were significantly higher in HFD (0.2 ± 0.16 , $n=7$) compared to ND (5.2 ± 0.98 , $n=8$). No difference was found in CIA-severity between HFD and ND. However, CIA induction increased the number of CLS in HFD (2.77 ± 1.07 , $n=6$) and interestingly in ND animals (8.14 ± 0.23 , $n=5$) compared to healthy ND (0.45 ± 0.03 , $n=4$) and healthy HFD mice (2.57 ± 0.53 , $n=4$) without CIA. As expected, HFD led to a significant increase in systemic leptin in healthy animals in both models. Interestingly, CIA and DMM induction decreased systemic leptin levels significantly in ND and HFD, which was more prominent in CIA. The systemic effect was not reflected by local leptin distribution in the joints (CIA) which were not altered by diet. 5. and 7 weeks after CIA induction HFD led to a reduction in local adiponectin and visfatin expression, which were not reflected in systemic levels.

Conclusion: The data show that HFD deteriorates OA, which is similar to observations in humans. In contrast, HFD induction showed no significant difference in CIA severity compared to ND. Furthermore, CIA reduced local adipokine expression under HFD at later time points but not under ND. According to high numbers of CLS in ND/CIA animals and the strong reduction of leptin in CIA with HFD, CIA onset and severity seems to be obesity independent and more dependent on inflammation while OA appears to be directly influenced by obesity. However, HFD-induced obesity seems to alter local and systemic adipokine expression also in CIA. Interestingly, local adipokine distribution in affected joints was independent from systemic adipokine levels.

Disclosure of Interests: Hani Manfred Sauermlach: None declared, Marie-Lisa Hülser: None declared, Carina Schreyöck: None declared, Yubin Luo: None declared, Aline Bozec: None declared, Georg Schett Speakers bureau: AbbVie, BMS, Celgene, Janssen, Eli Lilly, Novartis, Roche and UCB, Ulf Müller-Ladner Speakers bureau: Biogen, Elena Neumann: None declared

DOI: 10.1136/annrheumdis-2020-eular.2898

THU0069 MIMICKING ARTHRITIS IN VITRO TO TEST DIFFERENT TREATMENT APPROACHES

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Background: Our ultimate goal is to study potential drug candidates in an experimental setting of arthritis. Therefore, we aim to develop a valid human *in vitro* 3D joint model mimicking features of joint inflammation by applying inflammatory conditions namely immune cells and pro-inflammatory cytokines. Our *in vitro* 3D joint model consists of different components including an osteogenic and chondrogenic part, the joint space filled with synovial fluid, and the synovial membrane. Developed as an alternative experimental setup to animal experiments, our 3D joint model will enable us to study efficiently the effects of potential drug candidates in a human-based *in vitro* model.

Objectives: Here, we aimed to demonstrate the suitability of our human-based *in vitro* 3D osteochondral model by analyzing the influence of the main cytokines involved in the pathogenesis of RA as well as the impact of a specific therapeutic intervention.

Methods: Based on human bone marrow-derived mesenchymal stromal cells (hMSCs), we developed 3D bone and cartilage tissue components that were characterized in detail (e.g. cell vitality, morphology, structural integrity) using histological, biochemical and molecular biological methods as well as µCT and scanning electron microscope (SEM). In brief, to establish the osteogenic component, we populated β-tricalcium phosphate (TCP) – mimicking the mineral bony part – with hMSCs, while the scaffold-free cartilage component was generated by cellular self-assembly and intermittent mechanical stimulation (fzmb GmbH). Subsequently, we co-cultivated both tissue components for three weeks to generate an interconnected 3D osteochondral model. To test the suitability, we applied a cocktail of TNFα, IL-6 and MIF using concentrations reported from RA synovial fluid alone or in combination with specific therapeutic drugs and analyzed their impact by qPCR.

Results: We verified the osteogenic phenotype of our 3D bone tissue component by demonstrating an increase in mineralized bone volume and the induction of bone-related gene expression (*RUNX2*, *SPP1* and *COL1A1*) as compared to the corresponding control. Secondly, we verified the chondrogenic phenotype of our cartilage tissue component by HE and Alcian Blue staining as well as by the reduced expression of *COL1A1* and an abundant expression of *COL2A1*. Interestingly, co-cultivation of both components for up to 3 weeks demonstrated colonization, connectivity and initial calcification implying a transitional bridging area. Cytokine stimulation with a cocktail of TNF, IL-6 and MIF leads to an upregulation of the metabolic marker *LDHA* and the angiogenic marker *VEGF* in both bone and cartilage. The inflammation

markers *IL8* and *TNF* are also upregulated in both components, while *IL6* is downregulated in bone compared to the unstimulated control. In addition, a cytokine-induced upregulation of matrix-metalloproteases was observed especially in the cartilage component. All these cytokine-related effects could be antagonized with a cocktail of therapeutics (milatuzumab, adalimumab and tocilizumab).

Conclusion: The results of our study showed cytokine related effects of both tissue components, which can be therapeutically antagonized. By combining the components in a 96 well format, we aim to provide a mid-throughput system for preclinical drug testing.

Acknowledgments: This project is funded by the Federal Ministry of Education and Research (BMBF)

Disclosure of Interests: Alexandra Damerau: None declared, Moritz Pfeifferberger: None declared, Annemarie Lang: None declared, Timo Gaber: None declared, Frank Buttgerit Grant/research support from: Amgen, BMS, Celgene, Generic Assays, GSK, Hexal, Horizon, Lilly, medac, Mundipharma, Novartis, Pfizer, Roche, and Sanofi.

DOI: 10.1136/annrheumdis-2020-eular.3051

THU0070 DEFINING SYNOVIAL SIGNATURES IN THE RAT CIA MODEL: WHAT CAN WE LEARN ABOUT RA PROGRESSION?

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Background: Patients showing inadequate or no response to current therapies represent a key unmet need in rheumatoid arthritis (RA). To address this, novel or combination therapies are of high clinical interest. Identification of novel therapeutic targets requires a greater understanding of the pathogenic molecular drivers in the RA synovium. However, our current knowledge of human molecular patterns that emerge as a result of disease progression is complicated by patient-to-patient heterogeneity and access to synovial tissue.

Objectives: Here we use the current knowledge of human synovial heterogeneity to conduct a longitudinal study of global molecular responses in the rat collagen-induced arthritis (CIA) model to better understand synovial biology, improve the preclinical modeling of human disease, and discover novel targets for RA.

Methods: A rat CIA model was performed as previously described.¹ RNA-Seq was performed on 56 knee synovial tissues collected at multiple time points throughout the course of disease. Differential gene expression was determined at each individual time point and longitudinally with disease progression. Published human synovial datasets were used to categorize these genes into myeloid, lymphoid, fibroid, and low inflammatory signatures.² Differentially expressed genes (DEGs) at each time point were compared to human synovial datasets of RA patients before and after treatment. In addition, we compared disease-driven genes in CIA to genes in RA patients that are unchanged following therapy to identify possible combination therapies.

Results: Disease pathology in the rat CIA natural history study progressed as expected: significant decreases were seen in body weight, as well as increases in ankle diameter, paw weight, and histopathology scores of joints in collagen-injected vs noninjected rats. There were 1900 DEGs identified between diseased and naïve rats over the course of disease, representing disease-induced gene signatures (Fig. 1). Comparing these DEGs to reported human RA synovial signatures, both the lymphoid and myeloid signatures were found to be highly upregulated. Interestingly, there were no significant DEGs representing the human fibroid and low inflammatory synovial signatures identified in the CIA rat model. This suggests that the rat CIA model most closely models RA patients with an immune synovial phenotype. In addition, we examined the overlap between disease-driven genes in CIA and genes in RA patients that are unchanged following therapy to identify signaling pathways that may be of utility in combination therapy. Of genes that were upregulated in CIA, 94% of genes that mapped to extracellular matrix-receptor pathways remained unchanged in the synovial tissue of RA patients following tocilizumab treatment.

Conclusion: Previous studies have shown that nearly 30% of treatment-naïve early RA patients exhibit a strong fibroid phenotype that correlates with less severe disease and a relatively poor response to disease-modifying anti-rheumatic drugs.³ These data indicate that the synovial biology associated with such patients (fibroid or pauci-immune) is not well captured in CIA, the most common preclinical RA model. To assess potential new therapies targeting these patients, it will be necessary to develop alternative animal models with more intact fibroid signatures. In addition to these findings, we also characterized the global molecular changes that occur with disease progression in the CIA rat and made a comparison to RA patients on treatment, providing an overall understanding of