Results: Blockade of SHH by GDC-0449 significantly alleviated the symptoms and decreased the synovial hyperplasia, inflammatory infiltration, cartilage and bone damage in ankles of CIA. The bone erosions in the area of the metatarsal and ankle joints and production of TNFα, IL-6 were decreased by SHH inhibition. In addition, the administration of GDC-0449 significantly decreased the number of TRAP positive cells and the expression of NFATC1. On the contrary, SHH overexpression led to increased severity of arthritis and pathological changes. We also observed the accelerated bone injury accompanied with increased number and activity of osteoclasts and increased production of serum IL-6 in mice with upregulation of SHH expression. Of note, the administration of p38 MAPK inhibitor reversed the effects of SHH overexpression, with a reduction of joint swelling and histological scores. Inhibition of p38 MAPK prevented the bone erosion and decreased the number of TRAP positive cells and the expression of NFATC1, which were promoted by SHH overexpression.

Conclusion: The study indicates that SHH promotes the synovial hyperplasia and bone erosion of CIA in a p38 MAPK-dependent manner. SHH-p38 MAPK signaling could be a potential target for the treatment of RA.

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THU0077 EXPRESSION PROFILE ANALYSIS OF LONG NONCODING RNAS INDUCED BY IL-15S IN RHEUMATOID ARTHRITIS FIBROBLAST-LIKE SYNOVIOCYTES

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Background: Long noncoding RNAs (lncRNAs) have recently emerged as important epigenetic regulators of gene expression and have been reported in various diseases including cancer, cardiovascular disease, and diabetes mellitus. However, the role of IncRNAs in the pathogenesis of rheumatoid arthritis (RA) remains unknown.

Objectives: Thus, we studied IncRNAs influenced by IL-1, which is one of the key mediators in the pathogenesis of RA, and also investigated whether regulation of NF-κB activation, which is known to be induced by IL-1, could lead to the changes of expression of those IncRNAs.

Methods: Fibroblast-like synoviocytes (FLS) were obtained from the knee joints of patients with RA. The next-generation sequencing (NGS) data were analyzed to identify differentially expressed IncRNAs between unstimulated RA FLS and IL-1-stimulated RA FLS. The expression levels of the top 5 candidates in NGS data were validated by RT-qPCR using extended number of unstimulated RA FLS and IL-1-stimulated RA FLS. IMD-0560, an inhibitor of IκB kinase (IKK) was used for the regulation of NF-κB activation. Activation and inhibition of NF-κB were confirmed by Western blotting. Changed expressions of the IncRNAs were identified by RT-qPCR.

Results: NGS analysis revealed up-regulated 30 IncRNAs and down-regulated 15 IncRNAs in IL-1-treated RA FLS compared with unstimulated RA FLS. Top 5 IncRNAs were selected among 30 IncRNAs up-regulated by IL-1 in RA FLS based on fold-change with P-value cutoff. The up-regulated IncRNAs including NR_046035, NR_027783, NR_033422, NR_003133, and NR_049759 were validated by RT-qPCR. IMD-0560 inhibited phosphorylation of IκB induced by IL-1 in RA FLS. Overexpression of IncRNAs induced by IL-1 was also inhibited by IMD-0560 in RA FLS.

Conclusion: Our study revealed that IL-1 increased the expression of NR_046035, NR_027783, NR_033422, NR_003133, and NR_049759 in RA FLS. In addition, the expression of these IncRNAs was regulated by inhibition of NF-κB activation. Thus, our data suggest that the IncRNAs might be involved in the pathogenesis of RA through NF-κB signaling pathway.

References:

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THU0078 THE MICROBIOME OF NEW-ONSET RHEUMATOID ARTHRITIS (NORA) PATIENTS DRIVES TLR4-DEPENDENT TH17 RESPONSES

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Background: Intestinal microbiota plays a prominent role in shaping the T cell immune response. Increasing evidence suggests that the gut microbiota is perturbed in patients with RA, and a variety of animal models demonstrated involvement of (mouse) microbiota in arthritis development. This underlines the necessity of understanding whether and how indigenous human NORA-associated microbiota may trigger RA.

Results: Mice were treated with anti-TLR4 F(ab)2 Fab fragment to block the TLR4-mediated innate immune response. The mice were treated with a low dose of TLR4 ligand LPS, which is known to cause arthritis in normal mice. In the group of mice treated with LPS, the arthritis was significantly suppressed in mice treated with anti-TLR4 F(ab)2 Fab fragment. In addition, the level of TNFα in the serum of the mice treated with anti-TLR4 F(ab)2 Fab fragment was significantly lower than that in the group of mice treated with LPS alone. These results suggest that the TLR4-mediated innate immune response plays a key role in the development of arthritis in NORA mice.

Conclusion: The TLR4-mediated innate immune response plays a key role in the development of arthritis in NORA mice. The results of this study suggest that the gut microbiota plays a significant role in the development of arthritis in NORA mice. Further studies are needed to clarify the exact mechanism of action of the gut microbiota in the development of arthritis in NORA mice.
**Objectives:** To comprehensively investigate the intestinal mucosa cytokine production and DC, T and B cell responses to human gut microbiota associated with new onset RA.

**Methods:** We utilized in vitro cultures of mucosal-like DCs (differentiated from bone marrow cells) and primary splenic DCs, as well as ex vivo cultures of healthy human intestinal biopsies, cultured in the presence of heat-killed fecal microbiota from either NORA or control donors. Furthermore, we performed studies in humanized mice carrying intestinal NORA microbiota, to study the effect on immune response during homeostasis and upon joint inflammation during collagen-induced arthritis (CIA).

**Results:** In 24h DC cultures, NORA fecal microbiota more potently induced the expression of co-stimulatory molecules CD40 and CD80, and this enhanced DC maturation was partially mediated through TLR4 as demonstrated using the TLR4 antagonist TAK242. Interestingly, HC and NORA fecal microbiota differentially induced IL-12 and IL-6 production, with significantly enhanced IL-6 and reduced IL-12 secretion by the NORA microbiome. Furthermore, in ex vivo cultures of human ileum biopsies, the production of IL-1 and IL-33, as well as IL-23/Tn17 cytokines IL-23, IL-22, and GM-CSF, were significantly increased by NORA-derived microbiome. Interestingly, in the small intestine lamina propria (SILP) of NORA-colonized mice, we observed enhanced Th17 polarization, increased innate GM-CSF expression and higher B cell CD40 and IgA levels during homeostasis. To study whether colonization with HC and NORA microbiota alters arthritis development, humanized mice and controls (mock, autologous, HC and NORA microbiota) were used in a CIA experiment. Macrophscopic scoring of the arthritis severity at weekly intervals demonstrated that arthritis severity was significantly enhanced in NORA-colonized mice compared to HC-colonization and mock controls.

**Conclusion:** Our data reveal that NORA microbiota, in addition to the previously described Th17 differentiation, induce higher levels of GM-CSF and B cell IgA in LP and have increased potential to aggravate arthritis through the activation of TLR4.

**References:**

**Disclosure of Interests:** Marije Koenders: None declared, Peter van der Kraan: None declared, Shahla Abdollahi-Marjorie Koenders: None declared, Heather Evans-Marije Koenders: None declared, Joyce Aarts: None declared, Parvathy Girija: None declared, Rebecca Rogier: None declared, Sergei Korolav: None declared, Julia Manasson: None declared, Peter van der Kraan: None declared, Shalah Abdollahi Roosdaz: None declared, Jose Scher Consultant of: Novartis, Janssen, UCB, Sanofi, DOI: 10.1136/annrheumdis-2020-eular.5269

**THU0081**

**PRECLINICAL CHARACTERIZATION OF TLL018, A NOVEL, HIGHLY POTENT AND SELECTIVE JAK1/TYK2 INHIBITOR FOR TREATING AUTOIMMUNE DISEASES**

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**Background:** Janus kinases (JAKs) are important regulators of intracellular responses triggered by many key proinflammatory cytokines and are clinically validated therapeutic targets for treating various autoimmune diseases. However, current approved JAK inhibitors failed to achieve maximal clinical benefit in part due to their unfavorable selectivity for individual JAKs such as JAK2 and/or JAK3, leading to dose-limiting toxicities or severe toxicities (e.g., thrombosis, anemia, immune suppression). Selective inhibition of JAK1 and/or TYK2 may minimize or avoid some of the toxicities and potentially offer a better therapeutic window for treating autoimmune diseases. No highly selective JAK1/TYK2 inhibitor has been reported to date.

**Objectives:** Discovery of a highly selective JAK1/TYK2 inhibitor that maximally avoids JAK2 and JAK3 inhibition. We described preclinical characterization of a novel, highly potent and selective JAK1/TYK2 inhibitor TLL018 and its potential utility in treating autoimmune diseases such as rheumatoid arthritis (RA).

**Methods:** Using predicting SAR, TLL018 was designed to achieve exquisite selectivity for both JAK1 and TYK2 while sparing JAK2, JAK3 and other human kinases, JAK2 or JAK3 is greater than 1 µM. Profiling against a panel of over 350 human kinase showed that TLL018 is exclusively selective for JAK1 and TYK2, with 90-fold selectivity against all other kinases tested. TLL018 exhibited potent cellular activity for JAK1-mediated IL-6 signaling (IC50 = 0.6 µM) with greater than 100-fold selectivity against JAK2-mediated cytokine (e.g., TPO) signaling in human whole-blood-based assays.

**Conclusion:** TLL018 is a highly potent and selective JAK1/TYK2 inhibitor that demonstrated excellent efficacy and tolerability in relevant mouse and rat arthritis models. The collective data of its preclinical pharmacology, PK and toxicology showed a favorable pharmaceutical profile, further supporting its development for treating autoimmune diseases including RA. Clinical evaluation of TLL018 is ongoing.


**THU0081**

**MIR-17-5P REDUCES INFLAMMATION AND BONE EROSIONS IN COLLAGEN INDUCED ARTHRITIS MICE AND DIRECTLY TARGETS THE JAK-STAT PATHWAY IN RHEUMATOID ARTHRITIS FIBROBLAST-LIKE SYNOVIOCYTES.**

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**Background:** micro-RNAs (miR) are strong regulators of gene expression. Their involvement in RA key cytokines pathway regulation entities them as important players in RA pathophysiology. The miR-17-92 cluster has been widely studied in cancer as they regulate cell apoptosis.

**Objectives:** The aims of this study were to screen miR-17-92 cluster’s expression in different RA phenotypes (erosive and non erosive), further elucidate the mechanisms and direct targets involved in miR-17-5p anti-inflammatory role and to investigate miR-17-5p therapeutic effect in arthritis.

**Methods:** A miR array was performed in synovial tissue from naïve erosive and non-erosive RA patients. Intra-articular delivery of miR-17-1-5p oligonucleotide was performed in collagen induced arthritis model mice. Clinical, histological and structural effects were studied over the course of arthritis. In depth studies of miR-17 mechanisms of action were performed in primary RA-FLS isolated from RA synovial tissue.

**Results:** Among others, miR-17-5p expression was reduced in erosive RA, miR-17 transfection in arthritic paws significantly reduced clinical inflammation. Moreover, synovial B cells, T cells, macrophages and polyfunctional neutrophils infiltrates were significantly reduced. Structural damage was also decreased as shown by a reduction in the number of osteoclasts and erosion score by CT analysis. Pro-inflammatory cytokines of the IL-6 family, STAT3 target genes and IL-15 expression were also significantly reduced, but not TNF-alpha. miR17 directly targeted the 3’-untranslated region of STAT3 and JAK1, STAT3 and JAK1 miRNA and protein expression were reduced in RA-FLS following miR-17 transduction. STAT3 and JAK1 miRNA and activation of STAT3 as assessed by immunohistochemistry were also reduced in injected paws.

**Conclusion:** We demonstrate an anti-inflammatory and anti-erosive role of miR-17 in vivo. This effect involves the suppression of the IL-6 family autocrine amplifying loop through the direct targeting of JAK1 and STAT3 as shown in RA-FLS.