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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