Objectives: To investigate the molecular mechanisms triggered by anti-MDA/MAA autoantibodies during osteoclastogenesis.

Methods: OCs were generated from monocyte-derived macrophages in the presence of the cytokines RANK-L and M-CSF. The development of OCs was monitored by light microscopy following tartrate-resistant acid phosphatase (TRAP) staining and erosion area on synthetic calcium phosphate-coated plates. Three different recombinant human monoclonal anti-MDA/MAA antibodies, cloned from single synovial B cells of RA patients, control antibodies and Fab fragments of the antibodies were added to OC cultures. Glycolysis was inhibited by 2-deoxyglucose, and Fc-gamma receptor I or II by anti-CD64 or anti-CD16 neutralizing antibodies. IL-8 levels were measured by enzyme linked immunosorbent assay. Cellular metabolism was monitored using Seahorse XF Analyzer (extracellular acidification rate and oxygen consumption) and a colorimetric L-Lactate assay.

Results: Lactic acid production correlated with the osteoclastogenic effect of some but not all anti-MDA/MAA antibodies on OCs ($R=0.4758$, $p=0.0252$) suggesting an antibody-mediated regulation of glycolysis. Further, extracellular acidification (ECAR) and oxygen consumption (OCR) rate of the developing OCs were increased by the osteoclastogenic anti-MDA/MAA clones (maximum increase of 54% for the ECAR and 78% for the OCR by clone 146+01G07, and maximum increase of 28% for the ECAR and 39% for the OCR by clone 1103:01H05), but not by the non-osteoclastogenic anti-MDA/MAA clones or control antibodies. The glycolysis inhibitor 2-deoxyglucose completely abolished the osteoclastogenic effect of the anti-MDA/MAA clones at drug concentrations that did not influence baseline OC development. Fab2 fragments of the osteoclastogenic anti-MDA/MAA clones had no effect on OC development and metabolism. In accordance with this, Fc-gamma receptor I neutralizing antibodies completely abolished the osteoclastogenic effect of the anti-MDA/MAA clones. The osteoclastogenic effect of the anti-MDA/MAA antibodies was independent of IL-8 production. In contrast to anti MDA/MAA antibodies, ACPA-mediated osteoclastogenesis was independent of glycolysis and Fc-gamma receptors but dependent on IL-8.

Conclusion: Our results describe a novel glycolysis-dependent mechanism by which anti-MDA/MAA antibodies promote osteoclast development that is different from the one previously described for ACPA.

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SAT0018

THE THERAPEUTIC EFFECT OF STIMULATION OF THE SPLENIC NEUROVASCULAR BUNDLE IN MICE WITH COLLAGEN-INDUCED ARTHRITIS IS ENHANCED BY CONCOMITANT ANTI-TNF TREATMENT

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Background: Vagus nerve (VN) stimulation has shown the potential to improve the disease development in animal models of arthritis and in patients with RA. However, the VN can affect respiratory, cardiovascular, endocrine and gastro-intestinal physiology. The splenic nerve (SpN) has been confirmed to be the principal effector nerve for the VN-mediated immune control. Previous studies have shown that stimulating the splenic nerve resulted in an increase of norepinephrine in the spleen, as well as a significant reduction in LPS-induced TNF (1).

Objectives: To test the therapeutic efficiency of splenic nerve stimulation (SNS) in collagen-induced arthritis (CIA) in mice alone or in combination with anti-TNF treatment.

Methods: CIA was induced in DBA1/J mice by immunization with bovine type II collagen at days 0 and 21. At day 11, mice were implanted with micro-cuff electrode (CorTec) onto the SpN or VN. From day 16 to day 45, SNS were applied as rectangular charged-balanced biphasic pulses with 650 μ A pulse amplitude, 200 μ s pulse width at 10 Hz frequency for 2 min 1 or 6 times a day using a Plexon stimulator. In order to investigate the mechanism of action in more detail, propranolol, a beta-adrenergic receptor (β -AR) antagonist, was added to the drinking water of mice receiving SNS. In addition, a control group was treated with anti-TNF (etanercept, 3 times/week; 10mg/kg i.p.). In curative settings, SNS and/or anti-TNF treatment was applied starting when mice scored positive for 3 consecutive days. Clinical arthritis was determined by visual examination of swelling and redness of the paws and measurement of paw thickness. Sham mice were undergoing the same procedure but did not receive stimulation.

Results: In CIA in mice all sham animals developed arthritis, compared to only 14% following six times per day SNS ($p < 0.001$) in a prophylactic setting. In contrast, 85% of the animals developed arthritis ($p = 0.35$) when SNS was applied only once a day. In both stimulated groups a significant decrease in clinical scores and paw thickness was observed compared to unstimulated group ($p < 0.01$ and $p < 0.05$, respectively). While etanercept treatment reduced clinical scores ($p < 0.001$) an immediate rebound in clinical score was seen following treatment arrest, while mice with SNS were still partially protected 35 days after treatment discontinuation ($p = 0.013$, compared to sham). Propranolol inverted the effect of SNS in CIA mice. Finally, when SNS was applied as a curative treatment, clinical scores were significantly reduced ($p < 0.001$). Importantly, these clinical scores even further decreased when anti-TNF treatment was given to mice receiving SNS.

Conclusion: These studies demonstrate that SNS suppresses pro-inflammatory cytokine production, and reduces clinical symptoms in mice with CIA which is at least partially mediated by the β -AR. The additive effect of anti-TNF in reducing the clinical scores demonstrates that that mechanism of action of SNS is not primarily mediated by reducing TNF levels. Moreover, anti-TNF potentiating the inhibitory effect of SNS is supporting a combined use of these treatments, or even a combination of SNS with other biologicals, to treat RA, potentially getting more patients closer to remission. In conclusion, the data is providing compelling scientific rationale and pre-clinical evidence that splenic neuromodulation might be a new treatment modality for RA.

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SAT0019

HISTOPATHOLOGICAL CHANGES OF SYNOVIAL TISSUE IN RHEUMATOID ARTHRITIS PATIENTS TREATED WITH TNF INHIBITORS

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Background: Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by hyperplasia of synovial tissues [1]. Tumor necrosis factor (TNF)- α is one of the pro-inflammatory cytokines that play a crucial role in the pathogenesis of RA synovitis, and TNF inhibitors (TNFi) were reported to force the RA to go into remission or low disease activity and have brought revolutionary impacts on RA treatment [2]. TNFi have been shown to act on inflammatory cells and form the discoid fibrosis in the sublining layers [3,4]. However, the changes of synovial tissue and the cause of discoid fibrosis in RA patients treated with TNFi has not been determined in detail.

Objectives: The purpose of this study is to demonstrate the histological changes and the types of cells around discoid fibrosis in RA synovium treated with TNFi.

Methods: Synovial tissues were obtained from 30 patients with RA during joint surgeries. 6 patients were treated with TNFi (1 patient with golimumab, 3 patients with etanercept, 2 patients with infliximab). As a control, synovial tissues were obtained from 6 patients who were treated only with csDMARDs (6 patients with MTX). The frozen sections were stained by hematoxylin and eosin (HE). To detect the apoptosis, TdT-mediated dUTP nick end labeling (TUNEL) was performed. The immunohistochemical characterization of the synovial cells was performed by using following antibodies: CD20 and CD3 for detecting B and T lymphocytes respectively, CD163 and CD86 for detecting M1 and M2 macrophage respectively.

Results: In the sections stained with HE, the formation of discoid fibrosis and the other characteristic changes including hydropic degeneration, vacuolation, sclerosis of small vasculature, and the number of multilayered synovial cells was decreased in synovium from RA patients treated with TNFi. In the sections with TUNEL stain, apoptosis of lining cells around the discoid fibrosis was detected in RA synovium treated with TNFi (Figure 1a, 1b). In the sections with immunohistochemistry stain, CD86 expression increased in lining layer of RA synovium treated with TNFi. CD163 positive cells showed diffuse expression in RA synovium treated with TNFi. In contrast, CD20 and CD3 positive cells decreased around discoid fibrosis compared to control sections. These results showed indicated that the types of cells in lining and sublining layers were mainly macrophages and that the apoptosis of macrophages might form the discoid fibrosis in lining layers.

Conclusion: This study showed the apoptosis of lining cells derived from macrophages resulted in the formation of the discoid fibrosis. These findings indicated TNFi might induce apoptosis of macrophage leading to the suppression of RA synovitis.

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Fig.1 a Discoid fibrosis in sublining layers. **b** TUNEL stain positive cells are around Discoid fibrosis

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SAT0020

MOMELOTINIB, A JANUS KINASE 1/2 AND ACTIVIN RECEPTOR 1 INHIBITOR, AMELIORATES JOINT INFLAMMATION, SYSTEMIC TH17 DIFFERENTIATION AND ARTHRITIS-LINKED ANEMIA IN PRE-CLINICAL AUTOIMMUNE RA

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Background: Janus kinases (JAKs) serve as signaling hubs orchestrating inflammation, innate and adaptive immunity and erythropoiesis. Unfortunately, some of these agents cause suppression of JAK-dependent erythropoiesis, thereby exacerbating inflammation-associated anemia, leading to potential under-dosing and reduced therapeutic benefit. We previously showed that the JAKi momelotinib (MMB) can correct anemia in a rat model of RA, an effect that has been clinically reproduced in myelofibrosis patients treated with MMB. Subsequently, the molecular basis for MMB's anemia benefit was determined to be a consequence of its potent inhibition of Activin Receptor Type 1 (ACVR1), resulting in decreased hepcidin and, as a consequence, increased systemic iron availability and improved erythropoiesis.

Objectives: The goal of the current study was to investigate the effects of MMB on arthritis in pre-clinical RA models.

Methods: The anti-arthritis activity of daily administration of MMB was assessed in Streptococcus cell wall-induced arthritis in Lewis rats (PG-PS model) and in collagen antibody-induced arthritis (CAIA) in DBA/1 mice. Consecutive assessment of arthritis was performed by joint thickness measurements and paw scoring. Following 3 weeks of treatment, synovial immune cell infiltration and T cell subset differentiation was quantified. Cytokine gene expression was profiled by quantitative rt-PCR. Anemia was assessed by determination of blood hemoglobin and serum, spleen and liver iron levels.

Results: MMB reduced inflammatory granulocyte and macrophage infiltration in synovial tissue by more than 60% at all tested doses as compared to vehicle treatment in PG-PS animals. Importantly, MMB treatment effectively decreased arthritogenic Th17 cell differentiation and overall CD4+ T cells in the synovia beginning at the lowest tested dose and coincided with complete remission of joint swelling at 25 mg/kg. Anti-arthritis activity of MMB was confirmed with significant reductions in arthritis scoring, which demonstrated non-inferiority versus the TNF- α inhibitor, etanercept, in the CAIA model. Consistent with its inhibitory

activity on the ACVR1-hepcidin axis, MMB reduced circulating hepcidin levels and mobilized systemic iron, resulting in substantial improvement of the RA-associated anemia in rats.

Conclusion: MMB is a highly efficacious anti-arthritis agent that ameliorates local joint inflammation and reduces the systemic differentiation of major arthritogenic effector cell population, Th17 lymphocytes. In accord with our previous report, MMB is distinct from other JAKi due to its ability to inhibit ACVR1 signaling leading to decreased plasma hepcidin, improved iron homeostasis and increased erythropoiesis. The dual anti-inflammatory and anemia-improving pharmacologic activities of MMB position it as a promising and differentiated therapeutic agent for the treatment of RA and other inflammatory diseases with an anemia component.

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SAT0021

STRUCTURAL EFFECTS OF LOCAL CRYOTHERAPY IN RHEUMATOID ARTHRITIS: A STUDY IN ADJUVANT-INDUCED ARTHRITIS

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Background: The control of joint destruction caused by rheumatoid arthritis (RA) is a key issue in the treatment of this disease. Recent evidence showed that radiographic progression of joint damage occur despite a sharp decrease in disease activity and the use of aggressive Disease Modifying Anti-Rheumatic Drug (DMARD) therapies [1]. Whether alternative treatments such as cryotherapy may have beneficial effects on joint destruction at the early stages of the disease remains to be demonstrated, but such strategy would be of interest as it would not interfere with DMARDs treatment.

Objectives: The aim of this study was to evaluate the effect of a 14-days-treatment of local cryotherapy on radiological outcomes in rat adjuvant induced arthritis.

Methods: Adjuvant-induced arthritis (AIA) was induced in 6-weeks old male Lewis rats by injection of *Mycobacterium butyricum* in Freund's incomplete adjuvant at the basis of the tail. A control group received saline. At the onset of arthritis, AIA rats were treated or not by application of cryotherapy on paws using either a cold spray or ice, twice a day for 14 days. Arthritis score and paws skin temperature was daily monitored. At the end of treatment, radiological exam of hind paws was performed and a score taking into account (swelling, osteoporosis, cartilage destruction, bone erosion, bone destruction and new bone formation) was assigned, according to Ackerman *et al* [2]. Circulating levels of cytokines (IL-6, IL-1 β , TNF- α) was measured by Magpix Luminex kit.

Results: Compared to untreated AIA, local cryotherapy significantly reduced the progression of arthritis score, whatever the modality ($p < 0.05$), and to the same extent (reduction of arthritis score at day 24 post-immunization: -38% with cold spray, $p < 0.01$, -37% with ice, $p < 0.01$). Radiological score was significantly reduced by both treatments with no difference between the two treatments (-33% with cold spray, $p < 0.01$, -44% with ice, $p < 0.01$). All the items of the radiological score were equally reduced by ice and cold spray except swelling that was significantly reduced only by ice. Interestingly, the use of the cold spray induced a greater decrease in the skin temperature than the ice treatment (18.32 ± 0.07 °C vs 20.46 ± 0.08 °C, $p < 0.001$). Conversely, cryotherapy did not significantly change the level of cytokines. No correlation was found between radiological score and arthritis score or cytokine levels.

Conclusion: These data demonstrated that local cryotherapy had positive effects on structural damage in adjuvant-induced arthritis. The mechanisms involved remain now to be determined. These results suggest that local cryotherapy would be an interesting complement to conventional DMARDs in early RA.

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