develop particularly severe bone fragility accompanied with high risk factors for fragility fractures^{1,2}. Although bisphosphonate is recommended for treatment of osteoporosis related RA and glucocorticoid-induced-osteoporosis³, there are more frequently fractures than expected from bone mineral density based prediction even after used bisphosphonate.

Objectives: To clarify the pathological mechanisms of bone fragility of these RA patients, we investigated bone biopsy samples obtained from RA and normal postmenopausal patients.

Methods: We examined 10 female postmenopausal RA patients receiving glucocorticoid and bisphosphonate therapy (RA group) and 10 age-matched female patients with postmenopausal osteoporosis (Ctl group) selected from patients who required autologous iliac bone grafts. Analyses of clinical data, bone mineral density, serum metabolic markers, bone quality and material mechanical property of biopsy sample were performed.

Results: Although bone mineral density didn't show significant differences, RA group had significantly higher score of fracture risk assessment tool (FRAX), number and severity of existing vertebral fractures.

	Control	RA	p
FRAX score (%)			
Major osteoporosis fx.	10.7±4.8	33.4±8.0	<0.001
Femoral neck fx.	2.8±2.4	9.9±6.1	0.002
Clinical fragility fx.			
Number	4	16	
Severity (# x grade)	4	29	
Mechanical strength (N/mm)	218.8±24.6	164.9±36.5	0.001

RA group exhibited significant bone quality abnormalities including deterioration of the bone microstructure, decreased calcification of the bone matrix, increased osteocyte atrophy and empty lacunae (Figure), and an impairment bone material strength properties.

Figure: Histological mechanism of deterioration of bone quality in RA patients receiving GCs. (A-D) Representative photomicrographs of HE stained sections of the specimen. Images show sections from Control (A, C) and RA groups (B, D). Boxed areas in B and D are shown at higher magnification in the indicated images. (E, F) Representative micrographs focused on osteocytes of Control (E) and RA groups (F) with toluidine blue staining. Osteocytic lacunae (red arrows) are out of shape and emptiness in RA patients. (G) Comparison of ratio of empty osteocytic lacuna is shown. (H, I) Representative micrographs of Control (H) and RA groups (I) with immunostaining of sclerostin (black arrow). (J, K) Representative micrographs of Control (J) and RA group (K) with von Kossa staining. Black arrows show the cement lines. (L) Comparison of serum BAP is shown. (M, N) Representative micrographs of Control (M) and RA groups (N) with immunostaining of sclerostin ALPase. (O) Comparison of serum TRACP-5b is shown. (P, Q) Representative micrographs of Control (P) and RA group (Q) with TRAP staining. Osteoclasts are stained red. Values shown are means ± SD (* p<0.05).

Conclusions: Our findings showed that RA patients receiving glucocorticoid treatment have severe bone fragility regardless of increased bone quantity by using bisphosphonate. The functional deteriorations of the osteocyte system and the abnormalities of bone quality might induce bone fragility fracture. Therefore, management of osteoporosis associated with RA should be targeted about bone quality as well as bone quantity.

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FRI0076 LACTATE/SLC5A12-INDUCED METABOLIC SIGNALLING NETWORK: A NEW TARGET IN RHEUMATOID ARTHRITIS

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Background: RA is a systemic-autoimmune-disease characterized by chronic inflammation of the synovial-joints. Up to 30–40% of patients do not respond to treatment and current biomarkers are largely insensitive at predicting disease progression and response to treatment. It is well recognised that RA-synovitis is a heterogeneous pathology with different histological phenotypes i.e. lymphoid-(L), myeloid-(M) and fibroid-(F). As a result of inflammation the RA synovial microenvironment is hypoxic and acidic partly due to the accumulation of lactate, the end product of anaerobic glycolysis. Exposure of CD4⁺ T cells to lactate inhibits their migratory capabilities and induces their shift toward a Th17 phenotype^{1,2}. These effects are mediated via the interaction of lactate with its transporter SLC5A12, which is expressed on the CD4⁺ T cell surface^{1,2}.

Objectives: To characterize whether the newly identified lactate/SLC5A12-induced metabolic signalling pathway can be harnessed in the stratification of RA patients in the different histological phenotypes and to modulate inflammatory responses *in vitro* and *in vivo*.

Methods: RA peripheral blood cells, RA synovial tissues (ST, 21 lymphoid and 8 myeloid) and mononuclear cells from tonsil of patients undergoing tonsillectomy were included in the analysis. RNA sequencing of RA-ST was performed and data analysed for the expression of metabolic genes. SLC5A12 expression and IL17 production was performed by flow-cytometry. Cytokines and transcription factors mRNA relative expression was evaluated by RT-PCR. Seahorse and western blot analysis was performed for the evaluation of metabolic pathways. Transwell plates were used for migration assays. Human-glucose-6-phosphate-isomerase (hG6PI) induced arthritis model was used to evaluate the impact of anti-SLC5A12 on the clinical and histological score.

Results: We showed that: i) the expression of SLC5A12 is up-regulated by CD4⁺ T cells upon inflammation; ii) SLC5A12 is up-regulated in anti-CD3 stimulated RA CD4⁺ T cells cultured in autologous synovial fluid; iii) the lactate/SLC5A12 induced metabolic pathway is differentially activated in RA patients with distinctive synovial "pathotypes"; iv) SLC5A12 antibody reduces lactate-induced pro-inflammatory cytokines, limits Th17 and follicular helper T cell differentiation, reverses lactate impaired CD4⁺ T cell migration and restores lactate-mediated inhibition of glycolysis *in vitro*; v) antibody-mediated blockade of SLC5A12 ameliorates the clinical course in human-glucose-6-phosphate-isomerase (hG6PI)-induced arthritis.

Conclusions: Targeting lactate/SLC5A12-induced metabolic signalling pathway may provide a novel therapeutic strategy to reduce inflammation in RA patients.

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FRI0077 GUT COMMENSALS MODULATE INFLAMMATION VIA T REGULATORY CELLS

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Background: Role of environmental factors in predisposition to develop rheumatoid arthritis (RA) have gained interest due, in part, to the studies showing an association of gut microbiota with immune homeostasis. Although the etiology of RA is unknown, recent studies on the role of gut microbiota in inflammatory adaptive immune response have led to the concept that interaction between the host microbiome and genetic factors influences autoimmunity. We have recently shown an association of Collinsella aerofaciens with RA

Objectives: In this study, we aimed to determine how human gut commensals modulate arthritis phenotype in humanized mice expressing RA-susceptible HLA-DQ8.

Methods: DQ8 mice following immunization with type II collagen develop arthritis and antigen-specific cellular and humoral response. DQ8 mice orally gavaged with RA-associated Collinsella aerofaciens and non-associated Prevotella histicola on alternate days for one week and were induced for arthritis. Gavage with microbes continued for 4 weeks. Mice were monitored for onset and progression of arthritis. Th17 regulatory network and cells involved in regulation were analyzed. DQ8 mice were gavaged with fecal homogenates supernatants from arthritis-resistant mice, induced for arthritis and monitored for disease.

Results: Mice gavaged with C. aerofaciens enhanced disease severity while P. histicola protected mice from arthritis. While treatment with P. histicola reduced intestinal permeability by increasing expression of tight junction proteins, C.

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.

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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 synovial 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 polarographically 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 oxygen 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 toxic 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

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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 outcome¹. 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 κ B 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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FRI0080 CD11B+GR1^{dim} TOLEROGIC 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 CFSE-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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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)