antlers of sika deer and can be chemically synthesized from glycerol, palmitic acid, and linoleic acid. Research using LPS-induced acute lung injury murine model has reported that EC-18 stimulates a more rapid resolution of LPS-induced lung inflammation. In addition, it has been reported that in a murine model of collagen-induced arthritis, EC-18 treatment ameliorated arthritis, with down-regulation of IL-6 level by regulating the activity of STAT3 in the synovium. Curdlan-administered SKG mice develop ILD spontaneously followed by peripheral arthritis, which resembles RA-ILD.

Objectives: We evaluated the modulatory effect of the EC-18 on arthritis and ILD in autoimmune arthritis animal model.

Methods: Male SKG mice were obtained from Dr. S. Sakaguchi. We injected curdlan (3mg/mice) in 8-week-old SKG mice and identified the presence of ILD by histological analysis at 20 weeks post-injection. Arthritis score was measured every week for up to 20 weeks. EC-18 (250mg/kg body weight/day, Enzychem Lifesciences Co., Daejeon, Korea) was administered every day orally. At 20 weeks post-injection, lung sections were stained with H&E and Masson's trichrome. Using the Opal method, multiplexed immunofluorescent staining of lung tissue was performed. According to the scale by Ashcroft et al., fibrosis severity of lung sections was assessed by a system of eight grades. Analysis of serum cytokines by the luminex multiplex cytokine assay was performed at 20 weeks post-injection.

Results: Oral administration of EC-18 decreased arthritis score significantly until 8 weeks post-injection and remained unchanged thereafter. At 20 weeks post-injection, histological analysis showed severe pulmonary destruction, including bronchial alveolar tissue damage and massive leukocyte infiltration, and fibrosis in the curdlan-administered mice, which was attenuated in EC-18 treated mice. In particular, 67% of curdlan-administered mice showed ILD-like phenotype, whereas the incidence rate in EC-18-treated mice was 17%. Furthermore, immunofluorescent-staining showed both IL-17A and neutrophil accumulation in lung in curdlan-administered mice; these were decreased in EC-18-treated mice. Interestingly, at 20weeks post-injection, EC-18 treatment down-regulated serum levels of IL-6 and TNF- α and up-regulated sIL-7R α (anti-fibrotic molecule).

Conclusion: Taken together, EC-18 exerts an anti-arthritis effect in early phase, but a long-term effect was not indicated. We emphasize the effect on ILD prevention of EC-18 via up-regulation of sIL-7R α and inhibition of neutrophil accumulation, suggesting a therapeutic agent potentially for RA-ILD.

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SAT0010

ANTI-CD30 IMMUNOTHERAPY AMELIORATES BONE AND CARTILAGE DESTRUCTION IN EXPERIMENTAL MODEL OF RHEUMATOID ARTHRITIS IN MICE

M. Matsuhashi¹, K. Nishida¹, Y. Nasu¹, R. Nakahara¹, M. Watanabe¹, Y. Hotta¹, T. Ozaki¹. ¹Department of Orthopaedic Surgery, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan

Background: CD30 is a member of the TNF-receptor family and commonly expressed on lymphocytes of Hodgkin lymphoma and anaplastic large cell lymphoma. It has been reported that levels of soluble CD30 in serum and joint fluid is significantly elevated in rheumatoid arthritis (RA). Although RA patients may develop lymphoproliferative disorders (LPD) as a result of immunosuppression by MTX or bDMARDs, safety medications after the regression of LPD for RA have not yet been established.

Objectives: To explore the potential of CD30 targeting therapy for RA.

Methods: (1) Immuno-histological staining of CD30 was performed for fresh synovial tissues of RA and osteoarthritis (OA). In addition, double immunofluorescence staining of CD30 with CD3, CD20, CD68, CD138 were performed on RA synovial tissue. (2) Brentuximab vedotin (BV) is an anti-CD30 antibody conjugated with monomethyl auristatin E, designed to induce apoptosis of CD30 expressing cells. A multiple myeloma cell line (RPMI8226) was used as a non-lymphoma cell line and plasma cell-like cell line. Immuno-cytological staining for CD30 was performed on RPMI8226. Cells were cultured and harvested on days 0, 1, and 3 to evaluate the effects of BV (50 μ l / ml per well). Cytospin specimens were stained by May-Grunwald-Giemsa (MGG) staining for cell counting and by FIFC-terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining for detection of apoptosis. (3) Collagen antibody induced arthritis (CAIA) was induced in DBA/1 mice by arthritogenic cocktail of monoclonal antibodies against type II collagen. BV was administered to the treatment groups (30mg/kg and 70mg/kg n=4 each) and evaluated clinical score, histological findings and levels of SAA, IL-6, and TNF α in serum by ELISA. Student's t-test (two-tailed) was used to determine statistical significance for analysis of synovial tissues and cell line assay. Two way ANOVA with Dunnett's post hoc analysis was used for multiple comparisons of mice model.

Results: (1) The number of CD30-positive cells was significantly higher in RA synovial tissue than in OA synovial tissue ($p<0.01$) (Fig. 1). CD30-positive cells were detected around the lymphoid follicles. Double immunofluorescence

showed CD30 and CD138 double-positive cells in the synovial tissue of RA, suggesting CD30 is predominantly expressed by plasma cells. (2) RPMI8226 cells expressed CD30. BV caused apoptosis of RPMI8226 cells, and the number of cells treated with BV decreased to 95% compared to controls. (3) All control mice (n=4) developed severe arthritis, and their scores reached a peak (score: 13.3) on day 10. In the mice of treatment group of 30mg/kg, paw swelling was slightly decreased, their clinical score reached a peak (score: 9.3) on day 10. In contrast, paw swelling was significantly reduced in the 70mg/kg treatment group. The peak of the clinical score was 4.3 on day 10 (Fig.2). Histological score evaluated synovitis with infiltration of inflammatory cells, pannus formation, and erosion of bone and cartilage. Histological score of hind paws were 3.0 ± 0.8 for the control group, 2.7 ± 1.0 for 30mg/kg group, and 0.7 ± 1.1 for 70mg/kg group ($p<0.01$), respectively. The serum levels of SAA and IL-6 of treatment group were lower than those of no treatment group ($p<0.01$).

Conclusion: We showed the expression of CD30 on synovial tissue of RA and the expression of CD30 on plasma cells. In addition, the current study provides the first evidence that BV depletion of CD30-positive cells suppressed arthritis and osteochondral destruction in CAIA mice. Our results may provide an important clue for the development of an effective treatment for RA with iatrogenic immunodeficiency-related LPD.

Fig. 1: Immuno-histological staining of CD 30 for fresh synovial tissues

Fig. 2: BV dose-dependently suppress paw swelling and the clinical score of CAIA

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SAT0011

COMBINED INHIBITION OF AUTOPHAGY AND GLUTAMINE METABOLISM SUPPRESSES CELL GROWTH OF RA SYNOVIOCYTES AND AMELIORATES ARTHRITIS IN SKG MICE

I. Naka¹, J. Saegusa^{1,2}, K. Uto², Y. Yamamoto¹, Y. Ichise¹, H. Yamada¹, K. Akashi¹, Y. Ueda¹, A. Onishi¹, T. Okano^{1,2}, S. Takahashi¹, S. Senda¹, A. Morinobu¹. ¹Rheumatology and Clinical Immunology, Kobe University Graduate School of Medicine, Kobe, Japan; ²Clinical Laboratory, Kobe University Hospital, Kobe, Japan

Background: Immunometabolism is now recognized to be crucial in the pathogenesis of rheumatoid arthritis (RA). We have recently shown that the expression of glutaminase 1 (GLS1), a key enzyme in glutaminolysis, is upregulated in fibroblast-like synoviocytes from RA patients (RA-FLS) and that GLS1 inhibition

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.

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SAT0012 ANTIBODY REACTIVITY AGAINST NATIVE PROTEINS IN RHEUMATOID ARTHRITIS

T. B. G. Poulsen¹, D. Damgaard², M. M. Jørgensen³, L. Senolt⁴, J. Blackburn⁵, C. H. Nielsen², A. Stensballe¹. ¹University of Aalborg, Translational Biomarkers for Pain & Precision Medicine, Aalborg, Denmark; ²Copenhagen University Hospital Rigshospitalet, Institute for Inflammation Research, Center for Rheumatology and Spine Diseases, Copenhagen, Denmark; ³Aalborg University Hospital, Department of Clinical Immunology, Aalborg, Denmark; ⁴Charles University, Institute of Rheumatology and Department of Rheumatology, Prague, Czech Republic; ⁵University of Cape Town, Department of Integrative Biomedical Sciences & Institute of Infectious Disease and Molecular Medicine, Cape Town, South Africa

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.

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SAT0013

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

N. Maney¹, H. De Paula-Lemos¹, B. Barron-Millar¹, A. Mellor¹, J. D. Isaacs^{1,2}, A. Anderson¹, A. Pratt^{1,2}. ¹Newcastle University Translational and Clinical Research Institute, Newcastle upon Tyne, United Kingdom; ²Musculoskeletal Services Directorate, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, United Kingdom

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