disease-relevant pathways in the synovium that may point to possible combination therapies.

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Figure 1. Differentially expressed genes from collagen-injected versus naïve rat synovial tissue were identified over the course of disease and mapped to biological processes.

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THU0071 CD5L IN RHEUMATOID ARTHRITIS: PROTECTIVE OR PROMOTER?

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Background: Rheumatoid Arthritis (RA) is an aggressive auto-immune disease characterized by synovial hyperplasia and chronic inflammation. The main players of RA pathogenesis are T-cell and B-cell dependent pathways and some myeloid cells are also abundant in the synovial tissue. However, how inflammation is initiated, propagated and maintained remains controversial. Unbiased proteomic reports revealed an enrichment in the scavenger receptor CD5L, a component of serum and synovial tissues of arthritic patients. Upon secretion, this blood circulating glycoprotein represses pathogenic Th17 cells, promotes M2 polarization and binds and aggregates Gram-negative and -positive bacteria. However, its mechanisms of action has not been established either in health or disease.

Objectives: We intend to clarify whether CD5L is an immune component that helps resolving RA or a factor that aggravates the disease.

Methods: We analyzed by ELISA the presence of CD5L in samples from RA patients covering different stages of the disease, and correlated with other markers of RA. In parallel, we experimentally induced collagen induced arthritis (CIA) in CD5L knockout (KO) mice to evaluate the incidence and severity of the disease. The differences between the cellular groups in circulation vs the composition on secondary lymph organs using flow cytometry were also investigated in KO and WT mice. The histopathology of the joints was examined, while cytokine concentrations at several timepoints and total Ig levels were measured by ELISA and cytometric bead assays, respectively.

Results: The samples from RA patients showed increased CD5L levels concomitant with the severity of the disease and a direct correlation with Sharp RTG Score or IL-6 serum levels, and inversely correlated with COMP levels. However, these correlations did not clarify whether CD5L helps to resolve RA or is a component that aggravates the disease. To clarify this aspect, we provoked CIA in CD5L KO mice and observed a higher incidence of RA, higher severity and a much lower recovery rate when compared with WT mice. To corroborate

these data, the H&E staining of sagittal section of fore- and hindpaws revealed histopathology consistency with RA, with notable inflammatory signs especially in KO mice. WT animals with RA also showed higher levels of CD5L when compared with the control group, which confirms the observations obtained for human samples. Total serum IgG levels did not correlate with the disease severity but KO mice presented higher quantities of IgG and IL-6 when compared with WT mice.

Conclusion: Overall, these data imply that CD5L is not a promoter of the disease but rather a fundamental protective molecule against inflammation.

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THU0072 THE RELATIONSHIP BETWEEN INFLAMMATION AND COGNITIVE IMPAIRMENT IN RHEUMATOID ARTHRITIS

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Background: The pathophysiology of cognitive impairment remains unclear, however, several studies have demonstrated that pro-inflammatory cytokines such as Interleukin-6 (IL-6), Tumor necrosis factor- α (TNF- α) and lipocalin-2 (LCN2) are related with cognitive impairment by activation of microglia and astrocyte in brain. Rheumatoid arthritis (RA) is a representative inflammatory disease; however, the association of pro-inflammatory cytokines and LCN2 with cognitive impairment has seldomly been investigated in RA.

Objectives: Here, we determined the effect of pro-inflammatory cytokines and LCN2 on cognitive impairment in collagen-induced arthritis (CIA) mouse model. In addition, we studied the effect of TNF- α inhibitor (etanercept) on cognitive impairment.

Figure 1. Comparison of learning and memory abilities in CIA mice.

For the Morris water maze, the learning phase was conducted four times per day over 5 days. (A) Escape latency to reach the platform during the training session (days 1-4), (B) escape latency to reach the platform, (C) time spent in the target zone, (D) number of the target zone crossing and (E) tracking figures in the final trial of the last training day was also shown. Normal; Healthy group, CIA; collagen-induced arthritis group, CIA/Etanercept; collagen-induced arthritis and etanercept treatment group. Values are the means $\pm$ SEM (n = 10 mice per group). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ and ns=not significant.

Figure 2. Effects of etanercept on expression of LCN2 and GFAP in the hippocampus of CIA mice. Double immunofluorescence of LCN2 and GFAP in the hippocampal CA1 region of CIA mice. Dual labeling (white arrow point) showed LCN2 (red) co-localized with GFAP (Green), demonstrating the close relationship between LCN2 and GFAP. Normal; Healthy group, CIA; collagen-induced arthritis group, CIA/Etanercept; collagen-induced arthritis and etanercept treatment group. Values are the means $\pm$ SEM (n = 4 mice per group).

Methods: We induced CIA mice and randomly divided into three groups: Normal (n=10), CIA group (n=10), CIA/Etanercept group (n=10). We evaluated severity of arthritis using clinical scoring system. Joint inflammation, cartilage damage, and osteoclastic bone resorption has checked. Level of pro-inflammatory cytokines (TNF- α , and IL-6) and LCN2 checked in mice sera using enzyme-linked immunosorbent assay (ELISA). The expression of pro-inflammatory cytokines and LCN2 in ankle and hippocampus analyzed using real-time PCR. The activation of glial fibrillary acidic protein (GFAP) and ionized calcium-binding adapter molecule 1 (Iba-1) in hippocampus analyzed using immunohistochemistry (IHC). The LCN2 evaluated by western blot using hippocampal tissue. Cognitive impairment determined by morris water maze (MWM) test.

Results: Compared to normal group, CIA mice showed increased severity of arthritis, inflammation and destruction of joint. In MWM test, CIA mice significantly exhibited increased escape latencies and escape time, reduced the time in the target quadrant, and the number of target zone crossings during five study days compared with normal group. The level of pro-inflammatory cytokines (TNF- α , IL-6) and LCN2 in both sera, ankle tissue and hippocampal tissue was significantly increased. We examined whether inhibiting inflammation can mitigate the severity of arthritis and cognitive impairment. Compared to CIA mice, CIA/Etanercept group showed decreased severity of arthritis and joint inflammation. In MWM test, CIA/Etanercept group showed reduced escape latencies and escape time, increased the time in the target quadrant and the number of target zone crossings. The level of pro-inflammatory cytokines and LCN2 significantly decreased in both peripheral tissue and hippocampal tissue. In addition, the expression level of GFAP, Iba-1 and inflammatory markers decreased after treatment of etanercept. The results indicated that inhibition of inflammation may improve cognitive impairment.

Conclusion: The results suggest that peripheral arthritis induced inflammation is a possible cause for cognitive impairment by increasing pro-inflammatory cytokines and LCN2 in both peripheral tissue and hippocampal tissue in RA. In addition, we indicate that early anti-inflammatory treatment using etanercept may mitigate or inhibit the progression of cognitive impairment in RA.

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THU0073

THE ANTI-ANGIOGENIC ROLE OF TOFACITINIB DURING EXPERIMENTAL MODEL OF ARTHRITIS.

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Background: During rheumatoid arthritis (RA), a chronic autoimmune disease, the loop existing between inflammation and angiogenesis, characterised by new vessels formation associated with the increased recruitment of inflammatory cells, via the abnormal neo-angiogenesis in the synovial tissues, is considered an early important pathogenic mechanism.

Tofacitinib, a potent and selective JAK inhibitor, showed a good profile of safety and efficacy in RA patients, slowing the radiographic progression of the disease. In the last years, many works confirmed that some pro-angiogenic genes are targets of STATs family, and among them, vascular endothelial growth factors (VEGF), a potent pro-angiogenic molecule, may promote the new vessels formation via JAK/STAT pathways.

Objectives: The aim of this work was to investigate the inhibiting role of tofacitinib, on the angiogenic mechanisms occurring during experimental model of arthritis.

Methods: Healthy control (HC) ECs were stimulated with VEGF and/or tofacitinib and assessed for tube formation and migration, by matrigel and Boyden chamber assay. Furthermore, after ethical approval the experimental model of arthritis was obtained, stimulating 32 mice with collagen (CIA) and 32 mice with PBS (control). At day-19, CIA and controls mice were divided in 16 mice receiving vehicle and 16 mice receiving tofacitinib. At day-35, the mice were sacrificed and the thickness of paw joints, the synovial vessels and the serum levels of VEGF and Ang-2 were evaluated

Results: *In vitro*, after tofacitinib-treatment, HC-ECs lose their ability to form vessels and to migrate. *In vivo*, tofacitinib significantly prevented the increase of paw thickness induced by the collagen administration and reduced the vessel density in synovial tissues of joints, when compared to CIA that did not received tofacitinib. Furthermore, the serum levels of VEGF and Ang-2 were higher in CIA mice, than in control mice. The administration of tofacitinib was able to prevent the VEGF and Ang-2 accumulation in CIA mice.

Conclusion: During the last decade, the biological analogies between solid tumors and synovial pannus, and the encouraging results of anti-angiogenic treatments in oncology, lead to increasing interest for angiogenesis as a possible therapeutic target in RA. The present study demonstrated the anti-angiogenic efficacy of tofacitinib, opening a new perspective application for this molecule and improving our therapeutic skill to control the clinical evolution of RA.

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