Methods: Micromass cultures of C28/I2 chondrocytes and C3H10T1/2 mesenchymal cells were employed to determine the ability of PGRN fragments to stimulate chondrogenesis. HT-29 and WEHI-164 TNF sensitive cells were used to evaluate the anti-TNF capacity of LAP-PGRN fragments. Co-immunoprecipitation was used to characterise interactions between PGRN fragments and TNFRII. DBA/1 fibroblasts transduced with lentivirus encoding LAP-PGRN fusions were delivered to DBA/1 mice with CIA to assess anti-arthritic effects.
Results: PGRN, LAP-PGRN, LAP-GRN A and LAP-Atsttrin were cloned and expressed in mammalian expression systems. PGRN elevated sulphated proteoglycan production in micromass cultures of C28/I2 human chondrocytes, and also stimulated proliferation of C3H10T1/2 cells. Furthermore, PGRN reduced TNF-mediated catabolism of extracellular matrix in established chondrogenic C3H10T1/2 micromass cultures. LAP-PGRN, LAP-GRN A and LAP-Atsttrin potentiated BMP2 mediated chondrogenesis in C3H10T1/2 micromasses. PGRN failed to protect WEHI-164 fibroblasts or HT-29 colorectal carcinoma cells from TNF-mediated cytotoxicity despite interacting with TNF receptor *in vitro* by co-immunoprecipitation. LAP-PGRN, LAP-GRN A and LAP-Atsttrin all failed to protect WEHI-164 cells from TNF-mediated cytotoxicity, even after MMP1 cleavage and release. CIA disease progression was reduced in DBA/1 mice treated with autologous fibroblasts overexpressing murine Etanercept or PGRN relative to control treatment. LAP-Atsttrin was more effective than LAP-PGRN and LAP-GRN A at reducing arthritic symptoms relative to LAP-Empty controls.
Conclusions: These findings suggest that PGRN could be used as a targeted dual-function chondrogenic and anti-inflammatory treatment for RA, but these effects are not elicited directly through the TNF pathway.
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THU0042 LONGITUDINAL IP-10 SERUM LEVELS ASSOCIATE WITH THE COURSE OF DISEASE ACTIVITY AND ACHIEVING DMARD-FREE SUSTAINED REMISSION IN RHEUMATOID ARTHRITIS

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Background: Although rheumatoid arthritis (RA) is a chronic autoimmune disease that is persistent in the majority of patients, 10–15% of the RA patients achieve disease modifying anti-rheumatic drugs (DMARD)-free sustained remission over time. Biological mechanisms underlying the persistence of inflammation in RA are yet unidentified. It is well established that increased serum levels of IFN-γ-induced protein 10 (IP-10) are associated with (acute) increased inflammatory responses against mycobacterial pathogens causing leprosy and tuberculosis, thereby providing useful diagnostic tools in these infectious diseases. Based on previous genetic susceptibility studies, we hypothesize that there is an overlap between inflammatory responses observed in these mycobacterial diseases and those observed in RA. Therefore, we determined the association between serial IP-10 serum levels and achieving DMARD-free sustained remission as well as disease activity scores (DAS)-remission.
Objectives: To 1) assess the association between IP-10 levels over time in patients that have persistent RA versus patients that achieve DMARD-free sustained remission, and 2) determine the association between IP-10 levels and DAS.
Methods: 139 serum samples of 34 RA-patients (1987-criteria), obtained at the time of diagnoses and at yearly intervals thereafter, were studied. 15 patients had persistent RA and 19 patients achieved DMARD-free sustained remission after a median follow up of 2.7 years. IP-10 serum levels were measured using a previously developed, user-friendly lateral flow assay. Baseline and change in IP-10 levels over time were compared between patients with persistent RA and those achieving DMARD-free sustained remission. The association between the change in IP-10 level and the change in DAS was studied; in addition the course of the absolute IP-10 levels and the DAS over time was plotted for individual patients.
Results: IP-10 serum levels varied from 316 – 53,685 pg/ml between RA-patients. Patients that had persistent arthritis or achieved DMARD-free sustained remission over time did not differ in baseline IP-10 levels (median persistent RA 1991 pg/ml and median DMARD-free sustained remission 3292 pg/ml, p=0.19). However, a significant decrease in IP-10 levels over time was observed in patients achieving DMARD-free sustained remission (p=0.003), whereas IP-10 levels remained stable in patients with persistent RA. Changes in IP-10 levels correlated well with changes in DAS scores (p=0.05). Also at the level of individual patients, a strong correlation between IP-10 levels and DAS over time was observed.
Conclusions: These longitudinal data indicate that IP-10 levels are associated with perseverance of RA as well as with disease activity. Rapid diagnostic tests measuring IP-10 levels can therefore be helpful in monitoring of RA patients.
Disclosure of Interest: None declared
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THU0043 TARGETED MEDICINE: THERAPEUTIC USE OF FUNCTIONAL CYTOKINE ASSAYS IN COMPLEX INFLAMMATORY ARTHRITIS

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Background: Functional cytokine assays have been limited to experimental models of inflammatory arthritis without translation into clinical therapy.
Objectives: To illustrate the successful use of functional cytokine assays in providing targeted treatment for complex inflammatory arthritis.
Methods: A 24 year old male presented in 2014 with a florid asymmetrical polyarthritis associated with an elevated acute phase response (ESR 50, CRP 42, ALP 132). He had no history of psoriasis, inflammatory bowel or eye disease and no family history.
Vasculitis and viral arthritis screen, ACE, ANA, Anti dsDNA, RF and CCP were negative. He was positive for HLA B27 without evidence of spondyloarthropathy on imaging. He was noted to have a monoclonal gammopathy of uncertain significance with normal bone profile, unremarkable urinary BJP and whole body CT scan. His joint symptoms responded to corticosteroid therapy with the addition of hydroxychloroquine and methotrexate. In 2015, his clinical condition had significantly deteriorated with worsening joint inflammation, 3 kg weight loss with a sudden rise in acute phase response, without proven infection. His functionality declined with long term sickness from work.
Biochemical tests revealed: Hb 123, MCV 87.2, Plt 393, Neutrophil 7.76, ESR 86, Alb 31, Calcium 2.68, ALP 387 (noted to be of bony origin), CRP 286, Ferritin 1161, PT 13, and APTT 39. Renal function, protein electrophoresis, blood film, haemolytic screen, viral and autoimmune liver screens were unremarkable. Hydroxychloroquine and methotrexate were temporarily with-held at this stage, although he was maintained on prednisolone 20 mg, with a partial response in his joint symptoms. An infiltrative pathology was a concern and was subsequently excluded.
The next therapeutic step would have been an anti-TNF agent. Nevertheless, due to his atypical and complex disease course, cytokine assays were performed to further characterise his disease profile and provide targeted treatment. Results showed an elevated IL6 production; IV Tocilizumab was selected for the treatment of his inflammatory arthritis. He has had a remarkable response in terms of his systemic and joint symptoms as well as considerable biochemical improvement. He was able to de-escalate his corticosteroid regime and return to work.
Results: TNF alpha, IL1 beta, and IL-6 are important inflammatory mediators and targets of intervention in the treatment of Rheumatoid arthritis. They may be induced *in vivo* and *in vitro* using Toll like receptor mediated innate activation. Active Whole Blood Cytokine Profiling was performed for the patient and compared to healthy controls. Innate activation of whole blood with various TLR agonists including LPS (TLR4), PAM3 (TLR1/2), Imiquimod (TLR7) revealed low induction of TNF alpha and IL1 beta in the patient when compared to controls. In contrast, the patient showed an elevated IL-6 production. Patient's cytokine levels in serum and in non-activated whole blood were normal.
Conclusions: Our case highlights the potential progression to personalised medicine in achieving optimal patient outcome delivered in a cost-effective way. Functional cytokine assays in the appropriate context and within selected patient groups can help to achieve disease remission by allowing targeted treatment.
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THU0044 CHONDROITIN SULPHATE INHIBITS MONOCYTE CHEMOATTRACTANT PROTEIN-1 RELEASE FROM 3T3L1 ADIPOCYTES: A NEW TREATMENT OPPORTUNITY FOR OBESITY-RELATED METABOLIC SYNDROMES?

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Background: Monocyte chemoattractant protein-1 (MCP-1) overproduction from inflamed adipose tissue is a major contributor to obesity-related metabolic syndromes. We have recently published that chondroitin sulphate (CS) can attenuate the monosodium urate (MSU) crystal mediated THP-1 macrophage inflammatory response reflected by reduced release of pro-inflammatory cytokines IL-1β and TNFα. We have also recently determined that the CS inhibitory effect is not acting at the inflammasome but upstream, most likely by inhibiting activation of NF-κB.
Objectives: We sought to determine whether CS had a similar inhibitory effect on MCP-1 release from lipopolysaccharide (LPS) stimulated adipocytes.
Methods: We cultured 3T3-L1 embryonic fibroblasts and induced their differentiation into adipocytes using an established protocol. We then treated the adipocytes with LPS to induce inflammation and thus MCP-1 release. At the same time we added varying concentrations of CS (Bioibérica, Spain) in a physiologically relevant range (10–200 μg/ml) and 24h after we measured MCP-1 release (R&D Systems, Minneapolis, MN, USA). We also cultured THP-1 monocytes and tested whether CS (200 μg/ml) could inhibit cell migration induced by human recombinant MCP-1. Monocyte chemotaxis in response to 24h exposure to

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 (1μg/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 25ng/ml and $P<0.0001$ at 100ng/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. PgE_2 derives from arachidonic acid (AA) synthesis, which is regulated by cytosolic phospholipase A2 ($cPLA_2$), 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 PgE_2 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). PgE_2 and IL-1β production were quantified by ELISA, gene expression by qRT-PCR, $cPLA_2$ by immunoblot. NF-κB activation was assessed in THP-1 cells containing a reporter gene under control of NF-κB p65 promotor. *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 PgE_2 and IL-1β by THP-1 cells and BMDM. PgE_2 production was associated with $cPLA_2$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 PgE_2 was not modified suggesting IL-1β- and NLRP3-independent pathways. Interestingly, CPP crystal-induced PgE_2 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 PgE_2 production and cellular infiltrate induced by CPP crystals.

Conclusions: PgE_2 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 PgE_2 production. The relations between these different pathways are under investigation.

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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: Resveratol (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 though 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) (100ng/ml) and stimulated with synthetic MSU (0.05mg/ml) and CPP (0.025mg/ml) crystals. RES and PD were added to cultures at, respectively, 100μM and 200μM. 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-1b induced by crystals both at extracellular