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POS0366

PRO-INFLAMMATORY EFFECTS OF HUMAN APATITE CRYSTALS EXTRACTED FROM PATIENTS SUFFERING FROM CALCIFIC TENDINOPATHY

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Background: Calcific tendonitis of the rotator cuff is due to carbonated apatite deposits in the shoulder tendons. During the evolution of the disease, an acute inflammatory episode may occur leading to the disappearance of the calcification. Although hydroxyapatite crystals-induced inflammation has been previously studied with synthetic crystals, no data are available with calcifications extracted from patients suffering from calcific tendinopathy. The objective of the study was to explore the inflammatory properties of human calcifications and the pathways involved.

Objectives: The objective of the study was to explore the inflammatory properties of human calcifications and the pathways involved.

Methods: Human calcifications were obtained from patients treated for their shoulder pain related to a calcific tendinopathy of the rotator cuff. Calcifications were extracted by ultrasound-guided lavage and aspiration as previously described [1]. Human calcifications and synthetic hydroxyapatite (sHA) were used *in vitro* to stimulate human monocytes and macrophages, the human myeloid cell line THP-1 and human tenocytes. The release of IL-1 β , IL-6 and IL-8 by cells was quantified by ELISA. Gene expression of pro- and anti-inflammatory cytokines was evaluated by quantitative PCR. NF- κ B activation and NLRP3 involvement was assessed in THP-1 cells using a NF- κ B inhibitor and a Caspase 1 inhibitor. The inflammatory properties were then assessed *in vivo* using a mouse air pouch model. The membrane thickness and infiltrate were assessed 6 and 24 hours after the injection of human calcifications or synthetic hydroxyapatite using hematoxylin and eosin staining. Macrophages, neutrophils and lymphocytes infiltrates were assessed by immunohistochemistry. Total RNA was extracted from the membranes and expression of IL-1 β , IL-6 and TNF β was quantified by PCR.

Results: Human calcifications were able to induce a significant release of IL-1 β when incubated with monocytes, macrophages and THP-1 only if they were first primed with LPS (lipopolysaccharide) for monocytes and macrophages or PMA (Phorbol 12-myristate 13-acetate) for THP-1. No IL-1 β was detected in tenocytes' supernatants. Stimulation of THP-1 by human calcifications led to similar levels of IL-1 β when compared to synthetic hydroxyapatite although these levels were significantly inferior in monocytes and macrophages. IL-6 and IL-8 levels were not increased in the supernatants after crystal stimulation. Patient's crystals enhanced mRNA expression of pro-IL-1 β , as well as IL-18, NF- κ B and TGF β when IL-6 and TNF α expression were not. IL-1 β production was reduced by the inhibition NF- κ B as well as Caspase 1 indicating the role of NLRP3 inflammasome. *In vivo*, injection of human calcifications or synthetic hydroxyapatite in air pouch led to significant increase in membrane thickness with an infiltrate mainly composed of macrophages. Significant overexpression of IL-1 β was only observed in the synthetic hydroxyapatite group.

Conclusion: As synthetic hydroxyapatite, human calcifications were able to induce an inflammatory response resulting in the production of IL-1 β after NF- κ B activation and through NLRP3 inflammasome. In some experiments, IL-1 β induction was lower with human calcifications compared to synthetic apatite. Differences in size, shape and protein content may explain this observation.

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POS0367

NLR4 AND FC- γ -R CROSSTALK ON CD1c+ DENDRITIC CELLS DIFFERENTIALLY CONTRIBUTES TO RHEUMATOID ARTHRITIS IMMUNOPATHOLOGY

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Background: Rheumatoid arthritis (RA) is an autoimmune disorder in which Th17 cells, B cells and inflammatory cytokines (1-3) contribute to joint tissue damage, however the role of specific myeloid populations to immunopathogenesis of RA remains unclear.

Objectives: To address this question, we studied transcriptional, phenotypical and functional characteristics of monocytes (Mo), CD1c+ and CD141+ conventional dendritic cells (cDC) from RA patients.

Methods: Frequencies and maturation patterns of Lin-CD14-HLADR+ plasmacytoid (CD11c-), CD1c+ and CD141+ cDC (CD11c+) subsets and CD14+ Mo from n=25 RA patients at baseline were analyzed by multicolor flow cytometry. In addition, longitudinal studies on the evolution of these populations after treatment initiation were conducted on a smaller group of RA patients. Moreover, CD1c+ and CD141+ cDC subsets and total Mo were sorted from the peripheral blood from n=4 untreated RA and healthy individuals and the synovial fluid from n=3 RA and chondrocalcinosis patients. Differential transcriptional patterns within each population were analyzed by RNAseq. Functional validation of targets were performed *in vitro* with cDC subsets isolated from the synovial fluid of RA patients. Finally, silencing of expression of NLR4 and NLRP3 on CD1c+cDCs was performed with specific siRNAs.

Results: Both CD1c+ (p=0.0001) and CD141+ (p=0.0008) cDCs were significantly depleted from the blood and enriched in the synovial fluid from untreated RA patients, but proportions of CD1c+ cDCs were more significantly recovered after treatment initiation and associated with improved clinical parameters. In addition, specific increased expression levels of the IgG-Fc receptor CD64 on CD1c+ cDC was associated with higher DAS28 (p=0.0002). Moreover, differential transcriptional patterns of circulating CD1c+cDCs from RA patients were characterized by genes linked to toll-like receptor, Fc-receptor, inflammasome pathways and elevated CCR2 expression (p=0.016), while CD141+cDCs transcribed interferon-related genes. Importantly, CCR2^{hi} CD1c+cDCs from the synovial fluid from RA patients transcribed proinflammatory cytokines such as IL1- β , CCL3 and IL-8, actively expressed the inflammasome mediator caspase 1 and were more effective activating pathogenic IFN γ IL-17⁺ CD4⁺ T cells *in vitro* than CD141+ cDC (p=0.0019). These functional profiles could be artificially induced stimulating CD1c+ cDCs with dsDNA in the presence of IgGs and was dependent on caspase 1 and the NLR4 inflammasome.

Conclusion: Our data provides novel insights about specific activation and functional patterns on CD1c+cDC contributing to RA pathogenesis and identifies new sensors that could represent novel therapeutic target to treat RA.

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