0.005) or HC (4.81%) ($p: 0.04$); between the 3 groups, pSTAT3 was differently expressed in CD4-lymphocytes and CD14-monocytes (figure 1). We also demonstrated a correlation between pSTAT3 level in CD14 +cells and ESR ($p: 0.026$; $r: 0.4625$). We found no correlation between pSTAT3 expression and Body Mass Index or disease duration.

Conclusions: In this study, STAT3 seems to be activated more in RA than SA or HC. Monocytes (as macrophage) and T-cells have important role in RA. Isomaki et al. demonstrated a correlation between IL-6 level and STAT3 activation so it might be suggested a possible role of pSTAT3 as a biomarker of disease activity. However, further studies are needed to explore the important role of JAK-STAT pathway in RA.

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AB0135 CIRCADIAN RHYTHMS OF IMMUNE CELLS IN HEALTHY INDIVIDUALS AND PATIENTS WITH RHEUMATOID ARTHRITIS

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Background: Clinical symptoms of rheumatoid arthritis (RA), such as joint stiffness, swelling and pain, manifest in circadian pattern with the highest intensity occurs at early dawn. This is known to correlate with the circadian expression of IL-6, which peaks before the onset of the symptom. Despite this finding, the circadian behaviour of immune system in cellular and molecular level in RA patients has not yet been extensively investigated.

Objectives: Our previous study suggested that immunological circadian rhythms in patients with RA were altered when compared to the healthy individuals. Currently, we are performing 24 hours study involving RA patients and healthy individuals to further monitor the dynamic occurrence of diverse immune cells in the periphery.

Methods: Up to this time five eligible RA patients aged 45–75 years and twelve eligible healthy controls were recruited to join the study. On the study day, the blood was drawn in two hours interval throughout 24 hours. The participants were provided with regular meal, allowed to eat snacks ad libitum and carry passive activities. The absolute number of circulating immune cells was determined using TruCount. RNA were isolated from CD14⁺ monocytes and analysed by real-time PCR.

Results: The major populations of immune cells in the periphery of healthy controls, including CD4 T cells, CD8 T cells, regulatory T cells, B cells and monocytes, displayed circadian rhythm that peaks during the rest phase. The rhythms are in general shifted a few hours later in the RA patients. Noteworthy, CD14 monocyte, which is one of the major sources of IL-6 in RA, showed a more pronounced rhythm with higher amplitude in RA patients compared to healthy individuals. Furthermore, the following clock genes are rhythmically expressed in CD14 monocytes of both groups: *Rorα*, *Per1*, *Per2*, *Per3* and *DBP*. The peak of *Rorα*, *Per1*, *Per3* *DBP* and *CRY1* is shifted a few hours later in RA patients. Interestingly, circadian variation is not observed in the expression of *RevErbα* in healthy individuals, while in the RA patients a rhythm is established.

Conclusions: In general, circadian rhythm of immune system in cellular and molecular level in RA patients appears to undergo phase shift and peaks a few hours later in comparison to healthy individuals. New established rhythms were also observed in cellular and molecular level. Another round of study involving seven RA patients is planned this spring to complete the project. Considering our data, we will continue to investigate circadian rhythms in expanded immune cell population using mass cytometry, immunoassay and microarray. Identification of immunological circadian rhythms in patients with RA and healthy individuals will help us to expand our knowledge in autoimmunity and provide an outlook on potential future implications.

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AB0136 THE EFFECT OF IL-6 RECEPTOR ANTIBODY FOR THE TREATMENT OF MCH-LPR/LPR-RA1 MICE THAT SPONTANEOUSLY DEVELOPED ANKYLOSING ARTHRITIS

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Background: Mch-lpr/lpr-RA1 (RA1) mice are new strain mice which spontaneously developed arthritis in ankle and finally leads to ankylosis. ¹ There is no report that drug treatment has been applied to these mice.

Objectives: To examine the effect of mouse IL-6 receptor antibody MR16-1 for the treatment of RA-1 mice.

Methods: Male RA1 mice were randomly divided into treated and control groups. MR 16-1 was applied from 10 weeks of age for the treatment group. Saline was applied for the control group. The drug was administered every two weeks with the initial dose of 2 mg, then 0.5 mg. The effects were evaluated by histological synovitis score, *in vivo* imaging using ICG-encapsulated liposomes and the expression of serum SAA and IL-6.

Results: The tissue evaluation was carried out at 14 weeks, 17 weeks and 20 weeks of age. The histological score of treated groups were significantly improved compared with control group at every age. The interclass correlation coefficient was 0.771. *In vivo* imaging using ICG-encapsulated liposomes showed that significant signal decrease in treated groups at 14 weeks, but no significant difference was observed after 16 weeks. Blood SAA was significantly improved at 17 weeks of age.

Abstract AB0136 – Figure 1

Conclusions: IL-6 receptor antibody is effective for the treatment of ankylosing arthritis of RA1 mice. IL-6 might be a new potential target of treatment for ankylosing arthritis.

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AB0137 ARTHRITIS-ASSOCIATED EGGERTHELLA LENTA MODULATE DISEASE VIA METABOLIC AND MICROBIAL ALTERATIONS

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Background: Role of environmental factors in predisposition to develop rheumatoid arthritis (RA) has 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 shown an association of rare lineage commensals, *Eggerthella lenta*, with RA.

Objectives: In this study, we aimed to determine how human gut commensal *E. lenta* modulates gut epithelial integrity and inflammation via metabolites in humanised mice expressing RA-susceptible HLA-DQ8.

Methods: DQ8 mice following immunisation with type II collagen develop arthritis and antigen-specific cellular and humoral response. DQ8 mice orally gavaged

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.

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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.

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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.

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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