suppresses RA-FLS proliferation (1). However, glutaminolysis has been known to suppress autophagy by activating mTORC1 or counteracting ROS production (2). Given the possibility of autophagy upregulation following glutaminolysis inhibition, therapies targeting both autophagy and glutaminolysis may be more effective in suppressing cell growth of RA-FLS, yet the relation between glutaminolysis and autophagy in RA-FLS has not been investigated.

Objectives: To examine the effects of inhibiting both glutaminolysis and autophagy on RA-FLS and autoimmune arthritis in SKG mice.

Methods: GLS1 inhibitor, compound 968 (C968), was used to suppress glutaminolysis, and Chloroquine (CQ) was used to inhibit autophagy. To detect autophagy, the expression of ATG5 and LC3B was measured by real-time PCR and the production of LC3-II was analyzed by Western blotting. The formation of autophagic vacuoles was identified by immunofluorescence. Cell growth was evaluated by BrdU assay. Apoptosis was analyzed by flow cytometry staining with Annexin V-FITC and PI. C968 and CQ were administered subcutaneously to Zymosan A-injected SKG mice.

Results: C968 upregulated the expression of ATG5 and LC3B, and increased the protein level of LC3-II in RA-FLS. C968 also facilitated autophagosome formation. These results suggested that inhibition of glutaminolysis promoted autophagy in RA-FLS. The combined treatment with C968 and CQ significantly suppressed cell proliferation of RA-FLS more strongly than did C968 or CQ alone. In addition, C968 combined with CQ increased the apoptosis rate, whereas either C968 or CQ alone did not. Furthermore, combination of C968 and CQ significantly attenuated the degree of arthritis in SKG mice, while C968 or CQ monotherapy did not (Figure).

Conclusion: The GLS1 inhibitor C968 promotes autophagy in RA-FLS. C968 in combination with CQ reduces proliferation and enhances apoptosis in RA-FLS, and ameliorates the arthritis in SKG mice. Suppressing C968-induced autophagy may be a promising therapy for arthritis.

References:

- [1] Takahashi S., et al. Arthritis Res Ther. 2017 Apr 11;19(1):76.
- [2] Villar VH., et al. Autophagy. 2015;11(8):1198-208.

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SAT0012 ANTIBODY REACTIVITY AGAINST NATIVE PROTEINS IN RHEUMATOID ARTHRITIS

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Background: The majority of patients with rheumatoid arthritis (RA) produce autoantibodies against proteins that have undergone post-translational modification, e.g. citrullination or carbamylation. There is growing evidence of their relevance and their potential utility to improve diagnosis, patient stratification, and prognosis for precision medicine. Investigating new autoantibody patterns may allow further stratification of patients and identifying subsets of patients that benefit from different treatment modalities. Following the discovery of high autoantibody reactivity against multiple modified proteins the interest in native targets decreased. Even though antibodies reacting with native proteins may also have a role in RA pathogenesis, their reactivity patterns are much less studied.

Objectives: To identify novel native autoantigens in RA patients and elucidate patterns within autoantibody reactivity against native autoantigens.

Methods: We investigated the reactivity of autoantibodies in plasma pools from 15 anti-CCP positive and 10 anti-CCP negative RA patients and 10 healthy donors against more than 1600 human proteins in native configuration using the Immunome high-density protein microarray.

Results: We identified 86 native proteins that were recognized by IgG antibodies from anti-CCP positive RA patients and 76 native proteins recognized by IgG antibodies from anti-CCP negative RA patients, but not by antibodies from healthy donors. Examples of proteins recognized by both patient subgroups are calcium/calmodulin-dependent protein kinase type II subunits, histone deacetylases, keratin, and vimentin. Reactivity against the ribonucleic protein SSB was observed in anti-CCP negative RA patients only.

Conclusion: Several human proteins in their native conformation are recognized by autoantibodies from anti-CCP positive as well as anti-CCP negative RA patients. In general, anti-CCP positive patients had higher autoantibody activity than anti-CCP negative patients and healthy donors.

References:

- [1] König, M.F., Giles, J.T., Nigrovic, P.A., Andrade, F., 2016. Antibodies to native and citrullinated RA33 (hnRNP A2/B1) challenge citrullination as the inciting principle underlying loss of tolerance in rheumatoid arthritis. Ann. Rheum. Dis. 75, 2022–2028.
- [2] Zheng, Z., Mergaert, A.M., Fahmy, L.M., Bawadekar, M., Holmes, C.L., Ong, I.M., Bridges, A.J., Newton, M.A., Shelef, M.A., 2019. Disordered Antigens and Epitope Overlap Between Anti-Citrullinated Protein Antibodies and Rheumatoid Factor in Rheumatoid Arthritis. Arthritis Rheumatol. art.41074.
- [3] Sirotti, S., Generali, E., Ceribelli, A., Isailovic, N., De Santis, M., Selmi, C., 2017. Personalized medicine in rheumatology: the paradigm of serum autoantibodies. Autoimmun. Highlights 8.

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SAT0013

PIM-1 KINASE IS A MEASURABLE MEDIATOR OF CD4+ T CELL DYSREGULATION AND THERAPEUTIC TARGET IN EARLY RHEUMATOID ARTHRITIS

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Background: As well as being an established oncoprotein and a therapeutic target in cancer, *Proviral Integration site for murine Moloney leukemia virus-1* (pim-1) has been implicated in human autoimmunity. We previously confirmed this serine-threonine protein kinase to be strikingly upregulated in circulating CD4+ T cells of untreated rheumatoid arthritis (RA) patients as a consequence of IL-6 signalling^{1,2}. Evidence for the relevance of pim-1 signalling in the disruption of RA synovial fibroblast (RASf) homeostasis³ further supports its candidacy as a therapeutic target.

Objectives: To investigate PIM1 and its family members (PIM2 and PIM3) as potential candidates for drug repurposing in RA.

Methods: A flow cytometric assay for PIM1 transcript measurement in circulating CD4+ T cells of early arthritis patients was validated against real-time PCR in paired cells isolated by bead selection. Synovial protein expression in tissue from the same cohort of untreated RA patients and disease controls was determined by quantitative multiplex immunofluorescence. The functional consequences of manipulating pim kinase family expression in freshly purified T cell receptor (TCR)-stimulated CD4+ T cells from early RA patients was explored. The impact of pim-1 specific and pim-1-3 (pan-pim) kinase inhibition on progression of the IL-6 dependent collagen-induced arthritis (CIA) model was assessed.

Results: The percentage of circulating CD4+ T cells positive for *PIM1* transcript by flow cytometry proved a faithful surrogate for gene expression in early arthritis (Figure 1A), distinguishing RA from other pathologies (Figure 1B). Pim-1 protein expression was increased in the synovium of untreated RA compared with disease controls, including amongst infiltrating CD4+ T cells