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 QKCitAA 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 synoviocyte (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].

Conclusion: Preliminary data seem to indicate that abatacept can promote the in vitro shift from the M1 into the M2 macrophage phenotype, by upregulating specific markers (CD163, CD204, CD206) in cultured M1-MDMs from RA patients and in M1 macrophages induced from HS.

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Disclosure of Interests: Samuele Tardito: None declared, Stefano Soldano: None declared, Emanuele Gotelli: None declared, Paola Montagna: None declared, Sabrina Paolino: None declared, Vanessa Smith: None declared, Maurizio Cutolo Grant/research support from: I received grant/research support from Bristol-Myers Squibb, Boehringer, Celgene.

DOI: 10.1136/annrheumdis-2021-eular.2524

AB0058

ASSOCIATION BETWEEN HLA-DRB1*04:01, RHEUMATOID NODULES AND PARTICULAR EPITOPES OF CITRULLINATED FIBRIN IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Background: Rheumatoid arthritis (RA) is associated with HLA-DRB1 genes encoding the shared epitope (SE), a 5 amino acid motive. RA is usually preceded by the emergence of anti-citrullinated protein antibodies (ACPAs) detected by anti-CCP2 tests. Citrullin is a neutral amino acid resulting from post translational modification of arginin by Peptidyl Arginyl Deiminases (PADs). ACPAs recognize epitopes from citrullinated human fibrinogen (Fib-cit) and can be specifically detected by the AhFibA assay. Five peptides derived from Fib-cit together represent almost all of the epitopes recognized by patients with ACPA-positive RA: β 60–74cit, α 36–50cit, α 621–635cit, α 501–515cit and α 171–185cit. As RA is a pleiomorphic disease, whose evolution is difficult to predict, the use of antibody fine specificity as a marker of clinical phenotypes has become a major challenge.

Objectives: Our objective was to study whether clinical characteristics and HLA-DRB1 genetic background were associated with a specific reactivity against these epitopes.

Methods: 184 ACPA positive RA patients fulfilling the 2010 ACR/EULAR criteria were studied. Patients characteristics, including HLA-DRB1 genotype, were collected from their medical files. Anti-CCP2, AhFibA, Rheumatoid Factors (RF), and antibodies against the five major Fib-cit peptides were analyzed using ELISA assays.

Results: Anti-CCP2 and AhFibA titres were strongly correlated (rs: 0.7037; $p = 5.69 \times 10^{-29}$, Pearson's). Anti- α 505–515cit antibodies were associated with HLA-DRB1*04:01 (OR = 5.52 [2.00 – 13.64]; $p = 0.0003$). High level anti- α 505–515cit antibodies were significantly associated with rheumatoid nodules (OR = 2.71 [1.00 – 7.16], $p = 0.044$). Anti α 501–515cit antibodies were associated with RF (OR=2.31 [1.10 – 4.78], $p = 0.026$).

Conclusion: Immune complexes containing anti- α 501–515cit antibodies and rheumatoid factors might be involved in the development of rheumatoid nodules on the HLA-DRB1*04:01 background. These findings highlight the role played by the HLA-DRB1*04:01 molecule and its rapid intracellular route into the lysosomes, enabling original antigen processing. Finally, purifying these epitope specific antibodies might be a new therapeutic opportunity for rheumatoid nodules.

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2021-eular.2568

AB0059

STUDY OF THE ANTI-INFLAMMATORY EFFECT OF CINNAMOMUM VERUM IN RATS

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Background: Inflammatory response is a common physiological response that protects the host of many harmful stimuli such as toxins, local injuries and

pathogens. Today, non-steroidal anti-inflammatory drugs are the most commonly prescribed for the treatment of many inflammatory treatments. However, long term administration of these NSAIDs causes many adverse effects, including gastrointestinal ulcers and bleeding and kidney damage. Therefore, the need for new anti-inflammatory drugs with fewer side effects is required.

Objectives: The aim of our study was to evaluate the anti-inflammatory activity of *Cinnamomum verum* (C. Verum) on rats while testing the dose effect and the typical effect of the solvent.

Methods: The edema was induced by injection of carrageenan solution (CARR) in paw of rats according to the method of Winter et al, 1963. The measurements of volumes of right hind paw of each rat were taken before induction of edema and each 1h, 2h, 3h, 4h and 5h after the injection of carrageenan. 7 groups of 6 rats received, one hour before the injection of the carrageenan, different treatment: G1= witness, G2=CARR + saline solution, G3= CARR+ Acetylsalicylic acid, G4= CARR + C. verum- MeOH 100, G5= CARR + C. verum- MeOH 300, G6= CARR + CARR + C. verum- EtOH 100 and G7= CARR + C.verum- EtOH 300.

Results: For this test, the volume of edema increases with time, this increase is greater in the group treated with saline solution than with the extracts of C. Verum and with Acetylsalicylic acid. The extracts of C. Verum administered intraperitoneally one hour before the injection of the carrageenan, reduced the edema in the third hour after.

Conclusion: The interesting anti-inflammatory activity of *Cinnamomum verum* recorded in this work may at least in part support its popular use in traditional medicine [1, 2].

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Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2021-eular.2948

AB0060

INCREASED AGE/AUTOIMMUNE-ASSOCIATED B CELLS IN RA AND CLINICAL IMPLICATIONS

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Background: Age/Autoimmune-associated B cells (ABCs) are an emerging B cell subset that accumulate in aged and autoimmune-prone mice. Expansion of human ABCs has been observed in patients with autoimmune diseases like SLE and correlated with disease activity. However, it is less known whether ABCs contribute to the pathogenesis of RA.

Objectives: The aim of this work was to explore the role of ABCs in RA.

Methods: 83 RA patients who met the 2010 ACR classification criteria for RA, 42 sex and age matched healthy control (HC), 35 Spondyloarthritis (SpA) and 31 Osteoarthritis (OA) patients were enrolled and blood samples were collected. The proportion of circulating ABCs was detected by flow cytometry and association with clinical and laboratory parameters were analyzed. Expression of characteristic proteins and inflammatory cytokines on ABCs were examined by quantitative real-time PCR.

Results: The proportion of ABCs, defined as CD19⁺CD27⁺IgD⁺CD21⁺CD11c⁺, was significantly elevated in RA patients, compared with HC, SpA and OA patients. The frequency of ABCs was higher in patients with high disease activity (DAS28>3.2) compared with remission and low disease activity (DAS28<3.2). There was a positive correlation of ABCs with SJC, TJC, DAS28 whereas no association with RF and anti-CCP titer were observed. In addition, increased mRNA expression levels of T-bet, IL-21, MAF and IL-17 were noted on ABCs compared with CD19⁺CD27⁺CD11c⁺ B cells.

Conclusion: ABCs were expanded in RA patients and associated with active disease status. It might contribute to RA development by production of IL-17.

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Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2021-eular.3026