aerofaciens had a complete opposite effect. Suppression of arthritis by P histicola was dependent on an increase in the numbers of CD103+ dendritic cells, myeloid suppressors, CD11b+Gr-1, and T regulatory cells, CD4+CD25+FoxP3+, in the gut as well as systemically. This led to reduction in TH17 response while increasing IL-10. On the other hand, C. aerofaciens gavage increased expression of IL-17 and regulatory chemokines as compared to controls. DQ8 mice gavaged with intestinal microbes of arthritis-resistant mice developed arthritis with lower incidence and had skewed Th17/Th2 response.

Conclusions: Our studies suggest that gut commensals influence immune response in and away from the gut. Commensals and their products may provide novel targets for therapeutic strategies in arthritis.

Acknowledgements: Funds were provided by the Department of Defense and Mayo Center of Individualized Medicine

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.2307

FRI0078 ADENOSINMONOFOSFAT-AKTIVACTIVATING PROTEIN KINASE (AMFK) – THE BIOPOWER REGULATOR OF AN AUTOPHAGY IN RHEUMATOID ARTHRITIS (PA)

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Background: Rheumatoid arthritis is an autoimmune disease characterized by altered cellular homeostasis. A great role of an autophagy is expected in the pathogenesis of these changes.

Objectives: To assess the functional activity of AMFK as a strategic biopower regulator of an autophagy and specific indicator of red-ox potential of cells in the sinovial fluid (SF) of patients with rheumatoid arthritis (RA).

Methods: SF of knee joints of 7 RA patients with active synovitis and 5 donors were investigated. Activity of enzymes was measured in cytosol of SF cells after ultracentrifugation. Activity of AMFK was estimated with the Western blotting method. The consumption speed of oxygen by SF cells was recorded polarographycally using as the substrate glutamic acid (5 μ mol/ml in incubation fluid). Registration of the active forms of oxygen was carried out by EPR (electronic paramagnetic resonance). Levels of adenylic nucleotides were determined chromatographically.

Results: In RA SF we noted activation of AMFK (on average by 2,5 times) at the considerable increase of the AMP level and decrease in ADP and ATP.

Conclusions: These data demonstrate transition of cells of SF in RA to the energy saving mode of functioning that is followed by strengthening of oxidizing processes, deep dissociation of respiration with oxidizing phosphorylation and sharp increase of oxigen consumption speed by SF cells in vitro against the background of the progressing hypoxia of synovial cells and chondrocytes in vivo. Destabilization of mitochondrial and lysosomal membranes of SF cells appears against the background of shift of pH in RA in more acidic zone. It leads to the reinforced formation of the active forms of oxygen (short-lived toxiferous hydroxyl radicals), shift of red-ox potential of cells, activation of lysosomal hydrolyzing enzymes that forms biochemical mechanisms of development of an autophagy in RA.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.5025

FRI0079 TAS5315, A NOVEL BRUTON'S TYROSINE KINASE INHIBITOR, IMPROVE BONE MINERAL DENSITY (BMD) AND BONE EROSION VIA INHIBITION OF OSTEOCLAST ACTIVATION IN MURINE MODEL FOR RHEUMATOID ARTHRITIS

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Background: The erosions of bone and cartilage are a cardinal feature of rheumatoid arthritis (RA) and associated with disease severity and poor functional outcome[1]. Although several anti-inflammatory drugs improve symptoms of articular inflammation, they are less effective against bone erosion. The bone erosions in RA are associated with aberrant activations of osteoclasts induced by pro-inflammatory cytokines and receptor activator of nuclear factor κB ligand (RANKL)[2]. Bruton's tyrosine kinase (BTK), which is expressed in immune cells and mature osteoclast, is reported to be a key molecule in inflammatory response and bone resorption[3,4]. Thus, targeting BTK may be efficacious against not only inflammation but also bone erosion through direct regulation of activation of effector cells such as B cells, macrophages and osteoclasts in RA.

Objectives: In this study, we evaluated the effect of TAS5315, a novel BTK inhibitor, on *in vitro* osteoclasts activation and bone erosion in mouse collagen-induced arthritis.

Methods: Kinase selectivity of TAS5315 was assessed by available kinase assay panels. The effects of TAS5315 on macrophages and osteoclasts were assessed by examining phosphorylation of BTK, cytokine productions, osteoclast differentiation and bone resorptions. The effects of TAS5315 were investigated in mouse collagen-induced arthritis (CIA). Disease severity was evaluated by clinical score of paw swelling. Changes in bone mineral density (BMD) and bone erosion were assessed using microCT. TNF blocker was used as a control drug.

Results: TAS5315 selectively inhibited the enzyme activity of BTK and had less off target inhibition against other kinases. TAS5315 dose-dependently inhibited cytokine productions by macrophages, phosphorylation of BTK, osteoclastogenesis and bone resorbing activity in osteoclasts. In established mouse CIA, TAS5315 significantly ameliorated paw swelling in a dose dependent manner and the anti-inflammatory effect of TAS5315 (0.3 mg/kg, once daily) was comparable to that of TNF blocker. Most importantly, improvement of BMD and bone erosion were observed in TAS5315 treated mice at a doses of higher than 0.1 mg/kg within 13 days from treatment initiation, but not in TNF blocker-treated mice. The onset of action of TAS5315 on BMD and bone erosion was earlier and stronger compared with that of TNF blocker. These data suggest that TAS5315 had direct effect against osteoclasts function and led to improvement of bone erosion in murine model for RA.

Conclusions: Our study demonstrates that TAS5315, a novel BTK inhibitor, would be an ideal RA therapeutic agent that could inhibit bone destruction as well as inflammation.

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Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.1761

FRI0080 CD11B+GR1DIM TOLEROGENIC DENDRITIC CELL-LIKE CELLS ARE EXPANDED IN INTERSTITIAL LUNG DISEASE IN SKG MICE

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Background: SKG mice develop interstitial lung disease (ILD) resembling human rheumatoid arthritis (RA)–associated ILD. We identified a unique cell population of $CD11b^{+}Gr1^{dim}$ cells in the severely inflamed lungs in SKG mice.

Objectives: The aims of this study are to clarify the mechanism behind the lung pathology, and to elucidate the phenotype and function of $CD11b^{+}Gr1^{dim}$ cells in ILD-induced SKG mice.

Methods: We assessed the severity of zymosan A-induced ILD in SKG mice histologically, and examined lung-infiltrating cells by Giemsa stain and flow cytometry. Total lung cells and isolated monocytic myeloid-derived suppressor cells (M-MDSCs) were cultured in vitro with GM-CSF (and IL-4). The proliferation of CSFE-labeled naïve T cells co-cultured with isolated $CD11b^{+}Gr1^{dim}$ cells and MDSCs was evaluated by flow cytometry.

Results: MDSCs, Th17 cells, and group 1 and 3 innate lymphoid cells (ILC1s and ILC3s) were increased in the lungs; the proportion of these cells varied with ILD severity. In this process, we found that a unique cell population, $CD11b^{+}Gr1^{dim}$ cells, were expanded in the severely inflamed lungs (Figure). About half of the $CD11b^{+}Gr1^{dim}$ cells expressed CD11c and Giemsa stain revealed that they were morphologically dendritic cell (DC)-like. The $CD11b^{+}Gr1^{dim}$ cells were induced from M-MDSCs with GM-CSF in vitro and were considered tolerogenic because they expressed high levels of PD-L1 and suppressed T-cell proliferation. The $CD11b^{+}Gr1^{dim}$ cells have never described previously and termed $CD11b^{+}Gr1^{dim}$ tolerogenic DC-like cells ($CD11b^{+}Gr1^{dim}$tolDC-LCs). Th17 cells, ILC1s and ILC3s in the inflamed lung produced GM-CSF, which in turn could induce $CD11b^{+}Gr1^{dim}$tolDC-LCs to reduce inflammation.

Conclusions: We identified a unique cell population, termed $CD11b^{+}Gr1^{dim}$tolDC-LCs, in ILD-induced lungs in SKG mice.

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Acknowledgements: The authors thank Shino Tanaka (Department Rheumatology and Clinical Immunology, Kobe University Graduate School of Medicine) for providing technical assistance.

Abstract FRI0078 – Table 1 AMPK activity (units/mg of protein), oxygen uptake rate (OUR, natoms of oxygen/min/mg of protein), the level of active free radical form of oxygen (AFRF, units/mg of protein), the content of adenine nucleotides in SF (nmol/ml) and pH in SF in RA

	AMPK	OUR	AFRF	AMP	ADP	AFP	pH of SF
RA SF	2,5±0,4	86,3±4,3	32,8±4,6	120–135 (127,5)	97–123 (110)	735–990 (862,5)	66,4–6,97 (6,81)
Donor SF	1,1±0,2	42,4±3,7	10,9±2,1	45–81 (63)	157–294 (222,5)	1490–1547 (1518,5)	7,43–7,65 (7,54)

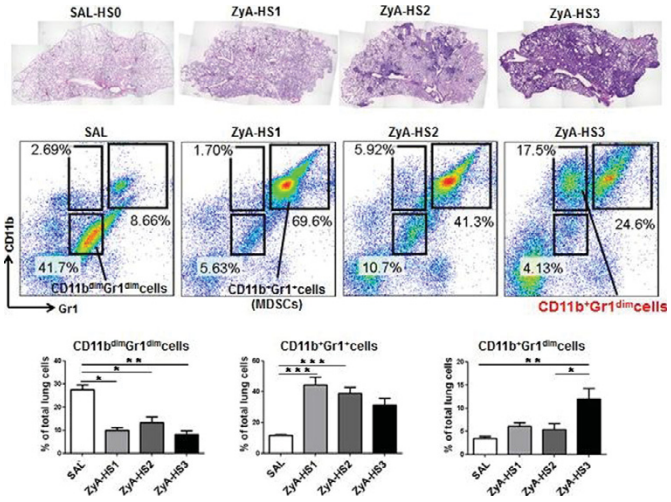

Figure 1

Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.1429

FRI0081 A MACAQUE MODEL OF RHEUMATOID ARTHRITIS BY IMMUNIZATION WITH CITRULLINATED PEPTIDES: LESSONS FOR THE HUMAN DISEASE

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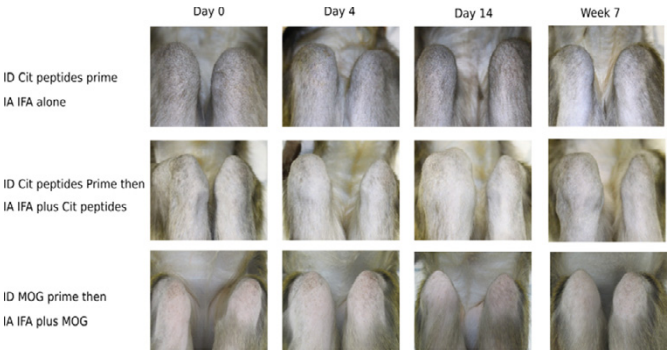
Background: Recent evolution in the understanding of rheumatoid arthritis (RA) mechanisms is the role of antibodies directed against citrullinated (cit) proteins (ACPAs). The shared epitope (SE) on the MHC class II is the main genetic risk factor of RA and favors presence of ACPAs. Mouse models dependent on cit peptides immunization require transgenic expression of the SE and are controversial. Non-human primates are ideal to study the interaction between ACPA and RA since 8% carry, similarly to humans, the SE called the H6 haplotype.
Objectives: The goal of this study was to develop a new animal model of RA based on immunization of genetically predisposed macaques against cit peptides to generate an ACPA-mediated model of arthritis.
Methods: Six macaques were intra dermally (ID) immunized with 4 peptides: vimentin (59–71) and (66–78), α fibrinogen (79–91) and aggrecan (89–103). H6 animals were immunized with either cit (n=2) or arginine (arg) (n=2) containing peptides. Two non H6 animals were immunized with cit peptides. These peptides are known to induce a T cell response in RA patients carrying the SE. T-cell response was assessed with Interferon γ ELISPOT and B-cell response by ELISA. An intra articular (IA) boost was done 30 weeks after initial immunization with either incomplete Freud's adjuvant (IFA) alone, IFA and cit peptides and IFA plus non relevant peptides.
Results: In the macaques, the T-cell response was specific to cit or arg peptides (depending on the peptides used for immunization). Surprisingly, the presence of the H6 epitope did not influence the response. Conversely the antibodies generated in response to the peptides were cross-reactive between the cit and arg peptides. Since no clinical response was observed, an IA boost was performed with the same 4 cit peptides and IFA adjuvant. This led to a prolonged neutrophil-rich mono-arthritis preferentially in H6 animals (Figure). Conversely, animals boosted with IFA alone only or with IFA plus myelin oligodendrocyte glycoprotein (MOG) peptides and previously immunized with MOG peptides presented with a transient mono-arthritis. Histological analysis revealed a local mononuclear infiltrate in one of the two animals that had prolonged knee monoarthritis. There was no clinical polyarthritis but 2 animals displayed synovial proliferation in 1 MCP and 1 MTP, respectively.
Conclusions: Immunization of macaques with cit peptides, then IA boost with the same cit peptides plus IFA, induced a prolonged monoarthritis. Shared epitope bearing did not restrict the T-cell response but seemed to favor the prolonged swelling after the IA boost. Neutrophil infiltration of the joint occurred similarly to what is seen in RA. Further use of neutrophil chemo-attractant might lead to a poly-articular disease. This macaque model of RA appears unique to study the events occurring during the pre-clinical phase of RA.



Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.2843

FRI0082 RAS SIGNALING INHIBITORS ATTENUATE ARTHRITIS IN ANIMAL MODELS OF RHEUMATOID ARTHRITIS BY DOWN MODULATING THE PATHOGENIC TH17 CELL RESPONSE

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Background: Ras-GTPases are vital for normal T cell activation, and downstream effectors of Ras include the MEK/ERK, PI3-kinase/AKT, mTOR/p70S6-kinase, and NF-kB pathways. Somatic mutations in *NRAS* cause an autoimmune lymphoproliferative disorder and T cells from Rheumatoid Arthritis (RA) patients exhibit perturbation of the Ras/MEK/ERK pathway. The small molecule Farnesylthiosalicylic acid (FTS) inhibits the interaction between Ras-GTPases and prenyl-binding chaperones vital for proper plasma membrane localization and downstream signaling [1]. Previous pre-clinical studies suggest that FTS has an immunomodulatory effect in various animal models of autoimmunity [2].
Objectives: To test in the Lewis rat adjuvant induced arthritis (AIA) and in the DBA/1 mouse collagen type-II induced arthritis (CIA) models the therapeutic immunomodulatory effect of FTS alone or combined with methotrexate (MTX).
Methods: Arthritis was induced in 8–12 week old male Lewis rats by complete Freund's adjuvant (CFA) injection and in male DBA/1 mice by collagen type-II (CII) immunization. Animals were treated prophylactically with once daily oral FTS (100 mg/kg); weekly i.p injection of MTX (0.5 mg/kg), oral FTS combined with MTX, or daily oral vehicle solution (0.5% carboxy methyl cellulose; CMC). Arthritis severity was scored daily from disease onset until study termination. In addition, we measured multiple disease- and drug-related immunological/molecular biomarkers.
Results: AIA severity was significantly reduced by FTS treatment compared to CMC controls (Figure 1A, $P<0.001$). Combining FTS and low dose MTX significantly increased its therapeutic efficacy compared to each drug alone (Figure 1A, $P<0.05$). FTS or FTS+MTX treatment also suppressed the upsurge in serum IL-17 and CRP compared to ailing controls. Global gene expression analysis of relevant splenic CD4+ T cells revealed that FTS is a potent inhibitor of pro-inflammatory and TH17 related gene networks. Next, our data from the mouse CIA model show that the therapeutic efficacy of FTS was non-inferior to MTX and it significantly reduced arthritis severity compared to controls (Figure 2, $P<0.001$). Importantly, FTS significantly inhibited the production of pathogenic anti-CII autoantibodies and upregulation of serum IL-6 and IL-17A compared to control arthritic mice. The in depth, multiplex, analysis of the effect of FTS on the T cell cytokine response to CII, revealed strong suppression of IL-22, IL-17, IL-9, GM-CSF and TNF production. Noteworthy, FTS therapy positively correlated with reduced Ras-GTP, p-ERK and p-AKT levels in splenic lymphocytes (drug related biomarkers).

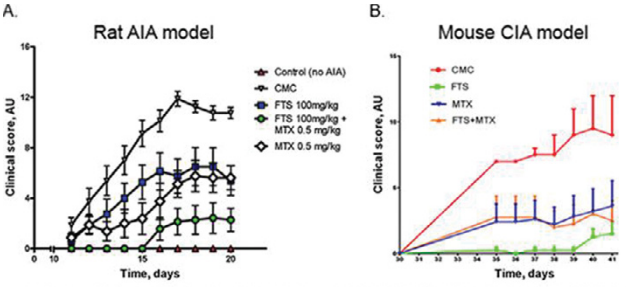

Figure 1. FTS attenuates arthritis severity in the (A) rat AIA and (B) mouse CIA models

Conclusions: FTS, a first-in-class oral selective Ras-GTPases inhibitor, exhibits a potent immunomodulatory effect in two classical murine model of arthritis, coupled with the inhibition of the TH17 response to relevant arthritogenic-antigens. Thus, Ras-signaling-blockade is a promising novel therapeutic approach for RA.

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