varying concentrations (0, 3.125–100 ng/ml) of recombinant human MCP-1 (R&D Systems) was tested using the CytoSelect 96-well cell migration assay (Cell Biolabs, San Diego, CA, USA). All experiments were run a single time with each treatment group run in triplicate. After normalization for cell viability, cell culture results were expressed as fold change from the media only negative control (no CS, no LPS). One-way ANOVA with Bonferroni's post-hoc test and post-hoc linear trend were performed on cell culture results using Graphpad Prism software (La Jolla, CA, USA).

Results: We found that LPS (1 µg/ml) caused a significant rise in MCP-1 release ($p < 0.0001$) from 3T3-L1 adipocytes. CS in physiologically achievable concentrations (100–200 µg/ml) produced a dose dependent reduction ($p < 0.01$ at 100 µg/ml and $p < 0.001$ at 200 µg/ml) of MCP-1 release from 3T3-L1 adipocytes in response to LPS. Recombinant MCP-1 (25–100 ng/ml) caused a dose dependent increase ($p < 0.001$ at 25 ng/ml and $p < 0.0001$ at 100 ng/ml) in cell migration of THP-1 monocytes. CS at the highest test concentration (200 µg/ml) had no effect on MCP-1 mediated THP-1 migration.

Conclusions: Our data demonstrate that CS inhibits the release of MCP-1 from 3T3-L1 adipocytes that have been stimulated with LPS, but has no effect on the chemotactic action of MCP-1 on THP-1 monocytes. Furthermore, our work data strongly suggests that it is the inhibition of MCP-1 release by CS that underlies this effect and not a direct inhibition of the chemotactic action of MCP-1 by CS. Given the importance of MCP-1 over-production in obesity-related metabolic syndromes, inhibiting the release of MCP-1 from adipocytes by CS, and thus blocking the recruitment of macrophages to adipose tissue, could provide a new treatment opportunity for these syndromes.

Disclosure of Interest: None declared

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THU0045 IL-21 AND IL-22 ARE INVOLVED IN BONE DESTRUCTION IN RHEUMATOID ARTHRITIS PATIENTS

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Background: Inflammatory process in bone marrow (BM) observed on MRI scans of rheumatoid arthritis (RA) patients (called bone marrow oedema) was shown to proceed joint destruction in RA. Our previous studies supported the concept that BM actively participate in the pathogenesis of RA by TLR triggered B cell activity (1), increased number of activated T cells and increased level of proinflammatory cytokines (2,3). Cytokines play a key role in the bone destruction of rheumatoid arthritis.

Objectives: To investigate the levels of IL-21 and IL-22 in RA BM plasma and their association with bone destruction.

Methods: BM samples were obtained from RA and osteoarthritis (OA) patients during total hip replacement surgery. Levels of IL-17AF, IL-21, IL-22, RANKL and cathepsin K in BM plasma were determined by specific ELISA tests. We analyzed pelvic radiographs of 22 patients with RA admitted to the NIGRR and subjected to total hip replacement. Radiographs were taken a day or two before surgery. In our study we assessed hip joint changes semi-quantitatively with the use of the proposed scoring system including primary RA (juxta-articular osteoporosis, axial joint space narrowing, inflammatory cyst presence, bony erosion) and late RA changes (axial migration of the femoral head, femoral head deformation, avascular necrosis of femoral head, femoral head subluxation).

Results: We found increased levels of activated T cell associated cytokines IL-21 (924.8 pg/ml vs 688.6 pg/ml, $p < 0.05$) and IL-22 (94.5 pg/ml vs 65.8 pg/ml, $p < 0.05$) in BM plasma of RA patients in comparison to osteoarthritis (OA) patients. Interestingly levels of both of these cytokines strongly correlated positively with concentration of osteoclastogenesis/osteoclast activity marker RANKL and cathepsin K. Surprisingly level of IL-17AF did not correlate with RANKL or cathepsin K. Furthermore, concentration of IL-21 was statistically significantly higher in patients with more severe radiologically assessed bone destruction. Median value of concentration of IL-21 in RA patients with small bone destruction was 797.4 pg/ml, with mild bone destruction was 1037.8 pg/ml, with severe bone destruction (1079.0 pg/ml).

Conclusions: Our results show an association between BM plasma levels of IL-21 and IL-22 and bone destruction, supporting the hypothesis that IL-21 and IL-22 are important pathogenic factors of this disease. Therapy targeting IL-21 and IL-22 may be of value in preventing bone erosions in patients with RA.

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THU0046 CALCIUM PYROPHOSPHATE AND MONOSODIUM URATE CRYSTAL-INDUCED PROSTAGLANDIN E2 PRODUCTION INVOLVES NF-κB ACTIVATION AND ROS PRODUCTION, INDEPENDENTLY OF INTERLEUKIN-1BETA AXIS

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Background: Monoclinic and triclinic calcium pyrophosphate dihydrated (mCPPD and tCPPD) and monosodium urate (MSU) crystals are responsible in human for relapsing acute arthritis. CPP and MSU crystal-triggered inflammation depends on several inflammatory mediators including interleukin (IL)-1β and prostaglandin (Pg) E2. IL-1β production is governed by NF-κB, NLRP3 inflammasome and caspase-1 activation. PgE2 derives from arachidonic acid (AA) synthesis, which is regulated by cytosolic phospholipase A2 (cPLA2), and cyclooxygenase-(COX) 2 activation. CPP crystal-induced IL-1β production is well documented, but CPP crystal-induced PgE2 production remains unclear.

Objectives: To evaluate how CPP crystals induce PgE2 production and the role of IL-1β in this process.

Methods: Synthetic and pyrogen-free m-CPPD, t-CPPD and MSU crystals were used to stimulate human monocyte cell line (THP-1 cells) and primary bone marrow-derived macrophages (BMDM) of wild type (wt) and NLRP3 inflammasome deficient (nlrp3^{-/-}) mice. Pharmacological inhibitors were used to assess the role of oxidative stress (N-acetyl-L-cysteine, NAC) and NF-κB pathway (Bay-117085). PgE2 and IL-1β production were quantified by ELISA, gene expression by qRT-PCR, cPLA2 by immunoblot. NF-κB activation was assessed in THP-1 cells containing a reporter gene under control of NF-κB p65 promoter. *In vivo*, CPP crystal-induced PgE2 production was evaluated in the air pouch model in the presence or not of NF-κB inhibitor or NAC.

Results: *In vitro*, m- and t-CPPD and MSU crystals rapidly induced the production of PgE2 and IL-1β by THP-1 cells and BMDM. PgE2 production was associated with cPLA2 and NF-κB activation along with increased expression of COX-2 (20 fold) and its receptor EP2 and EP4 genes. While CPP crystal-induced IL-1β production was abolished in THP-1 cells by treatment with caspase-1 inhibitor and in nlrp3^{-/-} BMDM, CPP crystal-induced PgE2 was not modified suggesting IL-1β- and NLRP3-independent pathways. Interestingly, CPP crystal-induced PgE2 production was completely abrogated by NF-κB inhibitor treatment and significantly decreased by the antioxidant NAC; in both case, COX-2 gene expression was dramatically inhibited. *In vivo*, treatment with NAC or Bay strongly inhibited IL-1β and PgE2 production and cellular infiltrate induced by CPP crystals.

Conclusions: PgE2 production mediated by CPP and MSU crystals is activated by NF-κB signaling, independently of NLRP3/IL-1β production axis. Moreover, ROS production, known as a NLRP3 activator in response to CPPD or MSU crystals, is also involved in the regulation of PgE2 production. The relations between these different pathways are under investigation.

Disclosure of Interest: None declared

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THU0047 RESVERATROL AND ITS PRECURSOR POLYDATIN INHIBIT CRYSTAL-INDUCED INFLAMMATION IN VITRO BY DECREASING OXIDATIVE STRESS AND IL-1BETA ACTIVATION PATHWAY

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Background: Resveratrol (RES) and its natural precursor polydatin (PD) are polyphenol compounds that display a broad variety of beneficial effects including anti-apoptotic and anti-inflammatory properties.

Objectives: This study aimed to investigate the role of RES and PD in the inflammatory process induced by monosodium urate (MSU) and calcium pyrophosphate (CPP) crystals *in vitro*. Their effect was evaluated through IL-1β, NLRP3 inflammasome, reactive oxygen species (ROS), nitric oxide (NO) and the phagocytosis index assessment.

Methods: A monocytic cell line (THP-1) was primed for 3 hours with phorbol myristate acetate (PMA) (100 ng/ml) and stimulated with synthetic MSU (0.05 mg/ml) and CPP (0.025 mg/ml) crystals. RES and PD were added to cultures at, respectively, 100 µM and 200 µM. Experiments were carried out either adding RES and PD along with crystals, or pretreating cells (2 hs) with polyphenols and removing them before crystal stimulation. IL-1β were evaluated in cell supernatants and at intracellular level by ELISA assay. IL-1β and NLRP3 expression was assessed by RT-PCR. Reactive oxygen species (ROS) and NO were measured by cytometric analysis using fluorogenic probes (CellROX Deep Red Reagent and DAF-FM, respectively). Crystal phagocytosis index was calculated at different time points using polarized light microscopy.

Results: RES and PD inhibited IL-1β induced by crystals both at extracellular

and intracellular level. This inhibition was more pronounced after polyphenol cell pretreatment. In this case cytokine production was completely suppressed both in control and in stimulated cells. Cell pretreatment was extremely effective also in reducing mRNA expression of IL-1 after crystal stimulation while NLRP3 expression was to some extent affected by RES. RES and PD had a slight inhibitory effect on crystal phagocytosis when added along with the stimulus, while pretreated cells did not show any difference in the phagocytosis index. ROS production induced by MSU crystals was more pronounced (4 fold increase) with respect to CPP crystals (2 fold increase). RES was more effective than PD in inhibiting ROS production ($p < 0.05$ crystals vs crystals+RES). The pretreatment showed a more marked decrease of ROS than the simultaneous treatment and the effect reached significance for both PD and RES. Similar inhibitory effects have been obtained when NO production was considered.

Conclusions: Our results demonstrated that RES and PD are effective in inhibiting crystal-induced inflammation affecting IL-1 production and NLRP3 expression and activation. Data obtained after cell pretreatment allow us to hypothesize that these polyphenols act on specific signaling pathway preventing inflammation, and that this action is independent from crystal phagocytosis.

Disclosure of Interest: None declared

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THU0048 SOLUBLE URIC ACID INCREASED THE EXPRESSION OF ABCG2 AND PDZK1 IN HUMAN INTESTINAL CELL LINES THROUGH THE TLR4/NLRP3/CASPASE-1 AND PI3K/AKT SIGNALING PATHWAYS

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Background: Hyperuricemia is the key pathophysiological basis of gout^[1]. The most common reason generating hyperuricemia is verified the obstacle of urate excretion by current researches^[2]. The intestine is known as the most important organ involved the excretion of uric acid besides the kidney^[1]. ABCG2 has an outsized genetic contribution to extra-renal underexcretion and causes a compensatory increase in urinary urate output^[3]. PDZK1 is a kind of an important structural protein regulated ABCG2 function, by potential molecular interaction with ABCG2^[4].

Objectives: The present study was undertaken to explore the effect and its related mechanisms of soluble uric acid on the urate excretion PDZK1 and ABCG2 in HT-29 and Caco-2 cell lines

Methods: HT-29 and Caco-2 cell lines were used as a well-established model of human intestinal epithelial cells. Cells were pretreated with or without inhibitors and then stimulated with soluble uric acid. siRNA transfection was used to assess the interaction between ABCG2 and PDZK1. qRT-PCR and western blotting were used to measure mRNA and protein levels, respectively. Subcellular fractionation methods and immunofluorescence were used to examine the proteins in different subcellular compartments. Flow cytometry experiments examined the function of ABCG2.

Results: Soluble uric acid significantly up regulated the expression of PDZK1 and ABCG2 in human intestinal cell lines. It also activated TLR4/NLRP3/caspase-1 inflammasome and PI3K/AKT signalling pathway through the phosphorylation of the ser473 pAkt. The increased expression of PDZK1 and ABCG2 was suppressed by a block of TLR4 (TAK-242) and caspase-1 inhibitor (acetyl-YVAD-chloromethylketone) and partly reduced by wortmannin, a specific inhibitor of PI3K. Additionally, lipopolysaccharide (LPS), the potent inducer of inflammatory responses mediated through TLR4, activated TLR4/NLRP3/caspase-1 inflammasome and up regulated the expression of ABCG2 and PDZK1. Besides, the stimulation of soluble uric acid facilitated the translocation of ABCG2 from intracellular compartment to plasma membrane and increased the transport activity. Furthermore, PDZK1 knockdown via siRNA significantly inhibited the expression and transport activity of ABCG2 regardless of the activation by soluble uric acid.

Conclusions: The PI3K/AKT and TLR4/NLRP3/caspase-1 signaling pathways modulate ABCG2 and PDZK1 expression stimulated by soluble uric acid in human intestinal cells. PDZK1 plays a pivotal role in the regulation of ABCG2.

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THU0049 29-KDA FIBRONECTIN FRAGMENT SUPPRESSED HMGB1-DEPENDENT AUTOPHAGY PATHWAY IN HUMAN ARTICULAR CHONDROCYTES

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Background: Fibronectin fragments found in synovial fluid induce the catabolic responses in cartilage. High mobility group protein Box 1 (HMGB1), a damage-associated molecular pattern (DAMP), resides in the nucleus, translocates into the cytosol in response to various stimuli, and is subsequently released into the extracellular space.

Objectives: In this study, we investigated the effect of 29-kDa fibronectin fragment (29-kDa FN-f) on HMGB1-mediated autophagy pathway in primary human chondrocytes.

Methods: Human articular chondrocytes were enzymatically isolated from articular cartilage. The mRNA and protein levels of HMGB1 were measured by quantitative real-time PCR (qRT-PCR) and Western blot analysis. The translocation of nuclear HMGB1 into cytoplasm was determined by western blot and immunofluorescence microscopy. Activation of mammalian target of rapamycin (mTOR), protein kinase B (Akt) and 1A/1B-light chain 3 (LC3) was measured by western blot analysis. Interaction of HMGB1 and Beclin-1 were evaluated by immunoprecipitation (IP). Release of HMGB1 into extracellular medium was measured by ELISA.

Results: HMGB1 was significantly reduced in human osteoarthritis (OA) cartilage compared to normal cartilage. qRT-PCR and Immunoblotting assay revealed that 29-kDa FN-f significantly reduced expression of HMGB1 at the mRNA and protein levels and also led to the translocation of the nuclear HMGB1 into the cytoplasm, together with decreased levels of Beclin-1 and phosphorylated Bcl-2. Using IP analysis, we demonstrated that in the presence of 29-kDa FN-f the association of HMGB1 and Beclin-1, which led to HMGB1-dependent autophagy pathway, was decreased, whereas the association of Bcl-2 and Beclin-1 was increased. In addition, prolonged treatment with 29-kDa FN-f significantly increased the release of HMGB1 into the culture medium. A variety of evidence, including down-regulated LC3-II, an autophagy marker, and increased phosphorylated 4EBP1 and p70S6K, substrates of mTOR, revealed that 29-kDa FN-f subsequently suppressed autophagy in primary chondrocytes by activating Akt/mTOR signaling pathway.

Conclusions: These results demonstrated that 29-kDa FN-f has a harmful impact on chondrocytes through suppressing HMGB1-dependent autophagy pathway and releasing HMGB1, DAMP, to extracellular space.

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THU0050 MICRORNA-365 INHIBITS IL-1 β -INDUCED CATABOLIC FACTOR EXPRESSION BY TARGETING HIF-2 α IN HUMAN PRIMARY CHONDROCYTES

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Background: MicroRNAs, small noncoding RNA molecules, are involved in the pathogenesis of various diseases such as cancer and arthritis. Endothelial PAS