the development of arthritis or are rather a consequence of the inflammatory processes. Furthermore, while both germ-free condition and administration of oral antibiotics prevent arthritis in mice, it is unclear whether modulation of the intestinal microbiota after the onset of arthritis may still suppress the disease.
Objectives: We aimed to assess potential alterations of the intestinal microbiome in the preclinical phase of inflammatory arthritis, and to determine the efficacy of microbiota modulations in the treatment of established disease in mice.
Methods: We sequenced fecal bacterial 16S rRNA to define the intestinal microbiome in mice before immunization with collagen and 21 days later before the booster injection for the induction of collagen-induced arthritis (CIA). To assess the efficacy of microbiota modulation during arthritis, mice with ongoing CIA were treated orally by a broad-spectrum antibiotic cocktail for one week to partially eliminate the intestinal microbiota. T cell differentiation and production of cytokines in intestinal lamina propria and joint-draining lymph nodes were assessed by flow cytometry and Luminex. Arthritis was assessed macroscopically and by histology. Serum-transfer arthritis induced by intraperitoneal injections of arthritogenic K/BxN mouse serum was used as a control, T cell-independent, model.
Results: The preclinical phase of inflammatory arthritis in mice was characterized by marked changes in the intestinal microbiome, represented by a significant increase of the phylum *Bacteroidetes* and a decrease of *Firmicutes* and *Proteobacteria*. Among the most abundant bacterial families, S24–7 and *Staphylococcaceae* were expanded, whereas *Lachnospiraceae* were reduced during the immune priming phase of CIA. Several operational taxonomic units associated with S24–7 family increased, while those assigned to *Lachnospiraceae* and *Ruminococcaceae* decreased in the intestinal microbiota before the clinical onset of arthritis. The abundance of intestinal lamina propria Th17 cells significantly correlated with the severity of CIA; however, intestinal Th1 cells were not correlated with the disease. Elimination of intestinal microbiota in mice with ongoing CIA specifically suppressed intestinal Th17 cell differentiation without affecting Th1 and Treg cells. Importantly, elimination of intestinal microbiota suppressed Th17 cell differentiation and IL-17 production in joint-draining lymph nodes, and reduced the severity of established CIA. In contrast, the T cell-independent serum-transfer arthritis was not affected by this strategy.
Conclusions: These observations suggest that perturbations of the intestinal microbiome may precede the development of inflammatory arthritis. Similar studies are warranted in human pre-RA or at-risk individuals to shed light on the functional role of the microbiome in the development of RA. Our studies also suggest that modulation of the intestinal microbiota after the onset arthritis may still provide opportunities to treat inflammatory arthritis.
Disclosure of Interest: None declared
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FRI0073 RAGE ACTIVITY INFLUENCES CO-DEVELOPMENT OF JOINT AND VASCULAR DISEASE IN RA

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Background: RAGE is expressed by many cells in blood and joints of RA and interacts with a variety of pro-inflammatory ligands, especially HMGB1 that are enriched in inflamed joint. The RAGE-ligand interaction leads to a sustained inflammatory response. Soluble RAGE (sRAGE) lacks the transmembrane and cytoplasmic domain of the cell surface full-length RAGE (flRAGE) and function as a decoy for RAGE ligand. Relatively little is known about factors that regulate sRAGE levels in human subjects and whether circulating levels reflect tissue RAGE expression and activity is unclear. The relationship between the up-regulation of flRAGE/HMGB1 and the level of "protective" sRAGE levels in RA is of obvious clinical interest.
Objectives: To elucidate the balance between the expression of flRAGE on peripheral blood monocyte and dendritic cell (DC), and the plasma concentration of sRAGE and HMGB1 in patients with active RA compare to controls; To ascertain whether flRAGE expression profiles, plasma sRAGE and HMGB1 level correlated with disease activity or inflammatory markers in RA patients.
Methods: 40 consecutive patients attending the rheumatology clinic at Prince of Wales Hospital, who fulfilled the 2010 ACR/RULAR classification criteria with active RA (28 joint disease activity score 28–4 [CRP] (DAS28)$>$3.2) and 40 age- and sex-matched healthy volunteers were recruited for this cross-sectional study. The expression profile of cellular transmembrane RAGE on peripheral blood monocyte ($ILT3^+CD123^-$), total DC ($ILT3^+CD14^-CD16^-$), myeloid DC ($ILT3^+CD14^-CD16^-CD123^-$) and plasmacytoid DC ($ILT3^+CD14^-CD16^-CD123^+$), plasma levels of HMGB1 and soluble RAGE in all RA patients and healthy controls were measured at baseline using flow cytometry and ELISA. In RA patients, associations with disease activity and severity variables were analyzed by simple and multiple linear regressions.
Results: Protein expression of flRAGE on peripheral blood monocytes ($ILT3^+CD123^-$), total DCs ($ILT3^+CD14^-CD16^-$) and myeloid DCs ($ILT3^+CD14^-CD16^-CD123^-$) were significant increased in RA patients with active disese compared with control subjects (all $p<0.01$) (no enough cells for flRAGE detection on plasmacytoid $ILT3^+CD14^-CD16^-CD123^+$ DCs). Also, the flRAGE was more common in patients with cardiac affection. There was no statistically significant difference of the plasma level of HMGB1 from active RA patients as compared healthy controls ($p>0.05$). The plasma sRAGE level was significantly lower in patients compared to healthy controls ($p<0.001$), which correlated negatively with serum levels of CRP, ESR, DAS28 and high-density lipoprotein (HDL) (all $p<0.05$). Linear regrssion analysis detected CRP as significant predictors for sRAGE level.
Conclusions: Autoimmunity-mediated inflammation might induce aberrant expression and activation of flRAGE while decreasing the plasma level of sRAGE in RA patients, subsequently leading to the augmented inflammatory response. Moreover, membrance flRAGE and sRAGE were associated not just with RA inflammation and autoantibody protein, but also with classical vascular risk factors for end-organ damage. These data suggest that RAGE activity influences co-development of joint and vascular disease in RA patients.
Disclosure of Interest: None declared
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FRI0074 ANTI-INFLAMMATORY EFFECT OF RESVERATROL IN VITRO; POTENTIAL ROLE IN MANAGING LOW DISEASE ACTIVITY IN ARTHRITIS?

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Background: Resveratrol (RSV), a non-toxic polyphenol found in grapes, certain nuts, roots etc., have received increased attention in the last decade due to its anti-inflammatory modulation of a number of pathways, including cyclooxygenase-1/-2, nuclear factor kappa-beta and cytokine production. In vitro, RSV has been shown to reduce production of interleukin 1-beta and tumor necrosis factor alpha in monocytes and inhibit T-cell activation and synoviocyte proliferation. In vivo, intra-articular injections of RSV have demonstrated anti-inflammatory and pannus inhibiting effects in rats with induced arthritis.
Objectives: Here, we tested whether the anti-inflammatory effect of RSV in arthritis patients depends on the degree of systemic inflammation and the cellular composition of extracted synovial fluid. Furthermore, we evaluated the anti-inflammatory effect of RSV in combination with methotrexate (MTX) and adalimumab.
Methods: Synovial fluid mononuclear cells (SFMCs) from patient with rheumatoid arthritis (n=7) and spondyloarthritis (n=7) were cultured in monoculture for 48 hours (in vivo activated lymphocytes and monocytes) or 21 days (spontaneous generation of osteoclasts). Cultures were either left untreated or treated with RSV (25 μM), methotrexate (0.5 μg/ml), adalimumab (5 μ g/ml) or in combination. Supernatants were analysed for the production of monocyte chemoattractant protein-1 (MCP-1) and matrix metalloproteinase 3 (MMP3). Osteoclast differentiation was analysed with a tartrate resistant acidic phosphatase (TRAP) enzymatic assay.
Results: In the SFMC cultures, RSV reduced MCP-1 production by 25% compared with untreated cells (P=0.032). When grouping results by c-reactive protein (CRP), i.e. $<$ median vs. $\geq$ median, the inhibitory effect of RSV was primarily seen in cultures from patients with CRP $<$21 mg/l. In this group, RSV reduced MCP-1 production by 63%. Combining MTX and RSV reduced MCP-1 production compared to MTX alone, but only in the group of patients with CRP $<$21 mg/l (P=0.002). Combining adalimumab and RSV seemed to reduce MCP-1 in the group of patients with CRP $<$21 mg/l, but increase production in patients with CRP $\geq$21 mg/l (P=0.03). Similar grouping based on lymphocyte count showed RSV, MTX and adalimumab, alone or in combination, all reduced MCP-1 significantly compared to untreated cells in cultures from patients with $\geq$62% lymphocytes in the synovial fluid. RSV, MTX and adalimumab did not affect MMP3 production in the SFMC cultures. In the osteoclast cultures, RSV alone did not affect MCP-1 or TRAP. However, the combination of RSV and MTX reduced MCP-1 compared to no treatment (P=0.004). Adalimumab alone or combined with RSV reduced TRAP compared with untreated cultures ($P<0.027$).
Conclusions: RSV exhibits an anti-inflammatory effect on SFMCs. Interestingly; our data suggest that this effect is most pronounced in patients with relatively low CRP. Further, RSV produces an additive anti-inflammatory response in combination with MTX in the group of patients with low CRP and a synovial fluid dominated by lymphocytes. Together, this suggests that RSV may possess an additive potential when added to MTX treatment of arthritis patients with low disease activity.
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FRI0075 RHEUMATOID ARTHRITIS PATIENTS RECEIVING GLUCOCORTICOID EXHIBITS SIGNIFICANT BONE QUALITY ABNORMALITIES, LEADING TO SEVERE BONE FRAGILITY REGARDLESS OF BISPHOSPHONATE TREATMENT

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Background: Patients with rheumatoid arthritis (RA) receiving glucocorticoids

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-osteoporosis[3], 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.

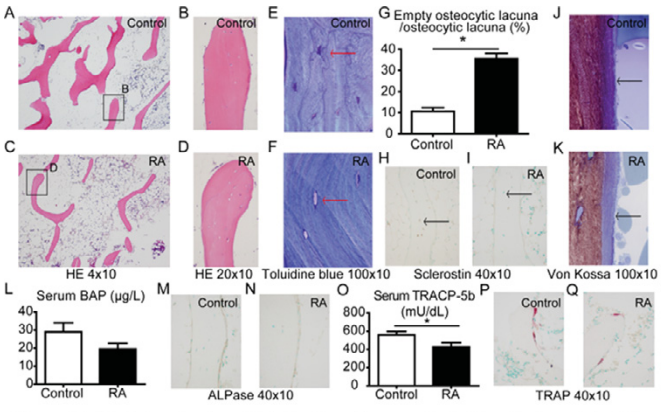

Figure: Histological mechanism of deterioration of bone quality in RA patients receiving GCs. (A-D) Representative photomicrographs of HE stained sections of the specimen. Images show sections from Control (A, C) and RA groups (B, D). Boxed areas in B and D are shown at higher magnification in the indicated images. (E, F) Representative micrographs focused on osteocytes of Control (E) and RA groups (F) with toluidine blue staining. Osteocytic lacunas (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 ALP ase. (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.

References:

[1] Kanis JA et al. J Bone Miner Res 2004.
[2] Takaha M et al. Arthritis Rheum 2012.
[3] Grossman, J.M. et al. Arthritis Care Res 2010.

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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.

References:

[1] Haas R. et al. PLoS Biology 2015.
[2] Pucino V. et al. Eur J Immunol 2017.

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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.