CD4⁺ T cells through histological and cellular and molecular biology techniques, and propose possible molecular regulatory pathways. Methods: Patients with RA from December 2019 to January 2022, and gender and age-matched control subjects (OA) and healthy controls (HC) were enrolled. Peripheral blood mononuclear cells (PBMC) and synovial fluid mononuclear cells (SFMC) were collected and CD4⁺ T cells were sorted by magnetic beads. The expression levels of different phenotypes of CD4⁺ T cells and important cytokines were analyzed by flow cytometry; the secretion levels of IL-10 and IFN-γ were detected by ELISA technology. In the intervention experiment, the CD4⁺ T cells were cultured with concentrations of atorvastatin to inhibit the cholesterol metabolism mevalonate pathway and supplement mevalonate acid, and then CD4⁺ T cell phenotype and cytokine expression were detected. The synovial tissues were obtained from RA and OA patients receiving joint replacement surgery, and non-affected distal interphalangeal joint tissues were obtained from refRA and ERA patients. Immunohistochemistry and multiplex immunofluorescence were used to detect the expression of inflammation signalling pathway of CD4⁺ T cells. CH25H was knocked down using small interfering RNA (siRNA), and the effects of knockdown of CH25H on inflammation and the effect on the transformation of CD4⁺ T cells were detected by the above methods. Results: (1) IL-10⁺CD4⁺ T cells in PBMC/SFMC of RA patients were significantly lower than HC/OA, as well as the expression of IL-10. After interfering the mevalonate pathway by atorvastatin, the ratio of IL-10⁺IFN-γ⁺CD4⁺ T cells and IL-10⁺INFN-γ⁺CD4⁺ T cells as well as the expression of IL-10 decreased in a concentration-dependent manner. The down-regulation effect induced by atorvastatin was compensated after the supplementation of mevalonate acid. (2) The expression of CH25H and LXR in CD4⁺ T cells of RA synovial tissue increased detected by immunohistochemistry and multiplex immunofluorescence, and the expressions of CH25H and caspase-1 in CD4⁺ T cells in synovial fluid were found increased by Western Blot, as compared with that of OA. (3) After the successful knockdown of CH25H confirmed by Real-time PCR and Western Blot, a significant decrease in the proportion of IL-10⁺IFN-γ⁺CD4⁺ T cells, and increase in proportion of IL-10⁺INFN-γ⁺CD4⁺ T cells were found in RA patients, accompanied with increase in IL-10⁺CD4⁺ T cells, while the proportion of IFN-γ⁺CD4⁺ T cells and expression of IL-10 decreased significantly. After supplementation with 25-HC, the siRNA-CH25H-mediated decrease in IL-10⁺CD4⁺ T cells was reversed and IFN-γ⁺CD4⁺ T cell formation was reduced. Meanwhile, the expression of NLRP3 and activated caspase-1 (caspase-1 p20) in peripheral blood CD4⁺ T cells was reduced, and could eliminate after supplementation with 25-HC. Conclusion: In peripheral CD4⁺ T cells in RA patients, 25-HC may activate the NLRP3 inflammasome through CH25H-LXR pathway, thereby inhibiting the phenotypic transformation of IFN-γ⁺CD4⁺ T cells to IL-10⁺CD4⁺ T cells, and eventually promoting the inflammatory process in RA. These findings provide new clue for the mechanism of CD4⁺ T cells in the pathogenesis of RA and suggest that the cholesterol metabolism pathway may become a new target of RA treatment. REFERENCES: [1] Nagy, G. et al. (2021). EULAR definition of difficult-to-treat rheumatoid arthritis. Annals of the Rheumatic Diseases, 80(1), 31. [2] Roödenrijn, N. M. T. et al. (2018). Characteristics of difficult-to-treat rheumatoid arthritis: results of an international survey. Annals of the Rheumatic Diseases, 77(12), 1705.

### Table 1

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Functional markers differences (refRA vs ERA): Table 1 shows functional markers expression across immune cell clusters in refRA compared to ERA, using linear mixed modelling (FDR <0.05). Acknowledgements: This study was supported by a UCSF Pharma PhD studentship (TB).

Disclosure of Interests: None Declared.

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incidence of arthritis, more severe clinical symptoms, and more pronounced joint inflammation and bone damage. NKp46 deficiency had no significant influence on the incidence and severity of arthritis in collagen-induced arthritis mice.

Conclusion: This study examined the proportion of NKp46+ ILC3-like cells in the peripheral blood, spleen, lymph nodes, and paw tissues in CIA mice and their correlation with disease severity. We confirmed that infiltration of NKp46+ ILC3-like cells in CIA joints positively correlates with arthritis progression, inflammation, cartilage erosion, and bone destruction. Most importantly, we revealed the pathogenic role of NKp46+ ILC3-like cells in rheumatoid arthritis through adoptive cell transfer. NKp46+ ILC3 cells were a major contributor to CIA arthritis. NKp46 may not be the primary actor in the pathogenic function of NKp46+ ILC3-like cells in CIA. Overall, our current work suggests that NKp46+ ILC3-like cells infiltrate in inflamed joints and participate in the pathogenesis of autoimmune arthritis.

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SERUM AMYLOID A CONNECTS LIVER AND JOINT TO PROMOTE MACROPHAGE ACTIVATION AND CHRONIC ARTHRITIS VIA NFAT5

Keywords: Innate immunity, Rheumatoid arthritis, Biomarkers

Background: The nuclear factor of activated T-cells 5 (NFAT5) is a member of the Rel family of transcription factors that can be activated by hypertonic stimuli [1]. It remains unclear, however, whether NFAT5 is required for damage-associated molecular patterns (DAMPs)-triggered inflammation and immunity. Serum amyloid a(SAA) is an endogenous toll-like receptor (TLR) ligand functioning as a DAMP responds to bacterial endotoxins [2]. In response to infection and inflammation, innate immune cells secrete pro-inflammatory cytokines, in particular IL-1β and TNF-α, to which the liver responds by producing acute-phase reactants [3]. Further identification of such pathologic process by acute-phase reagents will allow for better selection of therapeutic targets as well as a greater understanding of the mechanisms underlying chronic inflammation.

Objectives: To investigate SAA, an acute phase reactant as well as a TLR ligand, activates NFAT5 in macrophages of arthritic joints after being secreted from the liver and thereby promotes chronic inflammation.

Methods: SAA-induced upregulation of NFAT5 expression and activity in RAW264.7 cells, mice bone marrow derived macrophages and human peripheral CD14+ monocytes were assessed by western blot and/or luciferase reporter assays. SAA-activated arthritis in mice was generated by injecting SAA (5 μg x1) in the affected joint of mice with a suboptimal form of IL-1β-induced arthritis, which was induced by injection of methylated bovine serum albumin (mBSA, 200 μg x1) and/or IL-1β (250ng x2). Decrease in arthritis severity and inflammatory cell infiltration by NFAT5 and Tlr2/-/- mouse, specific knockout of myeloid cell infiltration by anti-citrullinated protein autoantibodies (ACPA) play an important role in the pathogenesis of RA and can be detected before the onset of classifiable or clinical RA. ACPA* individuals without clinical RA, or ACPA* At-Risk, are being studied to identify effective preventive interventions. B cells also contribute to disease through autoantibody-independent mechanisms, including production of RANKL and inflammatory cytokines. RANKL-producing B cell in clinical RA have

REFERENCES:

Figure 1.

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Disclosure of Interests: None Declared.

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ACTIVATED B CELLS ARE ENRICHED FOR RANKL AND PROINFLAMMATORY CYTOKINE PRODUCTION PRIOR TO ONSET OF CLINICAL RHEUMATOID ARTHRITIS

Keywords: Cytokines and chemokines, Adaptive immunity, Rheumatoid arthritis

Background: Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by inflammation of the synovial tissue lining the joint, leading to bone damage. Anti-citrullinated protein autoantibodies (ACPAs) play an important role in the pathogenesis of RA and can be detected before the onset of classifiable or clinical RA. ACPA* individuals without clinical RA, or ACPA* At-Risk, are being studied to identify effective preventive interventions. B cells also contribute to disease through autoantibody-independent mechanisms, including production of RANKL and inflammatory cytokines. RANKL-producing B cell in clinical RA have


