In vitro, exposure of TCR-stimulated early RA CD4+ T cells to pim kinase inhibitors restrained their activation and proliferative capacity; diminished pro-inflammatory cytokine production (IFN-g and IL-17) and an expanded CD206FoxP3+ regulatory T cell (Treg) fraction were also observed in treated versus untreated cells. Finally, administration of pim inhibitors robustly attenuated clinical scores of arthritis in the CIA model, with reduced cartilage loss observed in animals treated with a pan-PIM inhibitor compared with vehicle control (Figure 2).

References:

Disclosure of Interests: Nicola Maney Consultant of: Current employee of Eli Lilly, Henrique De Paula-Lemos: None declared, Ben Barron-Millar: None declared, Andrew Mellor Shareholder of: NewLink Genetics PLC, and has received patent licensing income from this source., John D Isaacs Consultant of: Pfizer, Roche, A. Corrales: None declared, J. M. Cifrín-Martínez: None declared, R. López-Mejías: None declared, M. A. González-Gay: None declared, 1Research Group on Genetic Epidemiology and Atherosclerosis in Systemic Diseases and in Metabolic Bone Diseases of the Musculoskeletal System, IDIVAL, Hospital Universitario Marqués de Valdecilla, Santander, Spain; 2Department of Functional Biology, Immunology Area, Faculty of Medicine, Universidad de Oviedo, Oviedo, Spain; 3Health Research Institute-IDIVAL, Santander, Spain; 4School of Medicine, Universidad de Cantabria, Santander, Spain; 5Cardiovascular Pathophysiology and Genomics Research Unit, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

Background: Interstitial lung disease (ILD) is one of the most significant comorbidities of rheumatoid arthritis (RA), increasing the mortality in these patients [1,2]. Although the pathogenesis of ILD associated to RA (RA-ILD) remains poorly defined [1], it is known that vascular tissue plays a crucial role in lung physiology [3]. In this context, a population of cells termed endothelial progenitor cells (EPC) is involved in vasculogenesis and endothelial tissue repair [4]. Previous reports suggest the implication of EPC in different conditions such as RA and idiopathic pulmonary fibrosis (IPF), the most common and destructive ILD [5,6]. Nevertheless, little is known about their specific role in RA-ILD.

Objectives: The purpose of this study was to shed light on the potential role of EPC in endothelial damage in RA-ILD.

Methods: Peripheral venous blood was collected from a total of 68 individuals (18 with RA-ILD, 17 with RA-ILD, 19 with IPF and 14 healthy controls). All subjects were recruited from the Rheumatology and Pneumology departments of Hospital Universitario Marqués de Valdecilla, Santander, Spain. Quantification of EPC was analyzed by the expression of surface antigens by flow cytometry. The combination of antibodies against the stem cell marker CD34, the immature progenitor marker CD133, the endothelial marker VEGF receptor 2 (CD309) and the common leukocyte antigen CD45 was used. EPC were considered as CD34+, CD45-, CD309+ and CD133+. All statistical analyses were performed using Prism software 5 (GraphPad).

Results: EPC frequency was significantly increased in patients with RA-ILD, RA-ILD and IPF compared to controls (p=0.001, p=0.002, p<0.0001, respectively). Nevertheless, patients with RA, both RA-ILD and RA-ILD, showed a lower frequency of EPC than those with IPF (p=0.048, p=0.006, respectively).

Conclusion: Our results provide evidence for a potential role of EPC as a reparative compensatory mechanism related to endothelial damage in RA-ILD, RA-ILD and IPF patients. Interestingly, EPC frequency may help to establish a differential diagnostic between patients with IPF and those who have an underlying autoimmune disease (RA-ILD).
SAT0016  DEVELOPMENT OF FIBROBLAST-LIKE SYNOVIOCYTE ASSAYS FOR TARGET DISCOVERY IN RHEUMATOID ARTHRITIS

D. Rueelas1, C. Chen1, H. Truong1, V. Lira7, Y. Moazami1, K. Currie1, J. A. Di Paolo1, H. Yu1, G. Min-Oo1. 1Gilead Sciences, Inc., Foster City, United States of America

Background: The rheumatoid arthritis (RA) synovium is characterized by an overabundance of fibroblast-like synoviocytes (FLS), which play a central role in the initiation and perpetuation of disease via multiple mechanisms. 2 FLS promote disease progression by producing high levels of proinflammatory factors, remodeling cartilage and bone, and promoting self-proliferation and resistance to apoptosis. Our current understanding of the molecular mechanisms that govern FLS-mediated pathology in the synovial joint remains incomplete. Importantly, almost 30% of treatment-naïve early RA patients exhibit a strong fibroblast phenotype that correlates with relatively poor response to disease-modifying antirheumatic drugs. 3 Yet, current therapies in RA are not directly aimed at FLS pathology, creating an opportunity for novel therapeutic target discovery.

Objective: Our objective is to develop a broad suite of screening assays in RA patient-derived FLS for the discovery of target pathways that control multiple pathological properties, including cytokine secretion, migration, and invasion.

Methods: A sensitive high-throughput RA-FLS secretion assay was developed to examine the ability of small-molecule inhibitors to block the production of interleukin (IL)-6 and matrix metalloproteinase (MMP)-3 in response to stimuli. To create a physiologically relevant stimulus, a surrogate synovial fluid cocktail (composed of 12 factors) was defined and titrated for optimal concentration selection. Small-molecule inhibitors (N=170) of diverse biological pathways were screened using the full cocktail or individual stimulation (TNFα, IL-1β, or IL-17) to characterize assay performance. In addition, an FLS platelet-derived growth factor (PDGF)-mediated migration screening assay was developed using a live cell imaging system (Incucyte) to quantify real-time FLS migration.

Results: Due to the variability and limited volume of synovial fluid, we developed a surrogate synovial fluid cocktail to mimic the relevant stimulation of RA-FLS in the inflamed joint. The surrogate cocktail was composed of 12 factors: TNFα, IL-1α, IL-17, IFNγ, OSM, LIF, GM-CSF, IP-10, VEGF, PDGF, AREG, and FGF2. Individual titration of these factors demonstrated that only 3 stimulatory factors (TNFα, IL-1α, and IL-17) resulted in a robust increase in IL-6 production. Importantly, when all 12 factors were combined, a synergistic increase in IL-6 and MMP-3 production by FLS was observed. Screening results identified several reference compounds, including an inhibitor of transforming growth factor-β-activated kinase 1 (TAK1), that was previously reported to block cytokine secretion in FLS. 4 Treatment with this compound showed complete inhibition of IL-6 and MMP-3 secretion. In addition to the cytokine secretion assay, treatment of FLS with this TAK1 inhibitor resulted in almost complete inhibition of migration (Fig. 1).

Conclusion: Novel FLS assays were developed to discover new targets and interrogate pathways involved in multiple disease-driving mechanisms of FLS in RA. In order to mimic the inflammatory environment present in the RA synovium, we developed a 12-factor surrogate synovial fluid cocktail. A synergistic release of both IL-6 and MMP-3 was demonstrated following cocktail stimulation compared to individual cytokines. This points to the important contribution that multiple factors play in the FLS pathogenic processes and will allow us to uncover pathway interactions that may not be captured with single stimuli. In addition, the development of a real-time, 96-well, imaging-based assay to interrogate FLS migration will allow us to identify targets that control this pathological function of FLS.

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2020-eular.5284

SAT0017  METABOLIC CHANGES INDUCED BY ANTI-MALONDIALDEHYDE/MALINDIALDEHYDE-ACETALDEHYDE ANTIBODIES PROMOTE OSTEOCLAST DEVELOPMENT

K. Sakuraba1, A. Krishnamurthy1, A. Circiumaru1, M. Sun2, V. Joshua3, M. Engström4, X. Zheng5, C. Xu1, K. Amana1, V. Malmström6, S. B. Catrina1, C. Grönwall1, B. Reth1, A. Catrina1,1Karolinska Institute, Department of Medicine, Rheumatology Unit, Stockholm, Sweden; 2National Hospital Organization Kyushu Medical Center, Department of Orthopaedic Surgery, Fukuoka, Japan; 3Karolinska Institute, Molecular Medicine and Surgery, Stockholm, Sweden

Background: Malondialdehyde (MDA) is a highly reactive compound produced by lipid-peroxidation in situations associated with oxidative stress. MDA can irreversibly modify proteins residues such as lysine, arginine and histidine. In addition, MDA adducts can further react with acetaldehyde to generate malondialdehyde-acetaldehyde (MAA) modifications. Such modifications can give rise to immunogenic neo-epitopes that are recognized by autoantibodies. In fact, anti-MDA/MAA IgG antibodies are significantly increased in the serum of patients with autoimmune diseases, such as rheumatoid arthritis (RA) 1 and systemic lupus erythematosus (2). Recently, we have shown that anti-MDA/MAA IgG antibodies are able to promote osteoclast (OC) differentiation in vitro (1).


DOI: 10.1136/annrheumdis-2020-eular.3752