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

S. Cao¹, X. Chen², G. Schett¹, A. Bozec¹. ¹Department of Internal Medicine 3-Rheumatology and Immunology, FAU and Universitätsklinikum Erlangen, Erlangen, Germany; ²Department of Rheumatology, Renji Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China, Shanghai, China

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.

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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

L. Chila^{1,2}, L. S. Rodriguez³, W. Bautista-Molano^{1,2}, J. M. Bello-Gualtero^{2,4}, C. Romero-Sánchez^{1,2,4}. ¹Universidad El Bosque, School of Dentistry, Cellular and Immunology Group, Bogotá, Colombia; ²Universidad Militar Nueva Granada, School of Medicine, Clinical Immunology Group, Bogotá, Colombia; ³Instituto de Genética Humana, Facultad de Medicina, Pontificia Universidad Javeriana, Bogotá, Colombia; ⁴Hospital Militar Central, Rheumatology and Immunology Department, Clinical Immunology Group, Bogotá, Colombia

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

Figure 2 association of carbamylated anti-peptide antibodies with the presence of HLA DRB1 SE and non-SE in FDR

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SAT0003

ELEVATED BASELINE AND INCREASING AUTOANTIBODY LEVELS ARE ASSOCIATED WITH INCREASED RISK FOR IMMINENT ONSET OF INFLAMMATORY ARTHRITIS IN A PROSPECTIVELY STUDIED ANTI-CITRULLINATED PROTEIN ANTIBODY POSITIVE COHORT: THE TIP-RA COLLECTIVE

K. Deane¹, G. Firestein², D. Boyle², J. Buckner³, E. A. James³, S. Posso³, W. Robinson⁴, L. K. Moss¹, J. Seifert¹, R. Gilmore¹, S. Barzideh¹, N. Rao⁵, F. Baribaud⁵, S. Nagpal⁵, A. Johnsen⁵, V. M. Holers¹. ¹University of Colorado Denver, Aurora, United States of America; ²University of California San Diego, San Diego, United States of America; ³Benaroya Research Institute, Seattle, United States of America; ⁴Stanford University, Palo Alto, United States of America; ⁵Janssen Research and Development, LLC, Spring House, United States of America

Background: The Targeting Immune Responses for Prevention of RA (TIP-RA) Collaborative prospectively studies individuals at high risk for developing RA because of serum ACPA positivity in absence of baseline inflammatory arthritis (IA). **Objectives:** The objective of the analyses presented herein is to evaluate the role of baseline and changing levels of ACPA and rheumatoid factor (RF) in relationship to incident IA/RA.

Methods: ACPA+ subjects and ACPA- controls were identified who did not have baseline historical or examination evidence of IA. ACPA+ was defined by serum elevation of anti-CCP3 ≥ 20 units (Inova). Subjects were evaluated annually or sooner if they had changes in joint symptoms. Factors including RFIgM and RFIgA (Inova) were also assessed, and relationships between autoantibody levels at baseline and over time and incident IA/RA were evaluated using t-tests, with paired testing where applicable.

Results: Baseline characteristics of ACPA+ and ACPA- subjects are in Table 1. Sixteen of the 94 (17%) ACPA+ subjects developed IA/RA a mean of 518 days from the baseline visit; 14 of these met 2010 ACR/EULAR criteria for RA at the time of detection of IA. There was a trend for ACPA+ subjects who later developed IA/RA to have higher baseline levels of anti-CCP3 compared to those who did not develop IA/RA (Table 2). In addition, those who developed IA/RA had significantly higher mean levels of RFIgM and RFIgA compared to those who did not. While not statistically significant, in longitudinal analyses in the ACPA+ subjects with incident IA/RA, anti-CCP3 levels increased from baseline to identification of IA (mean [SD] of 119 [102] to 126 [100], $p=0.42$). Furthermore, RFIgM levels increased from 36 [49] at baseline to 43 [51] at the time of IA ($p=0.31$), and RFIgA levels increased from 16 [29] to 21 [31] ($p=0.10$). In contrast, in ACPA+ subjects who did not develop IA/RA, anti-CCP3 levels increased only slightly over follow-up of a mean of 712 days: 75 [75] to 80 [76], $p=0.70$ while the levels of RFIgM and RFIgA decreased slightly during the same follow-up: for RFIgM mean [SD] levels went from 9 [22] to 8 [19], $p=0.74$; for RFIgA, 5 [16] to 3 [12], $p=0.67$.

Table 1. Baseline characteristics of ACPA+/- subjects

	ACPA- (n=162)	ACPA+ (n=94)	p-value
Age, mean	58	58	0.90
% Female	69	68	0.67
% Ever smoker	33	34	0.87
RF-IgM, mean (SD)	3.2 (10.0)	13.5 (30.2)	<0.01
RF-IgA, mean (SD)	0.3 (0.6)	6.5 (19.1)	<0.01

Table 2. Baseline characteristics of 16 ACPA+ subjects who developed incident IA/RA vs. 78 ACPA+ who did not

	Did not develop IA/RA (n=78)	Developed IA/RA (n=16)	p-value
Days from baseline to IA/RA or follow-up, mean (SD)	712 (124)	518 (295)	—
% Meeting 2010 criteria at time of IA	—	88	—
CCP3, mean (SD)	74.5 (75.3)	119.1 (102.1)	0.05
RFIgM, mean (SD)	9 (22)	36 (49)	<0.01
RFIgA, mean (SD)	4 (16)	16 (29)	0.03

Conclusion: In this prospectively followed cohort of ACPA+ subjects, higher levels of RFIgM and RFIgA at baseline were significantly associated with development of IA/RA within the follow-up period. Furthermore, there was a trend for rising levels of anti-CCP3 and RFIgM and A to be associated with development of IA/RA. These findings support the use of higher and/or rising levels of autoantibodies as additional features to predict imminent onset of IA/RA in ACPA+ individuals as well as potentially to use as outcomes of success of preventive interventions. Furthermore, the trend of increasing levels of RFIgM and RFIgA over time in individuals who developed IA/RA suggests that targeting pathways of RF development may lead to preventive interventions in a subset of RA.

References: None

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SAT0004

INCREASED M1 INFLAMMATORY PHENOTYPE OF CIRCULATING MONOCYTES IS ASSOCIATED WITH HISTORY OF CARDIOVASCULAR EVENTS IN RA PATIENTS

A. Goecke¹, C. Karsulovic¹, J. Guerrero², F. Tempio³, M. Lopez³. ¹University of Chile Clinical Hospital, Rheumatology, Santiago, Chile; ²Faculty of Medicine, University of Chile, Physiology, Santiago, Chile; ³Faculty of Medicine, University of Chile, Immunology, Santiago, Chile

Background: Cardiovascular (CV) Disease is the main cause of death in Rheumatoid Arthritis (RA). Current tools like Framingham or European SCORE underestimate CV risk in RA patients. Efforts to improve the assessment including RA biomarkers (disease activity) have been only partially successful. There is a need for better biomarkers to identify RA patients at high risk for CV disease. Monocytes have an important role in plaque development. Monocytes differentiate into 2 main phenotypes M1 and M2 (1). In RA and in post-MI patients M1 monocytes are expanded (2). mTORC influences monocyte phenotype *in vitro* and has been associated with development of atheromatous plaque (3).

Objectives: To evaluate the phenotype of circulating monocyte in RA patient with or without previous CV events (RA-CV(-)/RA-CV(+)), and its possible association with mTORC activity.

Methods: 9 RA-CV(+) patients aged between 18 and 65 yo with RA (EULAR/ACR 2010 criteria), were paired with RA-CV(-) patients. 6 healthy individuals (HI) were also studied. Pairing criteria were classic CV risk factors (AHA 2018), sex, age, years since RA diagnosis, comorbidities, number of DMARDs previously used and use of bDMARDs. M1 and M2 circulating Monocytes were evaluated in PBMC obtained from patients and controls by flow cytometry analysis. Intracellular inflammatory cytokines (IL1, IL6) and phosphorylated S6R (P-S6R) as a measure of mTORC activation was also evaluated. M1 was defined as CD14+HLA-DR+CCR2+ and M2 CD14+CD163+CCR2-. DAS28-RCP, DAS28-ESR and Lipid profile was also measured. The differences among groups was analysed using Mann-Whitney U nonparametric. The relationship between variables with Spearman rank correlation test.

Results: There were no differences in demographic, RA characteristic and CV risk factors between RA-CV (+) and RA-CV (-) patients. Male/Female 4/5, age 62±3 and 63±2 respectively. HI were younger than RA patients (32.5±7). CV events were 8 patients with MI and one Stroke. DAS28-RCP was 2.96±0.23 and 2.88±0.43 respectively. One patient in each group had failed to more than 2 sDMARDs