Background: STING (stimulator of interferon genes) is a cytosolic protein that is found in endoplasmic reticulum (ER) membrane, mitochondria and mitochondria-associated membranes. Although it is well established that STING plays an important role in innate immune responses, its potential involvement in rheumatic disease processes remains to be clarified (1).

Objectives: The aims of this study were to evaluate the levels of STING and its relationship with local inflammation in the synovial fluid (SF) of patients with psoriatic arthritis (PsA), rheumatoid arthritis (RA), gout, calcium pyrophosphate (CPP) crystal-induced arthritis (CPP-IA), osteoarthritis (OA) and OA with CPP crystals (OA+CPP).

Methods: SF was collected from the knees of 60 untreated patients: 10 with PsA, 10 with RA, 10 with gout, 10 with CPP-IA, 10 with OA and 10 with OA+CPP. SF was examined under optical light microscopy. White cell count (WBC) and the polymorphonuclear cell (PMN) percentage were determined in SF according to standard procedures. SF IL-8, IL-6, IL-1 β and extra- and intra-cellular STING levels were assayed by ELISA.

Results: The levels of WBC were higher in SFs from gouty patients ($27.7 \pm 20.56 \times 10^3/\text{mm}^3$), while OA and OA+CPP patients showed the lowest WBC count ($0.34 \pm 0.3 \times 10^3/\text{mm}^3$, $0.3 \pm 0.28 \times 10^3/\text{mm}^3$). SFs from inflammatory arthritis contained elevated percentages of PMN (gout: $85.5 \pm 10.86\%$, CPP-IA: $84 \pm 11.31\%$, RA: $80.33 \pm 8.14\%$, PsA: $42.6 \pm 35.97\%$). Extracellular STING was determined in OA ($440 \pm 413.31 \text{ pg/ml}$), OA+CPP ($225 \pm 205.06 \text{ pg/ml}$) and CPP-IA ($475 \pm 7.07 \text{ pg/ml}$) SF, while was not detectable in RA, PsA and gout. Intracellular STING levels were similar and the highest in SFs from gout ($96.4 \pm 35.44 \text{ pg/ml}$) and RA ($90.64 \pm 23.13 \text{ pg/ml}$), while remained under detection limit only in SFs from PsA. SF concentration of IL-6 was lower in OA ($354.87 \pm 377.56 \text{ pg/ml}$) and OA+CPP ($389.56 \pm 104.14 \text{ pg/ml}$) as compared with inflammatory arthritides (PsA: $3807.14 \pm 489.86 \text{ pg/ml}$; RA: $17354 \pm 2334.87 \text{ pg/ml}$; gout: $19359 \pm 84.85 \text{ pg/ml}$; CPP-IA: $20389.56 \pm 104.14 \text{ pg/ml}$). The patients with gout and RA had the highest levels of IL-8 ($2159.54 \pm 347.09 \text{ pg/ml}$; $2039.6 \pm 97.74 \text{ pg/ml}$) and IL-1 β ($35.93 \pm 20.46 \text{ pg/ml}$; $44.36 \pm 23.16 \text{ pg/ml}$), while OA showed the lowest concentrations (IL-8: $23.21 \pm 11.32 \text{ pg/ml}$; IL-1 β : $0.47 \pm 0.13 \text{ pg/ml}$). In the total group of patients, we found a negative correlation between extracellular STING and IL-8 ($r = -0.53$; $p = 0.004$) and IL-1 β ($r = -0.47$; $p = 0.012$). There was a positive correlation between intracellular STING and IL-8 ($r = 0.54$; $p = 0.017$), IL-1 β ($r = 0.77$; $p < 0.001$) and IL-6 ($r = 0.69$; $p = 0.009$).

Conclusion: This study is the first to define the presence of STING in SF of different arthritides. The high levels of extracellular STING in OA, OA+CPP and CPP-IA SFs may be due to the activation of factors that reduce its interaction with the ER. The effect of downregulating factors in PsA might explain the low concentration of intracellular STING in these patients.

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AB0061 INVOLVEMENT OF CELL-FREE DNA IN THE DIFFERENTIATION OF OSTEOCLASTS – IMPLICATIONS FOR CLINICAL RESEARCH

P. Škubica¹, J. Horinkova², M. Gregová², K. Pavelka², M. Husakova², P. Dankova¹. ¹Faculty of Science, Charles University, Department of Anthropology and Human Genetics, Prague 2, Czech Republic; ²Institute of Rheumatology and Department of Rheumatology, First Faculty of Medicine, Charles University, Prague 2, Czech Republic

Background: Cell-free DNA (cfDNA) is a molecule with undisputable immunogenic potential^[1]. Its role is well established in etiopathogenesis of systemic lupus erythematosus and many reports suggest it may play an important role in other rheumatic diseases^[2]. Patients suffering from rheumatic diseases are at higher risk of osteoporosis^[3], which may be a consequence of increased generation of osteoclasts (OC). Traditionally, increased formation of OC is attributed to changes in cytokine network^[4]. It is not yet known whether cfDNA can also act on mononuclear precursors and influence their differentiation into OC.

Objectives: The aim of this study was to assess role of cfDNA in differentiation of bone resorbing cells in rheumatic diseases. For this purpose, deoxyribonuclease I (DNase) was employed to eliminate cfDNA from sera of patients and healthy controls (HC) used for cell culture.

Methods: In order to evaluate effects of cfDNA on osteoclastogenesis, an *in vitro* experiment of peripheral blood monocytes (PBM) cultures was designed. Axial spondyloarthritis (AxSpA) was chosen as a model of rheumatic disease. Sera of

AxSpA patients (total n=13) and healthy controls (total n=13) were collected and subsequently used in cell cultures. PBM of 13 healthy donors were cultured in presence of 25 ng/ml macrophage colony-stimulating factor (M-CSF), 0.5 ng/ml receptor-activator of nuclear factor kappa B ligand (RANKL) and 10 % of pooled AxSpA or HC sera, respectively. Serum counterpart treated with DNase was used in parallel. After 14 days, cells were stained for tartrate-resistant acid phosphatase (TRAP) and counted. TRAP positive cells with 3 or more nuclei were recognized as OC.

Results: On average, differentiation of OC in presence of AxSpA sera led to higher osteoclastogenesis compared to DNase-treated counterpart (137 vs. 61; $p < 0.01$). In a similar way, treatment of HC sera with DNase greatly diminished osteoclastogenesis compared to untreated sera (17 vs. 111; $p < 0.05$). Overall, the effect of cfDNA depletion in HC sera regarding OC differentiation was more than 2.5x higher than the effect of cfDNA depletion in AxSpA sera ($p < 0.001$).

Conclusion: Results of our pilot study indicate possible involvement of cfDNA in osteoclastogenesis. Decrease in number of OC after cfDNA depletion in both AxSpA sera and HC sera suggests that cfDNA effect in osteoclastogenesis is non-specific with respect to healthy condition and may represent universal link between innate immunity and bone metabolism. Lower effect of cfDNA depletion in AxSpA sera on osteoclastogenesis is very possibly owing to presence of other inflammatory molecules, which influence osteoclastogenesis. Future research might identify cfDNA as useful therapeutic target to decrease osteoporosis risk not only in patients with rheumatic and autoimmune diseases, where pathological bone resorption is imminent, but also in general aging population.

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AB0062 CHARACTERIZATION OF IL-12 AND IL-23 REDUCTION BY TOFACITINIB IN MDCs

N. Vincken¹, C. Angioliilli¹, S. Cardoso¹, A. Lopes¹, M. Olde-Nordkamp¹, T. Radstake¹. ¹Center of Translational Immunology, Rheumatology and Clinical Immunology, University Medical Center Utrecht, Utrecht, Netherlands

Background: Psoriatic arthritis (PsA) is a chronic inflammatory auto-immune disease characterized by an excessive production of pathogenic mediators that cause inflammation of the skin, peripheral joints, entheses and the spine. Among these, interleukin (IL)-23, IL-12, the IL-17 family and TNF constitute key players in PsA pathogenesis.^{1,2} IL-23, consisting of IL23A (IL-23p19) and IL23B (IL-23p40) subunits, is predominantly produced by myeloid dendritic cells (mDCs). While the p19 subunit is unique to IL-23, the p40 subunit is shared with IL-12. Together, IL-12 and IL-23 play a crucial role in promoting the differentiation of naïve T lymphocytes into T helper (Th) interferon (IFN)- γ -producing Th1 or IL17-producing Th17 cells, respectively.³

Small-molecule inhibitors, such as the JAK/STAT inhibitor Tofacitinib, have recently shown promising therapeutic potential in PsA clinical trials.⁴ The inhibition of JAKs by Tofacitinib results in the direct suppression of multiple intracellular signaling pathways which constitute key hubs in the cytokine network.⁵ However, whether Tofacitinib is able directly target IL-12/IL-23 production by mDCs has not yet been documented. Suppression of these canonical inflammatory pathways would provide further evidence that Tofacitinib is an effective drug in halting both innate and adaptive immune responses.

Objectives: To evaluate the transcriptional and molecular events underlining IL-12 and IL-23 regulation by Tofacitinib in mDCs.

Methods: Peripheral blood mononuclear cells from healthy donors were isolated by Ficoll gradient. Monocytes and myeloid dendritic cells (mDCs) were isolated by using magnetic beads on autoMACS. Monocytes were cultured for 6 days in the presence of IL-4 and granulocyte-macrophage colony-stimulating factor (GM-CSF) to generate monocyte-derived dendritic cells (moDCs). MoDCs were harvested, washed and put to rest for 1 day prior to stimulation, while mDCs