AB0053

THE ROLE OF ANGIOPOIETIN-LIKE PROTEINS TYPES 3 AND 4 IN THE DEVELOPMENT OF RENAL DYSFUNCTION IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Background: Rheumatoid arthritis (RA) often contributes to the development of kidney disease. Angiotensin-like proteins can be target markers for studying cardiorenal complications of RA [1].

Objectives: Assessment of the correlation of serum concentrations of angiotensin-like proteins types 3 and 4 (ANGPTL 3 and 4) with the progression of renal dysfunction in RA patients.

Methods: 114 patients with reliable RA (90.4% of women, 9.6% of men) aged 21 to 80 years (mean age 55.4 ± 11.2 years old), disease duration - 11.18 ± 9.03 years, positive for rheumatoid factor (RF-IgM) - 63.2%, positive for anti-citrullinated protein antibody (ACPA) - 59.7% were examined. The laboratory examination included the determination of serum concentrations of angiotensin-like protein type 3 (Human Angiotensin-like Protein 3 ELISA, Bio Vendor, Czech Republic) and type 4 (RayBio Human ANGPTL4 ELISA Kit; RayBiotech, USA). To assess renal function in RA patients we used the calculated glomerular filtration rate (GFR) according to the CKD-EPI formula [2], taking into account the height and weight of a particular patient without indexing by body surface area. GFR values from 60 to 89 ml / min / 1.73 m² - as a slight decrease in global kidney function.

Results: The concentration of ANGPTL3 in the blood serum of RA patients (n = 158) was 641.9 ± 224.5 ng / ml, and that of ANGPTL4 (n = 158) - 3.15 [0.77; 12.1] ng / ml. 74.7% (n = 118) were considered positive for the presence of ANGPTL3; 49.4% (n = 78) of RA patients were recognized as positive for the presence of ANGPTL4. The average glomerular filtration rate in RA patients was 74.0 ± 18.6 ml / min. More than a half of the examined RA patients had GFR ranging from 89 to 60 ml / min / 1.73 m² (C1 - 21.5%; C2 - 58.9%; C3 - 19.6%). No sharp reduce of renal function (GFR <30 ml / min / 1.73 m²) corresponding to CKD C4-5 stages was recorded. Negative correlations of average strength were found between GFR and the level of ANGPTL 3 (r = -0.32, p < 0.001) and ANGPTL 4 (rS = -0.31, p < 0.001), as well as with age (rS = -0.28, p < 0.001), the duration of RA (rS = -0.22, p = 0.005) and blood pressure increase (rS = -0.25, p = 0.001). On the basis of GFR measurements, patients were divided into three groups: group I - optimal renal function (> 90 ml / min); group II - a slight decrease in renal function (89-60 ml / min); group III - reduced renal function (<59 ml / min).

Table 1. Content of ANGPTL 3 and 4 in RA patients with different GFR, ng / ml

	Group I (n=34)	Group II (n=93)	Group III (n=31)
ANGPTL 3	533.4±161.7 ^{I-III}	650.0±223.9	733.2±244.1
ANGPTL 4	0.77 [0.28;3.6] ^{I-II, I-III}	3.3 [0.93;12.1]	6.48 [1.52;19.3]

Note: upper case indicates intergroup differences at p < 0.05.

There was a significant difference in the content of ANGPTL3 in patients of the first group and the patients of the third group (H-test = 6.55, p = 0.032) and ANGPTL4 in the group of patients with normal renal function (group I) and groups of RA patients with decreased GFR (group I-II: H-test = 10.7, p = 0.001; groups I-III: H-test = 20.1, p < 0.001). ANGPTL4 indices also had intergroup differences (groups II-III: H-test = 7.2, p = 0.007) with GFR less than 90 ml / min.

Conclusion: Chronic rheumatoid inflammation potentiates the development of renal dysfunction according to our data in 78.5% of patients. It is also accompanied by an increase in the content of ANGPTL types 3 and 4 in the blood of RA patients. A better understanding of the actions and mechanisms of ANGPTL may be of paramount importance for the development of effective ways of treatment for cardiorenal complications in RA.

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Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2021-eular.2275

AB0054

IS TEA CONSUMPTION ASSOCIATED WITH RISK OF RHEUMATOID ARTHRITIS?

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Background: Only few studies have looked at the association between tea consumption and risk of rheumatoid arthritis (RA) with inconclusive results.

Objectives: To estimate the association between tea consumption and risk of RA in a large population-based case-control study.

Methods: We used data from the Swedish Epidemiological Investigation of RA (EIRA), a population-based case-control study including incident RA cases with 2 controls randomly matched from the general population, based on age, sex and residential area at the date of diagnosis. All participants filled in a comprehensive questionnaire on lifestyle factors, including a 124-item food frequency questionnaire (FFQ). Data from October 2005 - May 2018 was used.

Odds ratios (ORs) with 95% confidence intervals (CIs) were estimated using conditional logistic regression, overall and stratified by anti-citrullinated protein antibody (ACPA) and smoking status. We adjusted for smoking status, coffee and alcohol consumption, educational level and BMI. The dose-response trend of the association between tea consumption and risk of rheumatoid arthritis was estimated using restricted cubic spline with knots at 0, 0.29, and 2.29 cups per day. Missing tea values (44.8%) were imputed based on the zero-consumption assumption. A sensitivity analysis was performed to evaluate the influence of the assumption on the main result, with 70% of the missing randomly imputed as no consumption, and the remaining 30% randomly assigned to the other categories (10% per category).

Results: We included 2237 cases and 4661 controls. Controls were more likely to drink ≥2 tea cups/day compared to RA cases (22.1% vs. 19.7%). The crude odds of developing RA was 22% lower (OR=0.78, 95% CI: 0.66-0.92) among those who consumed ≥2 tea cups/day compared to those who drank <1 cup/day (irregular drinkers). After adjustment for covariates, the inverse association was less pronounced and only borderline significant (OR=0.85, 95% CI: 0.71-1.01). A similar OR was observed among non-tea consumer (OR=0.82, 95% CI: 0.70-0.95). The odds of developing ACPA positive RA (but not ACPA negative) was statistically significant lower among participants drinking ≥2 cups/day compared to irregular tea drinkers (OR=0.76, 95% CI: 0.62-0.94). In analyses stratified by smoking, an inverse association between tea intake and RA was found only among current smokers (OR=0.58, 95% CI: 0.37-0.92), while no association was found in never smoker. The sensitivity analysis showed results similar to the main analysis, although the OR in the no consumption category was not significant (OR=0.88, 95% CI: 0.76-1.02) while the OR in the highest category was statistically significant (OR=0.85, 95% CI: 0.73-0.99).

Overall tea consumption				
	0 cups/day	<1 cup/day	1-2 cups/day	≥2 cups/day
Overall				
Number	955/1941	453/847	388/845	441/1028
OR crude*	0.89 (0.77-1.03)	Ref	0.84 (0.71-1.00)	0.78 (0.66-0.92)
OR adjusted†	0.82 (0.70-0.95)	Ref	0.87 (0.73-1.04)	0.85 (0.71-1.01)
ACPA positive				
Number	637/1156	321/527	268/501	277/630
OR adjusted	0.81 (0.67-0.97)	Ref	0.88 (0.71-1.09)	0.76 (0.62-0.94)
ACPA negative				
Number	312/571	129/221	119/251	162/288
OR adjusted	0.87 (0.66-1.14)	Ref	0.87 (0.63-1.19)	1.07 (0.78-1.46)
Never smokers				
Number	290/859	164/436	142/433	213/563
OR adjusted	0.90 (0.72-1.14)	Ref	0.88 (0.68-1.15)	1.04 (0.81-1.33)
Current smokers				
Number	296/371	89/96	59/78	59/99
OR adjusted	0.81 (0.57-1.14)	Ref	0.83 (0.52-1.31)	0.58 (0.37-0.92)

Figure 1. Dose-response odds ratio for risk of rheumatoid arthritis (RA) by tea consumption.

Conclusion: In this large population-based case-control study, high tea consumption, as well as no consumption, was associated with a decreased risk to develop ACPA positive, but not negative, RA, when compared to irregular tea drinking (<1 cup/day). The inverse association between high tea consumption and RA was lowest in analyses restricted to current smokers.

Disclosure of Interests: Helga Westerlind: None declared, Ida Palmqvist: None declared, Saedis Saevarsdottir Employee of: part-time employee of deCODE genetics, Lars Alfredsson: None declared, Lars Klareskog: None declared, Daniela Di Giuseppe: None declared

DOI: 10.1136/annrheumdis-2021-eular.2423

AB0055

AUTOIMMUNE RESPONSE AGAINST THE SHARED EPIOTOPE SEQUENCE IN RHEUMATOID ARTHRITIS

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Background: Rheumatoid Arthritis (RA) is a systemic autoimmune disease, associated with hiperproduction of autoantibodies (AAb), in which the most specific are the AAb against citrullinated peptides (ACPA). RA is influenced by genetic factors, specifically, there is a strong genetic association with the shared epitope (SE), a five amino acid sequence motif in positions 70–74 of HLA-DRβ1 chains encoded by HLA-DRB1 alleles: QKRAA, QRRRA and RRRRA.

The present study aims to analyze whether SE-peptides (SE-p) can be a target of the autoimmune response in RA.

Objectives: To analyze the presence of AAb against the unmodified (Un) SE-p, citrullinated (Cit) SE-p and carbamylated (Car) SE-p.

Methods: Sera from consecutive 117 RA patients and 21 psoriatic arthritis (PsA) from our outpatient clinic were collected by venopuncture. Also 138 sera from blood donors were obtained as healthy controls (HC). All participants signed the informed consent.

We performed a homemade ELISA test using a sequence of 15 aminoacid peptides from positions 65-79 of HLA-DRB1 containing the 3 different SE sequences, in the Un, Cit and Car SE-p, synthesized in a linear and cyclized form. We established a 90% of specificity using a ROC curve obtained from HC and PsA for each ELISA test.

HLA-DRB1 polymorphism was performed using a HLA-DRB1 sequence specific oligonucleotide typing kit (Lifecodes) in 95 RA and in 15 PsA.

ACPA and RF were determined with commercial assays (Inova Diagnostics and Binding Site, respectively).

Results: The overall sensitivity of the different SE-p AAb tests ranged from 5.1-21.4%.

RRRAA SE polymorphism was associated with AAb against cyclized CitCitCitAA SE-p (p=0.025), QKRAA polymorphism was almost significantly associated with AAb against cyclized QKCitAA SE-p (p=0.067), whereas there was no association between QRRRA polymorphism and AAb against cyclized QKCitCitAA SE-p (p=0.690). On the other hand, there was no association between SE polymorphisms and AAb to any other peptide used in the ELISA test.

Significant differences were observed in the presence of AAb against lineal RRRRA, lineal CitCitCitAA and cyclized CitCitCitAA SE-p when comparing RA vs. HC patients (p=0.022, 0.044, 0.022, respectively). Moreover, there also were significant differences in the presence of AAb against cyclized CitCitCitAA SE-p between RA and PsA patients (sensitivity 21.4%, specificity 100%; p=0.014).

It must be highlighted that cyclized CitCitCitAA SE-p AAb were detected in 20.0% of RA patient sera that were negative for RF and ACPA.

There was no association between RF or ACPA with the presence of any SE-p AAb.

Conclusion: RA patients have autoantibodies against the Shared Epitope (SE). The cyclized CitCitCitAA SE peptide (SE-p) shows the best performance among all the peptides tested and could identify patients seronegative for ACPA and RF, both analyzed by commercial assays.

Additional studies must be performed to verify the diagnostic and utility of these new autoantibodies against SE-p in RA.

Acknowledgements: This work was granted by the 2018 call of the "Fundación Española de Reumatología" and the 2017 call grant "Fundació Parc Taulí".

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2021-eular.2470

AB0056

TNFR2 PROMOTES INFLAMMATORY PROGRAMS IN FIBROBLAST-LIKE SYNOVIOCYTES

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Background: TNF-mediated fibroblast-like synovocyte (FLS) activation is important for inflammation and joint destruction in rheumatoid arthritis (RA). The role of TNF-receptor 1 (TNFR1) in FLS activation has thoroughly been characterized. The functions of TNFR2 are, however, largely unknown.

Objectives: To investigate the contribution of TNFR2 to the TNF-mediated activation of FLS.

Methods: RA-FLS were transfected with TNFR2-targeting siRNA pools and transcriptional changes were determined by RNA-seq. QPCR, ELISA and immunoblotting were used to confirm the RNA-seq results and to gain insights into the pathways that regulate TNFR2-mediated changes in FLS.

Results: TNF stimulation of FLS resulted in a strong upregulation of proinflammatory cytokines, chemokines, tissue-degrading enzymes and other genes that are associated with synovial inflammation in RA. Silencing of TNFR2 markedly diminished the TNF-response of RA-FLS. Especially, "interferon"-stimulated-genes (ISGs) including putative master regulators of joint inflammation, such as the CXCR3 chemokines CXCL9, CXCL10 and CXCL11 were affected by the knockdown of TNFR2. Consistently, immunoblots showed that TNFR2 was required for the TNF-induced phosphorylation of the transcription factor STAT1, which is known to mediate the transcription of ISGs, such as CXCR3 chemokines.

Conclusion: TNFR2 regulates proinflammatory gene expression in RA-FLS via STAT1 and thereby contributes to the detrimental effects of TNF in synovial joint inflammation.

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2021-eular.2500

AB0057

IN VITRO EFFECT OF CTLA4-IGG ON M1-M2 SHIFT OF MACROPHAGES FROM RHEUMATOID ARTHRITIS PATIENTS

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Background: Among the cells involved in the inflammatory process of rheumatoid arthritis (RA) [1], macrophages play a key role through their capacity to polarize into "classically" or "alternatively" activated phenotypes (M1 or M2) and making macrophages important players for the inflammatory cascade or for the anti-inflammatory reaction, respectively [2]. CTLA4-Ig fusion protein (abatacept) has been shown to contribute to macrophage shift from M1 to M2 [3].

Objectives: We aimed to investigate the effects of abatacept to induce the polarization from the pro-inflammatory M1 phenotype into the anti-inflammatory M2 phenotype in cultured human macrophages obtained from RA patients' and healthy subjects (HS) circulating monocytes.

Methods: Cultured monocytes were isolated from peripheral blood mononuclear cells (PBMCs) of three early RA patients and ten HS, after signing informed consent and Ethics Committee approval. Cells were treated with phorbol myristate acetate (PMA) [5ng/ml] for 24 hours (hrs) to induce their differentiation into monocyte-derived macrophages (MDMs). Therefore, cultured HS MDMs were stimulated with lipopolysaccharides (LPS, 1mg/mL) for 4hrs [4] in order to induce their polarization into a pro-inflammatory M1 phenotype and then treated or not with abatacept at the concentrations of 100mg/mL and 500mg/mL for 3, 12, 24 and 48hrs. Cultured RA MDMs, were directly treated with abatacept as previous described. Cultured HS and RA MDMs without any pro-inflammatory stimuli and abatacept treatment were used as respective control.

The transition of MDMs from M1 to M2 phenotype was evaluated through gene expression and protein synthesis of M2 macrophage markers, namely scavenger receptors (CD163 and CD204), and mannose receptor-1 (CD206) by quantitative real-time polymerase chain reaction (PCR) and by Western blotting. The statistical analysis evaluation was carried out by GraphPad Prism 8 analysis software using the Wilcoxon non-parametric t-test. Any p-value lower than 0.05 was considered as statistically significant. Results were indicated as median±standard deviation (SD).

Results: In cultured RA MDMs (three cases), abatacept upregulated the gene expression of all investigated M2 markers, specifically after 12hrs of treatment with the concentration of 100mg/mL. In these cells, abatacept upregulated only the CD204 protein synthesis with more evidence at 24hrs of treatment and with the 500mg/mL concentration. In cultured HS MDMs (ten cases), abatacept upregulated the gene expression of M2 markers, significantly for that of CD206 [at 3hrs with 100mg/mL concentration, p= 0.0312] and CD163 [at 12hrs with 500mg/mL concentration, p= 0.0312]. Moreover, in these cells, abatacept significantly upregulated the protein synthesis of CD206 [at 48hrs with 500mg/mL concentration, p= 0.0195] and CD204 [at 24hrs with 100mg/mL concentration, p= 0.0156; both at 24 and 48hrs with 500mg/mL concentration, p= 0.0234].