with RA-associated *E. lenta* 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. Epithelial integrity was done by FITC-Dextran assay and citrulline levels in faecal and plasma samples were measured by liquid chromatography mass spectrometry

Results: Mice gavaged with *E. lenta* showed a much higher load of gut microbes compared to controls. Surprisingly, *E. lenta* did not augment gut permeability as it was similar to non-gavaged arthritic mice. *Eggerthella lenta* is involved in ornithine pathway leading to generation of citrulline. To test if gavage with *E. lenta* accumulates citrulline in the gut, we tested citrulline levels in faecal samples by liquid chromatography mass spectrometry. Surprisingly, mice gavaged with *E. lenta* had lower levels of citrulline in faecal samples as compared to mice naïve mice with no gavage or arthritic mice without gavage. One can speculate from these observations that there is an expansion of commensals, like *E. lenta* in RA, leading to production of citrulline. To determine if citrulline could be accumulated outside of gut, we measured citrulline in sera of mice in various groups, gavaged with *E. lenta*, naïve and mice induced for arthritis but no *E. lenta* gavage. Surprisingly, mice gavaged with *E. lenta* and induced for arthritis had lower levels of citrulline as compared to controls induced for arthritis but no gavage, $p < 0.05$. Naïve mice gavaged with *E. lenta* also showed significantly much lower levels compared to sera of naïve mice.

Conclusions: This data suggests that *E. lenta* may be a major player in determining certain metabolic pathways. If Citrulline is being converted to arginine and used in other pathways is currently under investigation. 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.

REFERENCE:

- [1] Chen J, Wright K, Davis J, Marietta E, Luckey D, Jeraldo P, Murray J, Nelson H, Matteson E, Taneja V. An expansion of rare lineage intestinal microbes characterizes rheumatoid arthritis *Genome Medicine* 2016;8:43. doi:10.1186/s13073-016-0299-7

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AB0138 THE BONE MARROW OEDEMA IS LINKED TO A OSTEOCLASTIC ENVIRONMENT IN BONE MARROW DURING COLLAGEN INDUCED MICE ARTHRITIS DEVELOPMENT

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Background: Bone erosion is a central pathogenic event in Rheumatoid arthritis (RA) and is associated with joint damage and poor functional outcome. The synovitis is traditionally regarded as the primary event of bone erosion in RA and synovial cells are usually thought to play a critical role in this pathologic process. However, the synovitis-centred concept has long been challenged by recent clinical finds. Bone erosion formation also could be found in joints without clinical traits of synovitis, suggesting that synovitis and joint erosion could be uncoupled. Moreover, recent study demonstrated that bone marrow oedema (BME) that identified by magnetic resonance imaging (MRI) is strongly associated with erosive progression, which independent of local synovitis in RA.

Objectives: BME is also called osteitis, which represent the replacement of adipose tissue with inflammatory cells in bone marrow. In this study we addressed 2 questions: 1) the course of BME and synovitis in a same joint during CIA development; 2) how BME cause the bone erosion in CIA.

Methods: Using collagen induced mice arthritis model, we compared the compared the time course of appearance of BME, synovitis and arthritic symptoms during the development of CIA. The changes RANKL, cytokines, osteoclast and immune cells expression in bone marrow during the development of CIA were analysed by flow cytometric analysis, immunofluorescence staining and RT-qPCR.

Results: MRI BME can be identified as early as 25 days after first immunisation, when there is no any histopathological changes and arthritis symptoms. Flow cytometry and immunofluorescence staining indicated that the proportion of pre-OCs were significantly increased in bone marrow after day 25 with the presence of BME. At day 25, accompanied by presence of BME and increased pre-OCs, the number of trabecular bone was significantly diminished as compared with those at day 20 and reached its lowest number at day 35. We then examined the transcription of several pro-inflammatory cytokines and chemokines related to OCs differentiation, migration and activation, such as RANKL, IL-17, TNF α , CCL3, CCL4, CCL12, CCR5 by Real-time PCR. The most prominent RANKL

change was observed at day 35. IL-17 and TNF α mRNA were elevated from day 25, reaching a peak at day 28 and thereafter gradually decreasing. Expression of CCL3 and CCL12 were only higher at day 28 and day 25, respectively, while there was no significant change in CCR5 and CCL4 mRNA in the bone marrow. The proportion of T cells and monocytes in bone marrow were significantly higher from day 25 to day 45. No differences in B cells and plasma cells were detectable at any time points. Bone marrow cells from the presence of BME showed significantly increased ability to formation OCs *in vitro*.

Conclusions: BME precede the synovitis and arthritic symptoms during the development of CIA. Accompanied BME appearance, we identified an altered bone microenvironment that favours OCs differentiation and survival in CIA mice. We proposed that BME linked to a unique "osteoclastic environment" in CIA disease progression. These findings provide a new perspective for comprehending the development of erosions in RA.

Disclosure of Interest: None declared

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AB0139 INVESTIGATION OF PREVOTELLA COPRI FROM RHEUMATOID ARTHRITIS PATIENTS

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Background: We have previously reported some of the rheumatoid arthritis (RA) patients had *Prevotella copri* in the intestine. By using germ-free (GF) SKG mice, we also showed that *Prevotella*-dominated gut microbiota contribute to the development of arthritis¹⁾. However, *P. copri* itself has not been isolated from RA patients and their molecular biology was unknown.

Objectives: Firstly, we planned to evaluate the intestinal microbiota in RA patients before and after the treatment. Second, we isolated *P. copri* strains from RA patients and healthy controls (HCs) and analysed whether RA patients-derived *P. copri* expanded in the intestine of GF mice.

Methods: We first examined whether RA patients have altered composition of microbiota. All the patients were diagnosed according to the American College of Rheumatology/European League Against Rheumatism 2010 classification criteria for RA. We collected faecal samples from 55 RA patients (61.5 $\pm$ 9.5 years, mean ages $\pm$ SD) and 33 HCs (56.2 $\pm$ 8.2 years) to investigate the microbiota by 16S rRNA-based deep sequence technique. We also analysed bacterial counts of *Prevotella* and *Bacteroides fragilis* by qPCR method. Moreover, we isolated *P. copri* from faecal contents of RA patients and HCs. GF mice were inoculated with *P. copri* from RA patients and HCs for further analysis.

Results: We found that 34.5% (19/55) of RA patients and 18.1% (6/33) of healthy controls have relatively high abundance of *Prevotella* (>4%) in the intestine. These results were compatible with our previous observations. When we focused on the patients who harboured high abundance of *Prevotella* in the gut, *Prevotella/B. fragilis* ratio was decreased after the treatment. The mean *Prevotella/B. fragilis* ratio were changed from 1.28 to 0.75 ($p=0.08$). Further analyses revealed that RA patients-derived *P. copri* successfully colonised to GF-mice and induced Th17 cells in the large intestine.

Conclusions: We found that alternation of microbiota composition was observed after the RA treatment. Moreover, we successfully isolated *P. copri* from RA patients and HCs. RA patients-derived *P. copri* efficiently expanded in the intestine of GF mice.

REFERENCE:

- [1] Maeda Y, et al. Dysbiosis contributes to arthritis development via activation of autoreactive T cells in the intestine. *Arthritis Rheumatol* 2016;68 (11):2646–2661.

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AB0140 THE ROLE OF RAF KINASE INHIBITORY PROTEIN IN RHEUMATOID ARTHRITIS

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Background: Rheumatoid arthritis (RA) is an autoimmune inflammatory disease of the joints and is characterised by immune cell infiltration, synovial hyperplasia, and destruction of cartilage and underlying bone¹. Raf kinase inhibitory protein

(RKIP), an endogenous inhibitor of the extracellular signal-regulated kinase (ERK) pathway, has been implicated as a suppressor of metastasis and NF- κ B pathway in cancers².

Objectives: The NF- κ B and ERK pathways are considered to be one of the most important pro-inflammatory signalling pathways in RA. Therefore, RKIP might be a potential therapeutic target for RA. However, whether and how RKIP regulates RA is not fully understood. The present study was performed to examine whether and how RKIP are differentially regulated in RA.

Methods: The expressions of RKIP were assessed in synovial tissue, fluid and fibroblast-like synoviocytes (FLS) from patients with RA and osteoarthritis (OA) by immunofluorescence staining and western blotting. RA- or OA-FLS were infected with either a recombinant adenoviral RKIP overexpressing vector (Ad-RKIP) or shRNA-expressing vector (Ad-shRKIP). Control cells infected with a GFP-targeted recombinant adenoviral vector (Ad-shGFP) (figure 1C). And then, we investigated the effects of RKIP on the migratory activity and invasion rates of FLS by transwell migration and invasion assay.

Results: Here, we show that RKIP expression is inversely correlated with RA. The levels of RKIP were significantly decreased in fibroblast-like synoviocytes (FLS), synovial fluid and synovium of RA patients compare to OA patients. And also find that migration and invasion of RKIP in RA were significantly increased by the inhibition of RKIP compare to OA-FLS. Knockdown of RKIP in RA or OA-FLS resulted in a dramatic increase of MMP3 and IL6. We also found osteoclastogenesis of RAW cells were increased by the knockdown of RKIP.

Conclusions: Our data identify a role of RKIP in RA and suggest that further studies on the potential involvement of RKIP will be beneficial in better understanding the pathology of and providing a new target for treatment for RA.

REFERENCES:

- [1] Ospelt C. Synovial fibroblasts in 2017. *RMD Open* 2017;3(2):e000471.
- [2] Yesilkalan AE, Rosner MR. Raf kinase inhibitory protein (RKIP) as a metastasis suppressor: regulation of signaling networks in cancer. *Crit Rev Oncog*. 2014;19:447–454.
- [3] Noh HS, Hah YS, Ha JH, Kang MY, Zada S, Rha SY, Kang SS, Kim HJ, Park J, Byun J, Hahm JR, Shin JK, Jeong S, Lee Y, Kim DR. Regulation of the epithelial to mesenchymal transition and metastasis by Raf kinase inhibitory protein-dependent Notch1 activity. *Oncotarget* 2016;7(4):4632–46.

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Spondyloarthritis – etiology, pathogenesis and animal models

AB0141 CERTOLIZUMAB PEGOL LIKE REDUCES INFLAMMATION AND BONE DAMAGE IN TMTNF TRANSGENIC MICE

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Background: Transmembrane (tm)TNF (TgA86) mice is a transgenic line that spontaneously develops peripheral arthritis and spondylitis at 4 weeks of age, mimicking human spondyloarthritis (SpA).

Objectives: The aim of this work is to understand the effect of TNF blockade in this SpA-like phenotype mouse strain, focusing on histological inflammation and bone damage.

Methods: (tm)TNF (TgA86) mice were treated with a certolizumab pegol like product mice equivalent (Ab501), 100 mg/kg twice a week intraperitoneal, or with vehicle (phosphate buffer saline), for 12 weeks, in a therapeutic (10 weeks of age) and preventive (4 weeks of age) experimental settings. A semi-quantitative score for the severity of histologic inflammation and bone damage, in the paws (infiltration, lining cells, erosions and global), and in the spine (inflammation, intervertebral disc destruction, cartilage damage, bone erosion and ectopic chondrocytes/chondrocyte) was applied to haematoxylin and eosin stained slides.

Results: 14 (tm)TNF (TgA86) mice (6M/8F) at 10 weeks of age (therapeutic group), and 15 (tm)TNF (TgA86) mice (10M/5F) at 4 weeks of age (preventive group), were treated with Ab501; and 30 (tm)TNF (TgA86) mice with the respective vehicles, for a period of 12 weeks. A statistically significant reduction of the inflammatory infiltrate ($p < 0.001$), lining cells layers ($p < 0.05$), erosions ($p < 0.05$) and global inflammatory score ($p < 0.05$) of the paws, was observed in the Ab501

treated group, when compared to the vehicle group, both in the 10 and 4 weeks of age treatment groups. In the spine, statistically significant reductions of the inflammation ($p < 0.001$), intervertebral disc destruction ($p < 0.05$), cartilage damage ($p < 0.001$), bone erosion ($p < 0.05$), and ectopic chondrocytes/chondrocyte scores ($p < 0.001$) was also depicted in Ab501 treated group in comparison with vehicle.

Conclusions: The certolizumab pegol like product mice equivalent reduced histologic inflammatory infiltrate in paws and spine of (tm)TNF (TgA86) mice. Bone damage, as defined by erosions in the paws and spine and ectopic chondrocytes/chondrocyte formation in the spine, also significantly improved in (tm)TNF (TgA86) mice.

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AB0142 A REGULATORY CCR10+ CD8+ T CELL POPULATION DIFFERENTIATES PSORIATIC ARTHRITIS FROM PSORIASIS LIMITED TO CUTANEOUS INVOLVEMENT

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Background: Few studies have compared the immune cell phenotype or function from patients with psoriatic arthritis (PsA) to patients with psoriasis limited to cutaneous involvement (Pso). Head-to-head comparisons of the immune cells in PsA and Pso can provide essential insights into the link between epithelial/cutaneous disease and rheumatic disease.

Objectives: Compare the immune cell phenotype and function from patients with PsA compared to Pso.

Methods: PBMCs were collected from 23 healthy controls, 21 patients with psoriasis in whom psoriatic arthritis was excluded by a rheumatologist (Pso), 23 patients with psoriatic arthritis (PsA) and 16 patients with ankylosing spondylitis. The Pso and PsA cohorts were free from immunomodulatory therapy and matched for demographics and skin severity. Extracellular and intracellular immunophenotyping using a highly standardised flow-cytometric approach detected over 110 different myeloid and lymphoid cell populations. Lesional and non-lesional skin biopsies and synovial fluid samples were collected from a subset of patients.

Results: The circulating immune cell composition showed a remarkable overlap in Pso and PsA, and only one specific cell population was different: CCR10 + CD8 + T cells, being enriched in PsA PBMCs as compared to Pso PBMCs. Further phenotypic and functional evaluation characterised them as effector memory, CXCR3-CCR6-, co-expressing the skin homing markers CCR4 and CLA, but lacking the gut homing marker $\alpha\beta 7$. Upon re-stimulation, CCR10 + CD8 + T cells showed low IFN γ production, high IL-10 production and were enriched for FOXP3^{high}CD25+ phenotype. Their frequencies in PBMCs were unrelated to joint disease activity and were stable throughout joint disease fluctuations. Synovial fluid displayed low frequencies of CCR10 + CD8 + T cells whereas skin contained the most abundant CCR10 + CD8 + T cell frequencies. Lesional psoriasis skin displayed relatively fewer CCR10 + CD8 + T cells compared to non-lesional skin.

Abstract AB0142 – Figure 1