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Novel insights in inflammatory mediators

OP0089 COMBINATION THERAPY OF SELECTIVE MMP9 AND TNF INHIBITORS ARE EFFICACIOUS IN THE MOUSE CIA MODEL OF RHEUMATOID ARTHRITIS

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Background: Matrix metalloproteinase-9 (MMP9) is highly expressed by infiltrating inflammatory cells, pannus tissue, and multinucleated cells in the synovium and subchondral bone tissue, including osteoclasts. MMP9 is implicated in rheumatoid arthritis (RA) by its involvement in joint destruction, activation of cytokines and chemokines, and promotion of tissue destruction by degrading the basement membrane of epithelia and vasculature. MMP9 knockout mice are protected from collagen-induced arthritis (CIA) disease progression. A potent, allosteric antibody inhibitor of MMP9 is currently being investigated in clinical trials. The ability of a functional murine analog of this antibody to reduce disease signs and symptoms in established, chronic mouse CIA model both as a single agent and in combination with anti-TNF, was investigated.
Objectives: We evaluated the efficacy and safety of selective MMP9 inhibition both alone and in combination with anti-TNF (etanercept), in CIA models of RA.
Methods: CIA was induced in male DBA/1J mice (n=15/group) and treatments were administered after disease establishment. Efficacy was assessed via metrics of joint injury including clinical score (erythema/ paw swelling, score 0–4) in addition to histopathological assessment of destructive joint remodeling (soft tissue changes: edema, necrosis, inflammatory cell infiltration, and fibroplasia, sum score 0–16; bone changes: cartilage damage, bone erosion, periosteal bone formation, synovitis, pannus formation, and joint destruction, sum score 0–24).
Results: All animals were included in the evaluation. In all endpoints assessed, treatment with each therapeutic agent, on its own or in combination, resulted in improvement with respect to body weight change, clinical score, and histopathological measures. The combination group provided the best overall trend for therapeutic benefit, although statistical significance as compared to each single agent alone was not met in most parameters. Body weight recovery was superior in combination as compared to single agent therapies (52% vs. 12–34%, relative to sham; $p<0.05$ combination vs. single agents). Clinical score and histopathology measures in soft tissue and bone changes were most improved in the combination therapy group, although it did not achieve statistical significance as compared to each single agent (26% vs. 17–21%; 1.5 vs. 1.5–1.8; and 7 vs. 7–9, respectively). Importantly, combination therapy resulted in a significant number of limbs with zero or mild disease as compared to single agents (no disease sign: 256% vs. 172–223%; mild disease sign: 178% vs. 138–141%). Analysis of complete blood count at the end of study revealed no abnormalities in any treatment group.
Conclusions: Selective inhibition of MMP9 was active in reducing disease severity in CIA models of RA. The combination of anti-MMP9 with anti-TNF was well tolerated and increased the number of limbs with no or mild disease compared to anti-TNF alone. Further studies are required to examine combination therapy of selective anti-MMP9 and anti-TNF therapies in a clinical setting.
Disclosure of Interest: S. Kim Shareholder of: Gilead sciences, Inc, Employee of: Gilead sciences, Inc, B. Carr Shareholder of: Gilead sciences, Inc, Employee of: Gilead sciences, Inc, L. Tong Shareholder of: Gilead sciences, Inc, Employee of: Gilead sciences, Inc, D. Jin Shareholder of: Gilead sciences, Inc, Employee of: Gilead sciences, Inc, R. Wang Shareholder of: Gilead sciences, Inc, Employee of: Gilead sciences, Inc, D. Marshall Shareholder of: Gilead sciences, Inc, Employee of: Gilead sciences, Inc, D. Gossage Shareholder of: Gilead sciences, Inc, Employee of: Gilead sciences, Inc, V. Smith Shareholder of: Gilead sciences, Inc, Employee of: Gilead sciences, Inc
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OP0090 THE HUMAN SAFE NLRP3 INFLAMMASOME INHIBITOR OLT1177 SUPPRESSES JOINT INFLAMMATION IN MURINE MODELS OF EXPERIMENTAL ARTHRITIS

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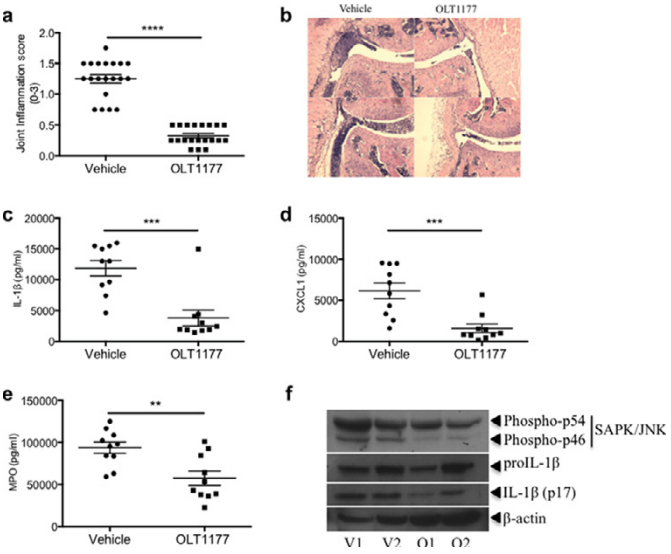
Background: NLRP3 is an essential component of the inflammasome, an intracellular macromolecular complex, which activates caspase-1 for the processing and release of active IL-1β and IL-18. Activation of the NLRP3 inflammasome takes place in inflammatory joint diseases, including gout. Although inhibition of IL-1β with either the IL-1 receptor antagonist anakinra or canakinumab, a neutralizing monoclonal antibody for IL-1β is highly effective for reducing the gout flares, a clinical need remains for a safe, oral treatment of recurrent gout flares refractory to standard of care. The small orally active and safe β-sulfonyl nitrile molecule OLT1177 is a specific inhibitor of the NLRP3 inflammasome, and currently being evaluated in a Phase 2 study for the treatment of acute gout flares.
Objectives: To investigate the anti-inflammatory effect of NLRP3 inflammasome inhibitor OLT1177 in mouse models of acute joint inflammation.
Methods: Two models of experimental arthritis were used in mice: zymosan- and monosodium urate (MSU)-induced arthritis. Knee joints of male C57BL/6 mice were injected intraarticularly with 180 μg heat-killed Zymosan or 300 μg of monosodium urate (MSU) crystals mixed with 200 μM C16.0 fatty acid and bovine serum albumin. Joints were assessed 24 and 4 hours after the zymosan or MSU/C16.0 injection, respectively, for clinical, histological and cytokine analyses.
Results: Intraarticular Zymosan induced a severe joint swelling with neutrophil infiltration, high levels of synovial IL-1β, IL-6 and the neutrophil chemokine CXCL1. Treatment with OLT1177 induced reduction of joint swelling and neutrophil infiltration ($P<0.0001$ and $P=0.006$ respectively) and suppression of IL-1β and IL-6 by 55% and CXCL1 by 30% compared to mice treated with the vehicle. Four hours following intra-articular injection of MSU crystals, an elevated clinical score for knee joint inflammation was observed in vehicle-treated mice (Fig. 1). Oral treatment with OLT1177 showed reduction in joint inflammation as depicted in Fig. 1a when compared to the vehicle group ($P<0.0001$). Histological analysis of the knee revealed suppression of the influx of inflammatory cells in the articular space with OLT1177 treatment ($P<0.05$; Fig. 1b). Treatment with OLT1177 showed significant reduction in synovial tissue extracts for IL-1β (69%; $P<0.001$; Fig. 1c); IL-6 (70%; $P<0.001$); CXCL1 (75%; $P<0.001$; Fig.1d) and MPO (39%; $P=0.006$; Fig. 1e). Mice were also treated with a single dose of OLT1177 1 hour after of intraarticular MSU and reduced joint swelling and IL-1β levels ($P<0.05$) were observed. OLT1177 treatment suppressed activation of the mitogen-activated protein kinases (MAPK) family, c-jun N-terminal kinase (JNK), which is implicated in the pathophysiology of rheumatoid arthritis and gout (Fig. 1f).



Conclusions: The orally active molecule OLT1177 is a potent inhibitor of IL-1β-driven inflammatory arthritis, particularly in a model of acute gout. Considering the outstanding safety profile in humans, OLT1177 is a potential therapeutic strategy to target NLRP3-driven IL-1β-mediated disorders.
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OP0091 S100A8 TRIGGERS A NOVEL IMMUNE-REGULATORY MECHANISM IN DEVELOPING DENDRITIC CELLS

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Background: S100A8/A9 heterodimers are well-known alarmins that, upon release from activated or necrotic phagocytes, promote inflammation by binding to Toll-like receptor 4 (TLR4). These proteins are highly expressed in synovial phagocytes during arthritis and proved to be reliable biomarkers for monitoring disease activity in RA. Interestingly, we now identify a novel immune-regulatory mechanism of S100A8 in human monocyte-derived dendritic cells (moDCs).
Objectives: This study aims to analyze immune-regulatory functions of S100 proteins in human DCs.
Methods: MoDCs are differentiated with or without exposure to S100A8 prior to maturation with LPS. After characterization of the activation status using flow cytometry, the ability of these cells to induce autologous $CD4^+$, $CD8^+$, and γδ T-cell proliferation is investigated. Cytokines, secreted during development are analyzed by Luminex cytokine arrays. The metabolic state of DCs is examined by using Seahorse XFp Analyzer assays. Finally, to identify molecular mechanisms leading to an immune-regulatory phenotype, the mRNA expression of moDCs is analyzed by genome-wide gene expression arrays.

Results: Our results demonstrate that early exposure to S100A8 interferes with *in-vitro* differentiation of moDCs. Compared to controls S100A8-exposed moDCs show dramatically reduced surface expression of co-stimulatory molecules upon LPS-induced maturation. In addition, early treatment of moDCs with S100A8 alters the secretion of immune-regulatory cytokines and chemokines depending on the developmental state of moDCs. S100A8-induced effects on moDC maturation are not limited to TLR4 stimulation but rather trigger a common state of unresponsiveness. Furthermore, mitochondrial respiration and glycolytic function is diminished in S100A8-treated moDCs.

As a consequence, S100A8-exposed moDCs have a reduced potential to induce autologous T-cell proliferation. We can show that these differences are mainly caused by reduced surface expression of co-stimulatory molecules on S100A8-treated moDCs.

Mechanistically, genome-wide gene expression analysis reveals dramatic differences in gene expression between S100A8-exposed and conventionally differentiated moDCs. We demonstrate that S100A8 pre-treatment of moDCs significantly blocks LPS-induced gene expression during moDC activation. Interestingly, *in-silico* analysis of transcription factor networks predicts NFκB and C/EBPδ as master regulators of S100A8-induced effects in developing moDCs. C/EBPδ on protein level, indeed, shows reduced expression in S100A8-differentiated moDCs prior and after LPS-induced maturation when compared to conventionally differentiated moDCs.

Conclusions: Taken together, our results demonstrate a novel regulatory mechanism of innate immunity to prevent overwhelming immune responses. Dysregulated repression of detrimental adaptive immune responses might very well contribute to the disease phenotype in auto-immune disorders with high systemic S100A8/A9 levels. Therefore, S100A8-differentiated immune-suppressive DCs potentially represent a promising therapeutic tool to treat auto-immune diseases in the future.

Disclosure of Interest: None declared

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OP0092 INTERLEUKIN 37 REVERSES THE METABOLIC COST OF INFLAMMATION, INCREASES OXIDATIVE RESPIRATION AND IMPROVES EXERCISE TOLERANCE

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Background: The IL-1 family cytokine interleukin 37 (IL-37) has broad anti-inflammatory effects and functions as a natural suppressor of innate inflammation and acquired immunity (1). We have reported that administration of recombinant human IL-37 to wild type mice or expression in mice transgenic for human IL-37 suppress proinflammatory cytokines and curb excessive inflammation in various conditions including inflammatory arthritis (2). Besides these anti-inflammatory effects, IL-37 also induces complex effects on metabolism. In particular, IL-37 can directly activate AMP-activated protein kinase (AMPK), a central regulator of cellular energy homeostasis and exercise-regulated metabolism (3).

Objectives: In this study, we evaluate the effects of IL-37 treatment on exercise tolerance in mice with systemic inflammation induced by LPS injection. We further investigate the effects of IL-37 on exercise tolerance in healthy mice, with specific focus on the metabolic changes induced by IL-37 administration and possibly responsible for a reduction in the metabolic costs of inflammation.

Results: Exogenous administration of IL-37 to healthy mice, not subjected to an inflammatory challenge, also improved exercise performance by 82% compared to vehicle-treated mice (p=0.01). Treatment with 8 daily doses of IL-37 resulted in a further 326% increase in endurance running time compared to the performance level of mice receiving vehicle (p=0.001). These properties required the engagement of the IL-1 decoy receptor 8 (IL-1R8) and the activation of AMP-activated protein kinase (AMPK), since both inhibition of AMPK and IL-1R8 deficiency abrogated the positive effects of IL-37 on exercise performance. Mechanistically, treatment with IL-37 induced marked metabolic changes with higher levels of muscle AMPK, greater rates of oxygen consumption and increased oxidative phosphorylation. Metabolomic analyses of plasma and muscles of mice treated with IL-37 revealed an increase in AMP/ATP ratio, reduced levels of pro-inflammatory mediator succinate and oxidative stress-related metabolites as well as changes in amino acid and purine metabolism.

Conclusions: These effects of IL-37 to limit the metabolic costs of chronic inflammation and to foster exercise tolerance provide a rationale for therapeutic use of IL-37 in the treatment of inflammation-mediated fatigue.

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OP0093 IL-38 OVEREXPRESSION INDUCES ANTI-INFLAMMATORY EFFECTS IN MICE ARTHRITIS MODELS AND IN HUMAN MACROPHAGES IN VITRO

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Background: IL-38 is a newly characterized cytokine that belongs to the IL-1 family. This cytokine is expressed in the rheumatoid arthritis (RA) synovial tissue (1) and IL-38 deficient mice have exacerbated arthritis (2).

Objectives: This work aims to analyze the effect of IL-38 overexpression in the joints of arthritic mice, in human macrophages and synovial fibroblasts *in vitro*.

Methods: Articular injections of an adeno-associated virus (AAV2/8) encoding IL-38 were performed in Collagen-Induced Arthritis (CIA), K/BxN Serum Transfer-Induced Arthritis (STIA) and Antigen-Induced Arthritis (AIA) in mice. The effect of IL-38 overexpression was evaluated through clinical scores, immunohistochemistry, microCT, Luminex and RT-qPCR analysis. THP-1 monocytes/macrophages were transduced with a lentiviral vector to overexpress IL-38. Effect of conditioned media from these transduced THP-1 cells was also tested on primary culture: M1 macrophages and fibroblast-like synoviocytes from RA patients (RA-FLS).

Results: Clinical inflammatory scores were significantly decreased after AAV IL-38 injection in joints of mice with CIA and STIA, but not AIA. This decrease was accompanied by reduced macrophage infiltration and a decreased expression of Th17 expressed cytokines (IL-17, IL-23, IL-22, TNFα). However, IL-38 overexpression had no effect on cartilage or bone destruction. *In vitro*, the THP-1 monocytic cell line expressed less IL-6, TNFα and IL-23 after IL-38 overexpression. Conditioned media from these cells, containing released IL-38, was also able to exert an anti-inflammatory effect on human primary M1 macrophages and RA-FLS by reducing their IL-23 (M1) and IL-6 (M1 and RA-FLS) expression.

Conclusions: This study shows for the first time that IL-38 overexpression attenuates the severity of experimental arthritis in two mice arthritis models. IL-38 may exert its anti-inflammatory effects by decreasing the production of pro-inflammatory cytokines by macrophages and synovial fibroblasts. This effect can lead to the development of novel treatment strategies in arthritis.

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OP0094 DEVELOPMENT OF A 37-CHANNEL MASS CYTOMETRY (CYTOF) PANEL TO PREDICT TREATMENT RESPONSE IN RHEUMATOID ARTHRITIS

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Background: It is well-established that reducing the inflammation of rheumatoid arthritis (RA) early leads to improved patient outcomes. Patients who fail to respond to conventional DMARDs move on to biologic drugs, however 30% will fail to respond to the first drug tried. Biologics are trialled for at least 3 months before their efficacy can be assessed, during which time irreversible joint damage may occur.

Currently there is no way to predict which drug will be effective in individual patients. It is therefore a research priority to develop a method to predict response to each class of biologic drug. I hypothesise that the immune phenotype of a patient will influence treatment response.

Objectives: This study aims to immunophenotype T cells using mass cytometry and to test novel unbiased methods of analysing high-dimensional data.

Methods: Ten healthy controls (HC) and 10 RA patients with established disease were included. T cells were isolated and stimulated for 4 hours using anti-CD3/anti-CD28 beads in the presence of monensin and brefeldin A. Cells were stained with a 37-channel mass cytometry panel including surface markers, intracellular antigens and transcription factors.

Analysis was performed by conventional biaxial gating alongside tools available on the MRC Cytobank platform, namely Visual t-distributed stochastic neighbour embedding (viSNE), and Cluster identification, characterization, and regression (CITRUS). The CITRUS analysis compared stimulated HC with RA T cells. To