neutrophils (PMN) promote tissue protection, and have been demonstrated to penetrate cartilage during inflammatory arthritis and provide protection to the tissue [1].