by qPCR. Their activity was measured using a Rac1, RhoA or Cdc42 Pulldown & G-Lisa Activation Assay Kit.

Results: 1) PGC-1 β knockdown inhibited lamellipodia and filopodia formations of RA-FLS compared with Lv-sh-GFP transfection group (cells with lamellipodia: 24% $\pm$ 7% vs. 40% $\pm$ 5%, $P=0.035$; cells with filopodia: 34% $\pm$ 9% vs. 49% $\pm$ 4%, $P=0.041$), while PGC-1 β over-expression promoted lamellipodia and filopodia formations of RA-FLS compared with Lv-GFP transfection group (cells with lamellipodia: 50% $\pm$ 4% vs. 34% $\pm$ 6%, $P=0.040$; cells with filopodia: 67% $\pm$ 7% vs. 52% $\pm$ 6%, $P=0.045$, Figure 1A).

2) PGC-1 β knockdown or over-expression did not affect the mRNA expression of Rac1, RhoA or Cdc42 (all $P>0.05$, Figure 1B). However, PGC-1 β knockdown suppressed the activity of Rac1, RhoA and Cdc42 (Pulldown assays: 65%~78% reduction; G-LISA assays: 28%~53% reduction, all $P<0.05$), while PGC-1 β over-expression significantly increased their activity (Pulldown assays: 1.55~1.72 folds; G-LISA assays: 1.43~1.68 folds, all $P<0.05$, Figure 1C, D).

Conclusion: Our findings revealed that elevated PGC-1 β in RA-FLS promoted pseudopodia formation by activating Rho family proteins which imply a novel target on regulating RA-FLS migration.

Figure 1 Effects of PGC-1 β on pseudopodia formation and the activity of Rho GTPases in RA-FLS. RA-FLS were transfected with lentivirus for PGC-1 β knockdown or over-expression. (A) Comparison of lamellipodia and filopodia formations by F-actin staining in RA-FLS. (B) The mRNA expression of Rac1, RhoA and Cdc42 was measured by qPCR. (C) The activity of Rac1, RhoA and Cdc42 was measured by Pulldown assays. (D) The activity of Rac1, RhoA and Cdc42 was measured by G-LISA assays. Data were representative as means $\pm$ SD, * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

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SAT0041

GENOTYPE OF THE RHEUMATOID ARTHRITIS SEVERITY LOCUS, RS26232, IS ASSOCIATED WITH INVASIVENESS OF RASFs IN VITRO

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Background: The single nucleotide variant rs26232 has been associated with both the susceptibility to, and severity of, rheumatoid arthritis (RA). There is an allele dose response between rs26232 and radiological damage, with the minor, T, allele being protective against disease severity. Rs26232 is situated in the first intron of C5orf30, which is a negative regulator of tissue damage and inflammation in RA.

Objectives: The objective of this study is to elucidate the mechanism by which rs26232 may mediate disease severity. We also aimed to determine the genotype-phenotype association of rs26232 in rheumatoid arthritis synovial fibroblasts (RASFs).

Methods: RASF were derived from knee biopsies of RA patients taken at arthroscopy (n=33). RASFs were used between passages 3-8. Matrigel-coated Boyden transwell chambers were used to measure invasion. Wound healing (scratch assays) were used to measure migration, whereas proliferation was measured via crystal violet staining of fixed RASF. Intracellular cytokine staining and cell surface markers were measured via flow cytometry. Secreted cytokines were measured in the supernatant of RASF using ELISA. Rs26232 genotype was determined by

PCR genotyping assay with allelic discrimination analysis. Anticitrullinated protein antibody (ACPA) status was measured for each participant. Quantitative real-time PCR was used to measure gene expression. Mann-Whitney U test was used to compare two independent groups, Kruskal-Wallis test was used to compare groups of greater than two.

Results: 49% (n=16) of our RASF cohort were homozygous for the major allele CC, 42% (n=14) were heterozygous CT, 9% (n=3) were TT. Rs26232 is associated with invasion of RASFs with the CC genotype being more invasive than the CT genotype ($p=0.021$). RASFs with the CC genotype had increased ICAM1 and VEGF cell surface expression compared to CT genotype ($p=0.001$, $p=0.05$ respectively). MCP1/CCL2 was decreased in CC compared to CT ($p=0.013$). No association was found between rs26232 genotype and migration, proliferation, or expression of MMP3, TIMP3, IP10 or MIP1a.

rs26232 is located in the first intron of C5orf30. There was no differential expression of total C5orf30 or with any of the 3 individual transcript variants in rs26232 genotype groups. In silico analysis of the region in which rs26232 is located on chromosome 5 identified a DNase Hypersensitivity cluster. Furthermore, mining of the Genotype-Tissue Expression (GTEx) expression quantitative trait loci (eQTL) database revealed a cluster of genes located upstream on chromosome 5 (102,850,000-103,150,000) associated with rs26232. This region upstream from rs26232 contains 4 genes (EIF3KF1, PPI5K2, PAM and GIN1), of which three are eQTLs of rs26232. In silico analysis revealed that the 3 eQTLs (EIF3KF1, PPI5K2 and PAM) also contain the active enhancer mark H3K27Ac, whereas GIN1 does not.

Conclusion: The CC genotype of rs26232 is associated with both increased invasiveness of RASFs and increased adhesion markers compared to CT genotype. Rs26232 does not mediate its affect via its nearest gene, C5orf30. Rather, in silico analysis predicts rs26232 may function as a distal regulator of EIF3KF1, PPI5K2 and PAM. Future work will test the hypothesis that rs26232 genotype phenotype association is mediated by EIF3KF1, PPI5K2 and PAM.

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SAT0042

TWO POPULATIONS OF PD-1^{hi}CD4⁺ T CELLS WITH DISTINCT B CELL HELPING CAPACITY, ARE ELEVATED IN THE PERIPHERAL BLOOD OF PATIENTS WITH EARLY RHEUMATOID ARTHRITIS

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Background: A novel population of B helper cells, phenotypically CD4⁺CXCR5⁺PD-1^{hi}, has been described in the synovial tissues and peripheral blood of seropositive RA patients with an established disease, and termed 'peripheral helper' (Tph) cells. Contrary to CD4⁺CXCR5⁺PD-1^{hi} follicular helper T (Tfh) cells, Tph cells are not located in lymphoid organs but accumulate in inflamed tissues. The frequency and kinetics of circulating Tph cells have not been examined in patients with early, untreated RA.

Objectives: To study the frequency of circulating Tph (cTph) cells, and also of circulating Tfh cell counterparts (cTfh), in patients with early RA (eRA).

Methods: Peripheral blood was drawn from DMARD-naïve early RA patients (2010 ACR criteria) with a disease duration < 24 weeks (n=48). For each patient, an age and gender-matched healthy control was also studied (HC, n=48). After isolation by Ficoll-Hypaque gradient, freshly isolated PBMCs were stained with antibodies to CD3, CD4, CXCR5, ICOS, PD-1 and CCR2, and examined by flow cytometry. Autologous cocultures of naïve or memory B cells were established with CXCR5⁺ (+) or (-) memory CD4⁺ T cells.

Results: Seropositive (RF+ and/or ACPA+, n=31) but not seronegative eRA patients (n=17), demonstrated increased frequencies and absolute numbers of cTph and cTfh cells. cTph but not cTfh cells expressed CCR2. Those eRA patients who experienced a significant clinical improvement at 12 months, demonstrated a marked reduction of their cTph whereas their cTfh cell numbers remained unchanged; at the same time, rheumatoid factor titres decreased significantly but ACPA (anti-citrullinated peptide antibodies) titres did not vary. Both CXCR5⁺ and CXCR5⁺CD4⁺ T cells were able to induce maturation of memory B cells, whereas only CXCR5⁺CD4⁺ T cells could differentiate naïve B cells.

Conclusion: Two populations of PD-1^{hi}CD4⁺ T cells with distinct phenotype and B cell helping capacity, are increased in the peripheral blood of

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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