RESULTS: Our findings revealed that elevated PGC-1 expression of Rac1, RhoA or Cdc42 (all Figure 1A). However, PGC-1 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 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 in RA-FLS promotes pseudopodia formation by activating Rho family proteins which imply a novel target on regulating RA-FLS migration.

Figure 1 Effects of PGC-1 on pseudopodia formation and the activity of Rho GTPases in RA-FLS. RA-FLS were transfected with lentivirus for PGC-1 knockdown or over-expression. (A) Comparison of lamellipodia and filopodia formations of RA-FLS compared with Lv-sh-GFP transfection group (cells with lamellipodia: 50% ± 4% vs. 34% ± 6%, P=0.040; cells with filopodia: 67% ± 7% vs. 52% ± 6%, P=0.045, Figure 1A).

2) PGC-1 knockdown or over-expression did not affect the mRNA expression of Rac1, RhoA or Cdc42 (all P>0.05, Figure 1B). However, PGC-1 knockdown increased Rac1, RhoA and Cdc42 mRNA expression in BV-2 (all P<0.05) compared to Rac1, RhoA and Cdc42 knockdown or over-expression. Furthermore, PGC-1 knockdown increased Rac1, RhoA and Cdc42 mRNA expression in BV-2 (all P<0.05) compared to Rac1, RhoA and Cdc42 knockdown or over-expression.

Conclusion: Our findings revealed that elevated PGC-1 expression of Rac1, RhoA or Cdc42 (all P<0.05, Figure 1B). However, PGC-1 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 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).

A) A novel population of B helper cells, phenotypically CD4+CXCR5-PD-1 hi, has been described in the synovial tissues and periphery of RA patients with seronegative RA and RA erosive disease, with both the susceptibility to, and severity of, rheumatoid arthritis (RA).

B) The single nucleotide variant rs26232 has been associated with 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 and PPIPSK2 and PAM. Future work will test the hypothesis that rs26232 genotype phenotype association is mediated by EIF3KF1, PPIPSK2 and PAM.

DISCLOSURE OF INTERESTS: None declared


SA0042

TWO POPULATIONS OF PD-1HI 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 erosive 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 D-MARD 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+) patients (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 early RA patients with erosive disease.
seropositive eRA patients. Whereas cTph are related with disease activity, cTph cells seem to be constitutively elevated.

**REFERENCE**


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**SAT0044**

**ANALYSIS OF THE ROLE OF RORγt-FoxP3+ T (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) T 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 with lymphocytes of non-immunized mice. 2) At 10 days after CII immunization, C-C chemokine receptor type 6 (CCR6), CD25, cytoxic T-lymphocyte antigen 4 (CTLA-4), and glucocorticoid-induced TNF-receptor (GITR) expression on Tr17, RORγt/Treg cells, and RORγt+Treg cells in LN were analyzed by flow cytometry. 3) Lymphocytes were harvested from FoxP3+GFP- reporter mice on 10 days after 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+FoxP3+ Treg cells was analyzed by flow cytometry and compared with that in RORγt+FoxP3+ cells or RORγt+FoxP3- 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+RORγt/Cre (conditional knock out; cKO) mice which deficient Tr17 cells were immunized with CII on days 0 and 21. Incidence and severity of CIA were analyzed and compared them with lymphocytes of non-immunized 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 CIA-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.

**REFERENCE**


**Disclosure of Interests:** Kotona Furuyama: None declared, Yuya Kondo: None declared, Masahiro Yokosawa: None declared, Masaru Shimizu: None declared, Hiroto Tsuboi: None declared, Isao Matsumoto: None declared, Takayuki Sumida Grant/research support from: Bristol-Myers Squibb, Speakers bureau: Bristol-Myers Squibb

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**SAT0044**

**HIGH THERAPEUTIC EFFICACY OF ORAL RAS INHIBITORS IN COLLAGEN INDUCED ARTHRITIS: INHIBITION OF RELEVANT MAP-KINASES AND THE CONSEQUENT INHIBITION OF AUTOINFLAMMATORY 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+ T cells. Importantly, 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).

**Conclusion:** 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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