lation between sputum NET complexes and multiple sputum ACPAs that was independent of smoking exposure. These data suggest that in the lung, NET formation may be associated with the production of multiple ACPA reactivities locally. Additional studies are needed to determine if NET-associated cit-proteins are an initial trigger or a self-perpetuating stimulus of sputum ACPA generation as well as contributions of other local mechanisms of citrullination.

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FRI0054 A NOVEL PHARMACOLOGICAL ACTION OF MTX THROUGH CIRCADIAN CLOCK GENES IN RA FIBROBLAST-LIKE SYNOVIAL CELLS

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Background: Methotrexate inhibits the proliferation of RA fibroblast-like synovial cells (RA-FLS) by folate anti-metabolism. We previously reported that disruptions of circadian clock genes were involved in the pathogenesis of inflammatory arthritis (1,2).
Objectives: To explore pharmacological effects of MTX on circadian clock genes of RA-FLS.
Methods: Under treatments of MTX on RA-FLS, cell viabilities were determined by WST-8 assay, expressions of circadian clock genes (*Clock, Bmal1, Cry1* and *Per2*), circadian transcriptional factor PAR bZip (*Dbp, Tef* and *Hlf*), and pro-apoptotic Bcl-2 interacting killer (*BIK*) were examined by real time PCR, and expressions of PER2 and CYTOCHROME C were examined by western blotting. The expressions of PER2, BIK, CYTOCHROME C and morphological changes of the nucleus were observed by fluorescent immunostaining. RA-FLS were transfected with *Per2* and *BIK* siRNAs and successively treated with MTX to determine cell viabilities by WST-8 assay.
Results: MTX (1,10 nM) treatment significantly decreased the cell viabilities. MTX (10nM) have increased mRNA expression of *Per2, Dbp, Tef, Hlf*, and *BIK*, and protein expressions of PER2 and CYTOCHROME C, as well. In fluorescent observations, PER2, BIK, and CYTOCHROME C were increased in apoptotic cells. Cytotoxicity of MTX was attenuated by knockdowns of *Per2* or *BIK* in RA-FLS.
Conclusions: The transcriptional factor PAR bZip binds to the D-box elements of *Per2* and *BIK* promoters (3,4). Here, we propose a novel action of MTX that up-regulates the expressions of *Per2* and *BIK* via PAR bZip to induce apoptosis in RA-FLS.

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FRI0055 HISTONE DEACETYLASE 1 (HDAC1): A NOVEL THERAPEUTIC TARGET IN RHEUMATOID ARTHRITIS

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Background: Autoreactive T cells have been shown to play a major role in Rheumatoid Arthritis, which drive the inflammatory process leading to an irreversible destruction of the joints. Gene transcription and regulation of proinflammatory cytokine production in T cells is regulated by epigenetic mechanisms. Histone deacetylases (HDACs) modify the epigenetic landscape by removing acetyl groups of lysine residues of histones, resulting in chromatin condensation and repression of transcription. The application of pan-HDAC inhibitors has been shown to be a potential therapeutic strategy. However, major side effects limited the clinical use and underline the need of more specific HDAC inhibitors.
Objectives: Our aim was to to investigate whether HDAC1 is linked with the development of autoimmune diseases. Therefore we were using the collagen-induced arthritis model (CIA) and the experimental autoimmune encephalomyelitis model (EAE).
Methods: Mice with a T cell specific deletion of HDAC1 (HDAC1cKO) were generated by using the CD4Cre/LoxP system. The clinical and the histological phenotype were assessed in the CIA and the EAE. Anti-collagen antibody levels were determined by ELISA. Qualitative and quantitative analysis of T cell subsets of the spleen and draining LN were assessed using flow cytometry. Additionally comparative RNA-sequencing of $CD4^+$ T cells from wild type (WT) and HDAC1cKO mice was performed.
Results: To address whether HDAC1 is involved in the pathogenesis of autoimmune diseases we induced the CIA and the EAE in WT and HDAC1cKO mice. Unexpectedly, HDAC1cKO mice did not develop any clinical or histological signs of inflammation, despite the presence of serum anti-CII antibodies. A similar protection against disease development was also observed in the context of EAE. A molecular analysis of HDAC1cKO $CD4^+$ T cells revealed increased STAT1 phosphorylation in activated HDAC1cKO $CD4^+$ T cells in comparison to WT cells. This was accompanied with an impaired expression of CCR6 in activated HDAC1cKO $CD4^+$ T cells, which is an essential chemokine receptor for the development of arthritis and EAE. In line with this finding we observed increased expression of CCR6 in $STAT1^{-/-}$ $CD4^+$ T cells. This indicates a negative role of STAT1 in the regulation of CCR6 expression.
Conclusions: Our data show the importance of HDAC1 as a key immune regulator in the pathogenesis of collagen induced arthritis. Therefore it might be considered as an interesting novel therapeutic target in RA.

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FRI0056 INCREASED EXPRESSION OF THE COLLAGEN INTERNALIZATION RECEPTOR ENDO180 IN FIBROBLAST-LIKE SYNOVIOCYTES OF PATIENTS WITH RHEUMATOID ARTHRITIS

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Background: Hyperproliferative and invasion are the characteristics of fibroblast-like synoviocytes in rheumatoid arthritis (RA-FLSs), which contribute mainly to RA disease progression and ultimately to joint destruction. Endo180 is a recycling endocytic receptor that has functions to regulate cell migration and bind and internalize collagens. However, the expression of Endo180 in RA-FLSs and its underlying mechanisms remain unclear.
Objectives: To examine the expression of Endo180 in RA-FLSs and the role it played in RA.
Methods: Tissues were collected from RA patients with joint replacement surgery or arthroscopy and RA-FLSs were obtained by tissue culture; Westren blot was used to measure the expression of Endo180 in RA-FLSs. Chemically synthesized small interference RNA (siRNA) specifically targeting Endo180 gene was transfected into RA-FLSs by cationic liposome; The interference efficiency of Endo180-siRNA on the production of Endo180 mRNA and protein was determined by RT-qPCR and Western blot respectly; The proliferative inhibition rate was examined by CCK8 assay and the migration of RA-FLSs were examined by Transwell assay.
Results: Expression of Endo180 was obviously higher in fibroblast-like synoviocytes in RA than OA and the traumatic patients. And its expression level has positive correlation with disease activity. The proliferative inhibition rate was obviously higher in the Endo180-siRNA group than the control groups ($P<0.05$) after transfection for 48h (8.31%±1.17%), 72h (15.93%±2.12)%), 96h (18.01%±2.78%). Transwell migration assay demonstrated the RA-FLSs through the transwell membrane in Endo180-siRNA group (21.27±6.35) were lesser than the NC-siRNA group (80.20±11.12) ($P<0.05$)and the blank control group (82.17±10.36) ($P<0.05$).
Conclusions: Endo180 may play a role in the regulation of proliferation and migration of RA-FLSs, which may provide beneficial therapeutic effects in RA.

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