seropositive eRA patients. Whereas cTph are related with disease activity, cTfh cells seem to be constitutively elevated.

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SAT0043

ANALYSIS OF THE ROLE OF RORγT+FOXP3+ (Tr17) IN THE REGULATION OF AUTOIMMUNE ARTHRITIS

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Background: RORγT⁺Foxp3⁺ regulatory T (Treg) cells, designated as Tr17 is one of the new subset of Treg cells, having the potential to regulate the development of experimental autoimmune encephalomyelitis (EAE) thorough a specific repression of Th17 mediated inflammation (1). The function of Tr17 remain unclear in the development of other autoimmune diseases such as collagen induced arthritis (CIA).

Objectives: To clarify the role of RORγT⁺Foxp3⁺ (Tr17) cells in the development CIA.

Methods: 1) Lymphocytes in draining lymph node (LN) were harvested from C57BL/6 mice on 10 days after immunization of type II collagen (CII) emulsified with complete Freund's adjuvant. The expression of RORγT in Foxp3⁺Treg cells was analyzed by flow cytometry and compared them with lymphocytes of non-immunized mice. 2) At 10 days after CII immunization, C-C chemokine receptor type 6 (CCR6), CD25, cytotoxic T-lymphocyte antigen 4 (CTLA-4), and glucocorticoid-induced TNF-receptor (GITR) expression on Tr17, RORγT⁺Treg cells, and RORγT⁺non-Treg cells in LN were analyzed by flow cytometry. 3) Lymphocytes were harvested from Foxp3^{RES-gfp} reporter mice on 10 days after first CII immunization. CD4⁺GFP⁺ Treg and CD4⁺GFP⁺ T cells were isolated and stimulated with anti-CD3 monoclonal antibody (mAb) and anti-CD28 mAb *in vitro*. The expression of IL-10 and IL-17 in RORγT⁺GFP⁺ Tr17 cells was analyzed by flow cytometry and compared with that in RORγT⁺GFP⁺ cells or RORγT⁺GFP⁺ cells. 4) After the induction of CIA, lymphocytes in inflamed ankle joints and LN were harvested from C57BL/6 mice. The expression of RORγT in both Foxp3⁺Treg cells and Foxp3⁺ non-Treg cells were analyzed by flow cytometry. 5) Foxp3^{cre}RORγT^{fl/fl} (conditional knock out; cKO) mice which deficit Tr17 cells were immunized with CII on days 0 and 21. Incidence and severity of CIA were analyzed and compared with Foxp3^{wt}RORγT^{fl/fl} (control) mice. 6) At 10 days post first CII immunization. Lymphocytes from control mice and cKO mice were cultured with or without 100 μg/mL of denatured CII for 72 h. IL-17 and IFNγ levels in supernatants measured by ELISA.

Results: 1) Tr17 cells in draining lymph nodes were significantly increased in CII-immunized mice compared with non-immunized mice. 2) CCR6, CD25, CTLA-4, and GITR expression was elevated on Tr17 cells compared with RORγT⁺Treg cells and Th17 cells. 3) IL-10 producing cells were significantly increased in Tr17 cells compared with RORγT⁺Treg cells (12.57 ± 1.135, p < 0.001). On the other hand, IL-17 producing cells was tended to be decreased in Tr17 cells in spite of the high expression of RORγT compared with RORγT⁺Th17 cells (2.376 ± 0.231, p = 0.176). 4) Tr17 cells and RORγT⁺non-Treg cells were increased and Foxp3⁺Treg cells were decreased in inflamed ankle joints compared with LN after the induction of CIA. 5) CIA tended to be exacerbated in cKO mice compared with control mice. 6) Production of IL-17 tended to be higher in cKO mice than in control mice.

Conclusion: Tr17 cells were increased in the course of CIA and infiltrated into inflamed joints. Moreover, Tr17 cells had the potential to regulate the development of CIA thorough the high expression of suppressive molecules such as IL-10 and CTLA-4.

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SAT0044

HIGH THERAPEUTIC EFFICACY OF ORAL RAS INHIBITORS IN COLLAGEN INDUCED ARTHRITIS: INHIBITION OF RELEVANT MAP-KINASES AND THE CONSEQUENT INDUCTION OF AUTOREACTIVE PATHOGENIC T CELLS AND AUTOANTIBODIES

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Background: The Ras family of GTPases plays an important role in signaling nodes downstream to T cell antigen receptor (TCR) and CD28, potentially lowering the threshold for TCR activation by autoantigens [1]. Somatic mutation in NRAS or KRAS may cause a rare autoimmune disorder coupled with abnormal expansion of lymphocytes. T cells from Rheumatoid Arthritis (RA) patients show excessive activation of Ras/MEK/ERK pathway. The small molecule Farnesylthiosalicylic acid (FTS) interferes with the interaction between RasGTPases and their prenyl-binding chaperones to inhibit proper plasma membrane localization and effective downstream signaling. Previous studies in the Lewis rat adjuvant induced arthritis show that FTS attenuates arthritis development and that the inhibition of pathogenic Th17-type cells is a central mechanism of action of this compound [2].

Objectives: To further study the therapeutic efficacy and molecular mechanisms that mediate the immunomodulatory effects of FTS in DBA/1 mouse collagen type-II induced arthritis (CIA) the pre-clinical model.

Methods: Arthritis was induced in 8-10 week old male DBA/1 mice by immunization with collagen type-II (CII) and complete Freund's adjuvant. Animals were treated semi-prophylactically with daily oral FTS (100 mg/kg); weekly i.p. injection of MTX (0.5 mg/kg); or daily 0.5% CMC vehicle solution (control treatment). Arthritis severity was graded daily by a validated clinical score (0-16 scale), starting at disease onset till study termination. In addition, multiple relevant immunological and molecular biomarkers were analyzed.

Results: We found that the clinical scores of mice in the FTS and MTX arms was significantly reduced (by ~80%, area under curve) compared to the control arm. Accordingly, FTS therapy significantly reduced joint pathology scores for inflammation, pannus formation, bone resorption, and cartilage damage. FTS also significantly inhibited the production of pathogenic anti-CII autoantibodies, anti-citrullinated peptide antibodies, and notably the de-sialylation of these autoantibodies as compared to control mice (Figure 1). The analysis of the effect of FTS on the T cell response to CII immunization, revealed strong attenuation of IL-22, IL-17, IL-9, GM-CSF, TNF, and IFN-γ producing pro-inflammatory CD4⁺ Th cells. Importantly, we found that *in vitro* FTS treatment during TCR-stimulation (anti-CD3/CD28 mAbs) significantly inhibited the ensuing phosphorylation of multiple critical MAP kinases such as Erk, Akt, p38, and mTOR.

Conclusion: We determined in the preclinical CIA model that FTS, a first-in-class oral Ras-GTPases inhibitor, is a potent immune modulator, via the inhibition of TCR/CD28/Ras-dependent activation of critical MAPKs, consequently attenuating the generation of pro-inflammatory autoreactive T cells.

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Figure 1: FTS therapy reduces the levels of pathogenic autoantibody and alters glycosylation of autoantibodies.

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SAT0045

A NOVEL SMALL MOLECULE, MBS2133, MODULATES OSTEOCLAST PRE-CURSOR METABOLISM TO INHIBIT OSTEOCLAST DIFFERENTIATION: AN ALTERNATIVE THERAPY FOR OSTEOLYTIC PATHOLOGY IN RA

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Background: Rheumatoid arthritis (RA) is a chronic inflammatory disease associated with substantial local and systemic bone loss. Despite the availability of several treatment options many patients do not reach low disease activity. Furthermore, current therapeutics generally target inflammation rather than erosive pathology. Thus, there remains a need for new therapies that can target both aspects of the disease. Prior studies have shown that biphenylcarboxylic acid small molecule derivatives not only inhibit murine osteoclastogenesis but also attenuate inflammation and bone destruction in murine models of RA^{1,2}.

Objectives: To evaluate a novel small molecule derivative, MBS2133, on human osteoblastogenesis, osteoclastogenesis and cellular function, and to investigate the *in vitro* mechanism-of-action.

Methods: Osteoblasts were derived from human mesenchymal stem cells. Cells were differentiated in the presence or absence of MBS2133 and mineralization assessed by Alizarin Red staining. Human CD14⁺ blood monocytes were differentiated into osteoclasts (OCs) with M-CSF and RANK-L, in the presence or absence of MBS2133, and/or metabolites. Mature OCs were stained with tartrate-resistant acid phosphatase (TRAP) and quantified by light microscopy. Osteolytic activity was assessed on mineral-coated surfaces. Western blot analysis was used to assess downstream signalling pathways. Changes in the metabolic profile of pre-osteoclasts following 4h exposure to MBS2133 was carried out by liquid chromatography mass spectrometry.

Results: MBS2133 had no effect on the differentiation and function of primary human osteoblasts. In comparison, exposure of RANK-L stimulated CD14⁺ monocytes to MBS2133 significantly reduced OC differentiation and osteolytic activity of mature OCs. Notably, exposure of pre-OCs to MBS2133 for 2h at initiation of osteoclastogenesis, was sufficient to significantly reduce subsequent OC differentiation. Evaluation of treated pre-osteoclasts revealed that RANKL-mediated phosphorylation of p38 was reduced. Metabolomic analysis of pre-osteoclasts revealed that MBS2133 induced a substantial reduction in a range of metabolites associated with glycolysis, oxidative phosphorylation and fatty acid oxidation pathway. Notably, L-carnitine, which facilitates the transportation of fatty acids to the mitochondrial matrix and enables processing and entry into tricarboxylic acid (TCA) cycle for further energy production, was significantly reduced. *In vitro* supplementation of L-carnitine inhibited the ability of the compound to switch off OC differentiation and osteolytic activity.

Conclusion: The results of this study demonstrate that MBS2133 specifically modulates the metabolome of myeloid cells, which has a substantial impact on their ability to differentiate into mature osteoclasts. These findings highlight the importance of modulating the glycolysis/oxidative phosphorylation axis in osteoclastogenesis and suggest that targeting the metabolic state of pre-osteoclasts could offer a new therapeutic approach to treat bone resorption in rheumatic diseases.

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SAT0046

ARE SENSE OF SOCIAL SUPPORT AND LOW DECISION LATITUDE AT WORK LINKED TO RISK OF RHEUMATOID ARTHRITIS, AND IF SO, HOW DO THEY RELATE TO OTHER RISK FACTORS? RESULTS FROM THE SWEDISH EIRA STUDY

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Background: The role of psycho-social factors in the development of rheumatoid arthritis (RA) is debated.

Objectives: We investigated whether psychosocial stress measured as low sense of social support, and low decision latitude at work, were linked to risk of RA, and whether they related to known lifestyle risk factors for RA.

Methods: The Swedish population-based EIRA study included incident RA cases (N=3724) and controls (N=5937), matched for age, sex and residential area. Responders filled in questionnaires regarding self-reported social support, decision latitude at work and life-style-factors.

The distribution of answers among controls were used to define exposure, thus for social support, those in the lowest quartile of social support were considered exposed to low social support and similarly for decision latitude, those in the lowest quartile were considered exposed to low decision latitude at work.

Using logistic regression, we first evaluated whether exposures associated with RA risk, considering potential confounding of established risk factors. Then, we investigated whether the frequency of those factors differed between individuals reporting low social support or low decision latitude at work or not, among cases and controls.

Results: There were 898 cases with low social support and 285 cases with low decision latitude at work (latter only available in first part of EIRA).

Low social support was not associated with RA risk in unadjusted analyses (OR= 1.05, 95%CI=0.95-1.15). Low decision latitude at work did associate with a higher RA risk in the unadjusted analyses (OR=1.52, 95% CI=1.20-1.94), but this association was no longer significant after further adjustment for smoking, obesity and university degree (adjusted OR=1.24, 95% CI=0.93-1.63). Associations between those life-style risk factors and RA were confirmed (no university degree, OR=1.50; smoking OR=1.71; obesity OR=1.15).

Next, we evaluated whether low social support or low decision latitude at work differed by previously established risk factors. Cases with RA reporting low sense of social support were more often men (OR=1.60, 95% CI=1.40-1.83), current smokers (OR=1.46, 95%CI=1.26-1.70), obese (OR=1.29, 95%CI=1.09-1.54), physically inactive (OR=2.78, 95%CI=1.98-3.90) and without a university-degree (OR=2.04, 95%CI=1.77-2.36); with similar pattern among the controls. For working-conditions, cases reporting low decision latitude at work were also more often current smokers (OR=2.05, 95%CI=1.33-3.16) and with no university degree (OR=8.23, 95%CI=5.13-13.22), but less often male (OR = 0.40, 95%CI=0.26-0.60). Again, the pattern was similar among controls. RF/ACPA-positivity did not associate with low social support or low decision latitude.

Conclusion: Neither low social support nor low decision latitude at work were associated with an increased risk of RA after adjustment for other known lifestyle risk factors for RA. An initial crude association between