Poster Presentations

SATURDAY, 06 JUNE 2020

Rheumatoid arthritis - aetiology, pathogenesis and animal models

SAT0001

L-ARGININE SUPPLEMENTATION AMELIORATES BONE EROSION IN RHEUMATOID ARTHRITIS THROUGH INHIBITION OF RANKL/RANK/TRAFF6 PATHWAY AND REPROGRAMMING OSTEOCLAST METABOLISM

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Background: L-arginine is of great importance in numerous biological procedures in human body, e.g. it participates in the urea cycle for detoxification of ammonia, and functions as a modulator in immune responses (1). Our previous investigation demonstrated that treatment with L-arginine reduced clinical symptoms, bone erosion and osteoclast numbers in serum-induced arthritis (K/BxN) (2). In addition, a decreased concentration of L-arginine has been observed in the serum of rheumatoid arthritis (RA) patients. Altogether, it is suggesting that L-arginine supplementation might be a potential treatment against RA.

Objectives: This study aims to investigate the treatment role of L-arginine supplementation in murine arthritis models. The project also plans to delineate the metabolic action of L-arginine supplementation during osteoclast differentiation in the presence of an inflammatory milieu.

Methods: Three murine arthritis models (serum-induced arthritis (K/BxN) model, collagen-induced arthritis model and hTNFg mice model) were applied in the presence or not of oral L-arginine supplementation. MicroCT and histomorphometry analyses were performed to quantify bone erosion and numbers of osteoclasts. In addition, *in vitro* osteoclastogenesis were performed in the presence of various amounts of L-arginine with or without treatment with 40ng/ml TNFα. OC differentiation was characterized by TRAP staining. Resorption activity was assessed by pit formation assay. Osteoclast markers and metabolic genes were determined with quantitative real-time PCR analysis and western blot. Dihydrorhodamine 123 staining was used to determine the level of intracellular ROS. Seahorse analyses and mass spectrometry metabolites analyses were conducted to address the metabolic condition.

Results: Arthritis severities were reduced after L-arginine supplementation in arthritis models. Moreover, an amelioration of bone erosion and reduced osteoclast numbers were observed in arthritic mice treated with L-arginine. *In vitro* treatment of L-arginine inhibited osteoclastogenesis, especially in the late phase of the differentiation, even with exposure to TNFα stimulation. The L-arginine induced osteoclast differentiation inhibition is likely due to an alteration in the RANKL/RANK/Traff6 pathway. L-arginine also boosted the intracellular production of ATP and ROS, promoting mitochondria-driven oxidative phosphorylations (OXPHOS), leading to the failure of activation and even death of the osteoclasts.

Conclusion: These data strongly suggested that L-Arginine ameliorates bone erosion in RA through the inhibition of RANKL/RANK/Traff6 pathway as well as reprogramming of the cellular metabolism during osteoclastogenesis. The immunometabolism action of L-Arginine might therefore help to reduce joint inflammation and destruction in RA.

References:

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SAT0002

ANTI-PEPTIDE ANTIBODIES SPECIFIC FOR THE HUMAN FIBRINOGEN B CHAIN COULD PLAY AN ESSENTIAL ROLE IN THE PROGNOSIS IN EARLY RHEUMATOID ARTHRITIS AND FAMILY RISK GROUPS

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Background: Rheumatoid Arthritis (RA) is a chronic systemic and autoimmune disease. The identification of antibodies has contributed to understanding RA. Recently, anti-carbamylated protein antibodies are the new autoantibodies present in RA and have been associated with severity and detected before the onset of RA. However, it is not clear the precise target antigen of anti-CaP antibodies

Objectives: To determine associations between the anti-carbamylated protein and peptides antibodies with genetic and rheumatic indexes in patients with early RA (eRA) and individuals with family history of RA (FDR)

Methods: A cross-sectional study involving 51 eRA patients, according to ACR/EULAR 2010 and 124 FDR matched by sex and age with healthy controls (HC). Two peptides of fibrinogen beta chain (Anti-Ca-Fib2 and Anti-Ca-Fib3) and a peptide without modification were synthesized. Antibodies against carbamylated peptides were measured using in-house indirect ELISA. RF and anti-CCP by turbidimetry and ELISA, hs-CRP by chemiluminescence. ESR by capillary photometry, disease activity was measured by SDAI, DAS28, RAPID3, and disability was assessed using MDHAQ. HLA-DRB1 (shared epitope SE) typing was made using the Luminex 200 TM. The comparisons were performed by McNemar and Wilcoxon tests. The chi-squared and Fisher's tests were used in categorical variables. The associations were evaluated by the Mann-Whitney U test or t-test and conditional logistic regressions

Results: In FDR anti-Ca-Fib2 and anti-Ca-Fib3 were more frequent than HC (25.0% vs 14.5%, 34.7% vs 15.3% and 33.1% vs 11.3%, respectively). The anti-Ca-Fib2 antibodies were associated with the HLA DRB1 SE* 1501 allele (p=0.03), with non-SE*0901 allele (p=0.01) (Fig. 1), the anti-Ca-Fib3 was associated with positive RF (p=0.0012). The FDR condition was associated with the presence of anti-Ca-Fib3 (OR=4.7; 95%CI=1.8-11.7; p=0.001) and painful joints (OR=2.2; 95%CI=1.01-4.68; p=0.045). In eRA anti-Ca-Fib2 were more frequent 47.1% vs. 13.7% in HC (OR 6.6 95% CI: 1.97-35.0 p = 0.0005) showing a risk of present anti-Ca-Fib2 antibodies six times higher in patients, without no differences in the frequency of anti-Ca-Fib3 between patients and controls. The anti-Ca-Fib2 antibodies were associated with positive RF (p = 0.022) and with high positive anti-CCP (p = 0.032) additionally we observed association with HLA-DRB1 SE* 0405 (p = 0.050) and non SE alleles* 1501 (p = 0.026) and* 0407 (p = 0.010)(Fig.2). Anti-Ca-Fib3 were associated with DAS28 ESR (p = 0.046) and with the HLA-DRB1 SE* 1402 allele (p = 0.050) and non-SE*0301 allele (p = 0.025). We found an association between eRA diagnosis with the presence of anti-Ca-Fib2 (OR 6.72 95% CI: 1.09-41.38 p = 0.040) and positive anti-CCP (OR 7.94 95% CI: 1.44-43.77 p = 0.017)

Conclusion: Anti-Ca-Fib3 and anti-Ca-Fib2 antibodies might have significant prognostic value because the relationship with joint inflammatory manifestations in FDR. These results suggest a role for these antibodies as early biomarkers of RA, probably including additional mechanisms related to other non-SE alleles, especially in high-risk individuals. Finally, the anti-peptide antibodies in the present study may represent a straightforward way to identify antibodies directed to a specific carbamylated target

Figure 1 association of carbamylated anti-peptide antibodies with the presence of HLA DRB1 SE and non-SE in eRA