FRI0069 NEUTROPHILS PLAY A KEY ROLE IN THE REGULATION OF THE CHRONIC INFLAMMATION ASSOCIATED WITH RHEUMATOID ARTHRITIS THROUGH EPIGENETIC MECHANISMS MODULATED BY ANTI-CCPs ANTIBODIES AND REVERSED BY BIOLOGIC THERAPIES

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Background: MicroRNAs (miRNA) are as a new class of modulators of gene expression, regulating inflammation, degradation of extracellular matrix and invasive behavior of the resident cells in rheumatoid arthritis (RA).

Objectives: 1) To investigate the miRNA expression profile in synovial and blood neutrophils in RA and its role in the pathogenesis of this disorder. 2) To study the effects of biological therapies on the miRNA profile in neutrophils.

Methods: Neutrophils were isolated from peripheral blood (PB) of 25 healthy donors (HD) and 25 RA patients. Neutrophils were isolated from paired synovial fluid (SF) of 15 RA patients. nCounter microRNA Assay was used to detect 800 human microRNAs simultaneously. Altered miRNAs were analyzed for potential mRNA targets using Ingenuity pathways analysis (IPA) software. mRNA targets and genes involved in miRNA biogenesis were evaluated. RA neutrophils were treated in vitro with tocilizumab and infliximab. Healthy neutrophils were treated in vitro with anti-CCPs isolated from RA patients alone or combined with tocilizumab or infliximab. RA neutrophils were cotransfected with the pre-miRNAs 126 and 148.

Results: 94 miRNAs were downregulated and 3 upregulated in PB neutrophils from RA patients compared to HD. Among the miRNAs deregulated in blood, 34 miRNAs were even more significantly reduced in SF neutrophils. Accordingly, RA neutrophils showed a downregulation of the proteins participating in miRNA biogenesis and upregulation of its mRNA targets. Altered miRNAs were mainly involved in immunological disease and inflammatory response, suggesting the abnormal activation of this cell subtype in RA. In vitro treatment of RA neutrophils with infliximab reversed the expression of miRNAs, genes involved in their biogenesis and reduced the inflammatory profile in these cells. In addition, treatment of healthy neutrophils with IgGs anti-CCPs isolated from RA patients decreased the expression of the miRNAs, proteins involved in the biogenesis machinery and increase its inflammatory targets. Finally, cotransfection of RA neutrophils with the pre-miRNAs 126 and 148 reduced the levels of their target proteins.

Conclusions: 1) RA neutrophils exhibit a defect in the miRNAs processing, showed by a decrease in most of the detected miRNAs and the downregulation of proteins involved in their biogenesis. This defect seems to be modulated by anti-CCPs antibodies. 2) miRNA downregulation is even more pronounced in synovial fluid neutrophils, which may contribute to the high inflammatory profile of these cells in the joint. 3) Infliximab reverses the altered miRNA profile and the defect in the biogenesis machinery, reducing the proinflammatory pattern in these cells. 4) miRNAs 126 and 148 regulate the expression of numerous inflammatory proteins in RA neutrophils. In sum, this study shows that the neutrophils play a key role in the regulation of the chronic inflammation link to RA. The effect, associated to epigenetic mechanisms, seems to be mediated by the anti-CCPs and is reversed by biologic therapies.

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FRI0070 ANGIOTENSIN II TYPE 2 RECEPTOR (AT2R) IS OVEREXPRESSED IN RHEUMATOID ARTHRITIS AND OSTEOARTHRITIS SYNOVIAL AND INCREASES STEADILY WITH INFLAMMATORY STIMULI: A POTENTIAL NEW TARGET FOR PAIN AND ANTI-INFLAMMATORY THERAPIES

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Background: Despite increasing evidence suggesting that angiotensin II type 2 receptor (AT2R) antagonism might be a feasible strategy for the treatment of chronic inflammation and pain, no study has yet analyzed the synovial expression of AT2R.

Objectives: To investigate the expression of AT2R in rheumatoid arthritis (RA) and osteoarthritis (OA) synovium and its possible modulation in fibroblast-like synoviocytes (FLS) by proinflammatory stimuli.

Methods: The expression of AT2R in RA and OA synovium was investigated by immunohistochemistry. AT2R expression in synovial T cells, B cells, macrophages and FLS was assessed by double immunofluorescence. FLS were isolated from healthy (H), OA and RA synovium and treated with tumor necrosis factor (TNF)- α and interleukin (IL)-1 β , alone or in combination. Immunocytochemistry and Western blotting were performed to study AT2R expression in cultured FLS.

Results: RA synovium showed stronger AT2R immunostaining than OA synovium in the lining and sublining layers. In RA synovium, AT2R was strongly expressed

in CD3+ T cells, CD20+ B cells, CD68+ macrophages and vimentin+ FLS. High levels of AT2R were found in OA-FLS and RA-FLS at baseline, while AT2R expression was negligible in basal H-FLS. AT2R expression was higher in RA-FLS than OA-FLS. Treatment with TNF- α and IL-1 β was able to foster the expression of AT2R not only in OA-FLS and RA-FLS, but also in H-FLS.

Conclusions: AT2R is strongly expressed in different cell types of the inflamed synovium and proinflammatory stimuli may foster the expression of AT2R in FLS. AT2R might represent a novel potential therapeutic target in chronic arthritides.

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FRI0071 TOFACITINIB RESTORES THE INHIBITION OF REVERSE CHOLESTEROL TRANSPORT INDUCED BY INFLAMMATION: UNDERSTANDING THE LIPID PARADOX ASSOCIATED WITH RHEUMATOID ARTHRITIS

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Background: Patients with active rheumatoid arthritis (RA) have increased cardiovascular mortality, paradoxically associated with reduced serum lipid levels. In fact, an inverse relationship between C-reactive protein (CRP) and circulating lipid levels has been observed [1,2]. The Jak inhibitor tofacitinib ameliorates systemic and joint inflammation in RA with a concomitant increase in serum lipids [3].

Objectives: Our aim was to analyze the effect of tofacitinib on the lipid and inflammatory profile of hyperlipidemic rabbits with chronic arthritis (CA), and on the regulation of reverse cholesterol transport (RCT) during chronic inflammation.

Methods: New Zealand male rabbits were randomly assigned to three groups: control group ($n=6$), chronic arthritic rabbits (CA, $n=9$) and CA rabbits receiving tofacitinib (CA+TOFA, $n=9$). All animals were fed *ad libitum* with a high-fat diet (0.5% cholesterol, 4% peanut oil). CA was induced in previously immunized rabbits by administering four intra-articular injections of ovalbumin. A group of rabbits received tofacitinib (10mg/kg/day) for two weeks. All animals were euthanized 6 weeks after challenge, when synovial and serum samples were collected. For *in vitro* studies, vehicle and ABCA1 siRNA-treated THP-1-derived macrophages were exposed to high lipid concentrations, and then stimulated with interferon gamma (IFN γ) in the presence or absence of tofacitinib, in order to assess their cholesterol efflux capacity and the involved mediators.

Results: CA rabbits showed lower levels of serum TC and LDL-C compared to controls ($p=0.001$, $p=0.012$), while TC/HDL-C ratio was higher in CA+TOFA rabbits when compared to CA animals (150 ± 15 vs 231 ± 17 , $p=0.004$). Synovial inflammation and CRP levels were increased in CA animals, and a significant reduction was shown in CA+TOFA rabbits in both parameters. We also observed an inverse correlation between serum TC and CRP ($R=-0.454$, $p=0.029$). Tofacitinib was able to reduce the lipid content within synovial macrophages up to a 58% ($p=0.041$), although it did not modify synovial macrophage density. In foam macrophages in culture, an inflammatory milieu induced by IFN γ further stimulated the intracellular lipid accumulation ($p=0.041$) along with a decrease in the protein levels of the nuclear factor LXR α (liver X receptor α) and the cholesterol transporter ABCA1 (ATP-binding cassette transporter 1) ($p=0.002$ and $p=0.0047$, respectively). Tofacitinib prevented the lipid accumulation within macrophages ($p=0.029$) by increasing LXR α ($p=0.047$) and ABCA1 synthesis ($p=0.004$) in a Jak/STAT-dependent manner, while it was unable to reduce lipid accumulation in ABCA1 silenced macrophages.

Conclusions: Our results suggest that active inflammation could be associated with lipid accumulation within macrophages – in the synovium and probably in other tissues – thus inducing a decrease in serum lipid levels. Tofacitinib may prevent this phenomenon, at least partially, by increasing RCT pathways in macrophages. Taken together, these findings further explain how serum lipid levels are diminished in RA and partially justify the effect of tofacitinib on the lipid profile in RA patients.

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FRI0072 ALTERATION OF THE INTESTINAL MICROBIOME IN THE PRECLINICAL PHASE OF EXPERIMENTAL ARTHRITIS AND THE EFFICACY OF MICROBIOTA MODULATION IN ESTABLISHED ARTHRITIS IN MICE

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Background: The composition of intestinal microbiota is perturbed in patients with new-onset rheumatoid arthritis (RA) and those with chronic RA. However, it is not known whether the changes in the intestinal microbiome precede

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.

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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 (fRAGE) 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 fRAGE/HMGB1 and the level of "protective" sRAGE levels in RA is of obvious clinical interest.

Objectives: To elucidate the balance between the expression of fRAGE 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 fRAGE 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 fRAGE 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 disease compared with control subjects (all p < 0.01) (no enough cells for fRAGE detection on plasmacytoid ILT3⁺CD14⁺CD16⁺CD123⁺ DCs). Also, the fRAGE 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 regression analysis detected CRP as significant predictors for sRAGE level.

Conclusions: Autoimmunity-mediated inflammation might induce aberrant expression and activation of fRAGE while decreasing the plasma level of sRAGE in RA patients, subsequently leading to the augmented inflammatory response. Moreover, membrane fRAGE 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 synovocyte 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 µg/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. ≥ 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 ≥ 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 ≥ 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 RSV 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