Background: Rheumatoid arthritis (RA) is a systemic autoimmune disease which mainly affects joints. [1] Macrophages often infiltrate in the inflammatory joints. Activated macrophages release TNF-α, IL-1β to accelerate tissue damage, one of the most important targets for RA intervention. The traditional drugs currently used commonly have some disadvantages cannot be bypassed[2], while DNA nanostructure is a new type of drugs have precise design, and likewise takes biological effect together[3]. We synthesized a DNA tetrahedron conjugated with HA which targeted to macrophage.

Objectives: To verify whether MTX-loading DNA tetrahedron can regulate the apoptosis and polarization of macrophage and finally improve the condition of CIA model mice by while decrease the side effect of MTX.

Methods: DNA TET was synthesized by mixing signal strand DNA in TM buffer and heated to 95 °C for 10 min, then cooling to 4 °C. Electrophoresis was applied to confirm the formation of TET. The absorbance of MTX solution was detected by microplate reader to analyze the loading efficiency of MTX into TET. Flow cytometry was used to detect the apoptosis and polarization. CIA model was established based on DBA/1 mice. Mice were randomly divided into five groups: normal group injected with NS; after established CIA model, CIA group injected with NS; MTX group injected with MTX and conjugated with HA which targeted to macrophage.

Results: We synthesized DNA tetrahedron (A) and used 8% PAGE electrophoresis to confirm the successfully synthesis(B). Then We found that when TET concentration fixed, the loading MTX concentration gradually increased and saturated at 190μM(C). While completely loading needed at least 4 hours(D). Fluorescence showed that single DNA strand cannot be taken by RAW, while TET can be easily taken by RAW(E). CCK8 showed that empty TET had no obvious effect on cells, while MTX and MTX-TET with equivalent concentration can obviously suppress the vitality(F). Similarly, the apoptosis trial showed that TET can slightly decrease the apoptosis of RAW, MTX and MTX-TET can significantly promote the apoptosis(G). Flow cytometry showed that the MTX-TET can decrease the expression of M1 marker CD80 (H).

At last, we treat mice with NS, TET, MTX and MTX-TET once a week after CIA model established, and found that TET have no significantly effect on mice, while MTX and MTX-TET can alleviate the inflammation symptom of paws(I).

Conclusion: Conclusions: We-synthesized MTX-loading DNA tetrahedron conjugated with HA, and found that the MTX-TET NP have the excellent ability of promote RAW apoptosis and relieve proinflammatory M1 polarization, while also can alleviate the symptom of CIA mice.

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through upregulating cellular activities involving iNOS-presenting macrophages and B cell receptor signaling, which may be associated with the pathogenesis of P.g.-triggered RA.

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POS0403

RISK FACTORS FOR PROGRESSION OF RHEUMATOID ARTHRITIS-ASSOCIATED INTERSTITIAL LUNG DISEASE: REASSURING IMPACT OF METHOTREXATE

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Background: Factors associated with rheumatoid arthritis-associated interstitial lung disease (RA-ILD) progression and prognosis are not well identified, especially the impact of methotrexate.

Objectives: Identify risk factors of ILD progression in RA-ILD patients in a longitudinal study.

Methods: RA patients with ILD confirmed in 2 high resolution computed tomography (HRCT) chest scans spaced at least 6 months apart (T0; date of the first HRCT chest scan describing ILD; Tx: date of the last HRCT chest scan available) were consecutively included in this retrospective multi-centric study from 2010 to 2020. HRCT chest scans were analyzed for each patient at T0 and Tx by 2 independent radiologists to determine ILD pattern (definite UIP, probable UIP, indeterminate UIP, non-UIP) and progression during the follow-up including variation of the fibrosis score (aggravated or non-aggravated). Characteristics of patients (demographic-clinical-biological findings, respiratory function tests, and treatments exposure) at ILD diagnosis and during the follow-up (T0-Tx) were analyzed as potential determinants of ILD progression through multivariable logistic regression analysis. Overall survival was analyzed using Kaplan-Meier method.

Results: 74 RA-ILD patients were included. During a mean duration between T0-Tx of 2.8 years ± 2.4, 28 patients (38%) had ILD progression. Thirty-three patients (45%) were treated by methotrexate at ILD diagnosis (T0) and 29 of them (39%) continued methotrexate during T0-Tx. Logistic regression in multivariate analysis revealed that a treatment by methotrexate at ILD diagnosis was protective against ILD progression (OR=0.14 [0.04-0.52]; p=0.0031). Non-UIP pattern at ILD diagnosis was also protective against ILD progression (OR=0.09 [0.02-0.46]; p=0.0005). The follow-up for survival analysis was 5.1 years ± 2.9. Thirty-three patients (35%) died, and the 3-year survival rate was 80%. Survival was better for non-aggravated ILD patients (HR= 3.5 [1.46-8.4]; p<0.004) and for patients treated by methotrexate during T0-Tx (HR=0.36 [0.15-0.84]; p=0.018) and worse for definite UIP patterns (HR=2.570 [1.078-6.128]; p=0.0332).

Conclusion: In RA-ILD patients, non-UIP pattern and methotrexate treatment are associated with better ILD evolution and prognosis.

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Spondyloarthritis - aetiology, pathogenesis and animal models

POS0404

VOLUNTARY WHEEL RUNNING MODEL IN MICE TO MECHANICALLY STIMULATE THE ENTHESIS OF THE ACHILLES TENDON

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Background: Excessive bone formation in the entheses is one of the features of peripheral spondyloarthritis. Biomechanical stress is proposed to occupy a central place in spondyloarthritis pathophysiology, but the precise molecular and cellular mechanisms underlying the pathological response of the entheses are still largely unknown [1]. Besides, physical therapy and exercise are recommended as non-pharmacologic therapies for patients. We focused on the effect of exercising on enthesis ossification.

Objectives: We aimed to develop and characterize an in vivo model in mice to study the impact of mechanical stimulation on the enthesis of the Achilles tendon.

Methods: DBA/1 mice were subjected to voluntary running exercise by the use of activity wheels for two weeks, and compared to mice housed in standard conditions (n=17 per group). The running performances were recorded.

Figure 1. Expression level of the mechanosensitive gene Sclerostin (Sost). It dropped in response to exercise in entheseal tissues, but not in long bones, revealing a tissue-specific response to mechanical stimulation.

mRNAs were extracted from the long bones (flushed tibia and femur) and the ankles’ entheses for real-time PCR analysis. μCT was performed on the femurs. Alkaline phosphatase activity was detected by histology on the anchorage of the Achilles tendon to the calcaneum, and by enzymatic assay in serum samples. Luminex analysis was also conducted on serum samples for II-6 and II-8/Kc detection.

Results: Free access to the activity wheel resulted in a running exercise of 5.5±0.8 km/day (approximately 80 km in total) at 14.5±0.5 m/min. No effect was detected on the femur architecture by μCT. Sclerostin (Sost) gene expression was monitored as a mechanosensitive marker. Its expression was expectedly reduced by half in entheseal tissues, but no modulation was observed in long bones (Figure 1). Similarly, exercise-induced regulation of Osterix and Runx2 expressions was observed only in enthesis samples. This tissue-specific pattern was also verified for key genes of the sphingosine-1 phosphate metabolic pathway, which we recently implicated in spondyloarthritis pathophysiology [2]. The in situ staining of alkaline phosphatase activity suggested the presence of more positive cells in the anchorage of Achilles tendon of running mice, compared to control ones. However, alkaline phosphatase activity in serum samples and its gene expression in rough tissue extracts were unchanged. No inflammatory response was detected as II-8/Kc serum levels were similar in the control and the exercising group (59±14 vs 57±14 pg/mL).

In addition, II-6 was not detected in the serum and its expression was very faint and constant in the tissue extracts.

Conclusion: This work is still in progress for a more complete characterization of the model. We believe that this experimental design will be useful to study the role of mechanical stimulation specifically in the enthesis and that it can help to better understand the spondyloarthritiphathophysiology.

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POS0405

GENETIC VARIANTS WITHIN PSORIATIC ARTHRITIS (PsA)-WEIGHTED GENES FBXL19 AND HLA-B*39 MAY SERVE AS A POTENTIAL LINK BETWEEN PsA AND OBESITY

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Background: PsA patients have been observed to have a higher body mass index (BMI) compared to individuals with a similar disease (e.g., rheumatoid arthritis) or healthy controls1. Approximately 45% of PsA patients are considered obese with BMI exceeding 30 kg/m2, and these patients have more severe articular disease and lower response to therapy2. A recent mendelian randomization study noted that higher BMI leads to higher risk of psoriasis when using genetic variants as instrumental variables for BMI2.

Objectives: To determine if known PsA-weighted genetic variants are overrepresented in an obese population.