Objectives: To investigate the presence and levels of mucosal and circulating IgA1 and IgA2 isotypes of ACPA in patients with RA, and to investigate their association to cigarette smoking habits.

Methods: Patients with established RA, mean disease duration of 12.2 years (n=196), and healthy controls (n=101), included in the Secretary Antibodies in Rheumatoid Arthritis (SARA) study were analysed by enzyme immunoassays regarding total IgA ACPA and the subclasses IgA1 and IgA2 ACPA in serum and saliva. The results are presented as delta-values of optical density (OD) between each IgA ACPA subclass and the corresponding arginine peptide.

Results: Serum IgA1 ACPA was detected in 44% of the RA patients and serum IgA2 in 39%. 10% of the RA patients had detectable salivary IgA1 and/or IgA2 ACPA. Both serum and salivary IgA2 levels were higher among smokers than never-smokers, while this association was not seen for IgA1 class antibodies.

Abstract AB0110 – Table 1. Levels of serum and salivary IgA anti-CCP subclasses among RA patients, comparing ever smokers and never smokers.

Antibody level	Smokers (n=85)	Never smokers (n=93)	T-test (p value)
Serum IgA aCCP, AU/mL (SD)	100 (211)	39 (59)	0.013
Serum IgA1 aCCP, AU/mL (SD)	101 (328)	33 (43)	0.061
Serum IgA2 aCCP, AU/mL (SD)	333 (745)	140 (156)	0.022
Salivary IgA aCCP* (SD)	0.31 (0.62)	0.21 (0.65)	0.271
Salivary IgA1 aCCP* (SD)	0.29 (0.55)	0.21 (0.46)	0.283
Salivary IgA2 aCCP* (SD)	0.24 (0.38)	0.14 (0.17)	0.026

aCCP=Antibodies to Cyclic Citrullinated Peptides, SD=Standard Deviation

*Delta value between Optical Density (OD) value for CCP (Cyclic Citrullinated Peptide) and OD for CAP (Cyclic Arginine Peptide), the corresponding arginine peptide.

Abstract AB0110 – Figure 1. Levels of IgA anti-CCP subclasses in RA patients and controls.

Conclusions: In this study of patients with established RA, IgA2 ACPA but not IgA1 ACPA was associated to cigarette smoking. As IgA2 predominate over IgA1 in mucosal secretions, this finding strengthens the hypothesis that smoking via mucosal ACPA production is one pathway to develop RA.

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Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.4131

AB0111 MEMBRANE TNF EXPRESSION ON MONOCYTES AND DIFFERENTIATION OF MONOCYTES INTO M2-M1 MACROPHAGES: 2 NEW BIOMARKERS OF RHEUMATOID ARTHRITIS

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Background: Three monocyte subsets have been described based on their CD14 and CD16 expression profiles, the subpopulation CD14⁺CD16⁺ being expanded in rheumatoid arthritis (RA) patients. Macrophages contribute *in situ* to the RA pathogenesis. They can display various states of activation or «

polarisation ». Two distinct states of polarisation for macrophages have been recognised: the “classically activated macrophage phenotype” (M1) and the “alternatively activated macrophage phenotype” (M2). To sum-up, M1 are considered to be pro-inflammatory and M2 to be regulatory and anti-inflammatory.

Objectives: Here, we have assessed monocytes subsets and their capacity of differentiation into M2 or M1 macrophages in RA patients and controls.

Methods: PBMCs were isolated and we first determined monocytes subpopulations and looked at expression of membrane TNF (mTNF). After negative selection of monocytes, Macrophages were Derived from Monocytes(MDM) by 7 days of culture in the presence of M-CSF (M2 differentiation) or GM-CSF (M1 differentiation). Expression of total macrophages markers (CD11b and CD71) and the M2 macrophage polarisation markers (CD163 and CD206) were evaluated.

Results: We have confirmed that the CD14⁺CD16⁺ monocytes subset was expanded in RA patients. For the first time, we have demonstrated that mTNF expression was significantly increased only in monocytes in RA patients (CD14⁺: Mean 5.6% HD versus 10.6% RA, CD14⁺CD16⁺: Mean 3.3% HD versus 11.1% RA, CD16⁺: Mean 2.4% HD versus 6.3% RA). Moreover, mTNF expression on monocytes correlated with the activity of the disease assessed by DAS28 CRP (Spearman r 0.548 and p=0.0021*). We have observed a significant decrease of macrophages induction by M-CSF in RA patients as shown by a decreased expression of CD11b-CD71 (Mean 87.2% HD versus 57.1% RA, P value ****). Among MDM, we have found a specific decreased level of M2 markers (CD206 Mean 78.1% HD versus 27% RA and CD163 56.2% HD versus 37.2% RA) suggesting an impaired maturation to M2 stage in RA patients.

Conclusions: Monocytes from RA patients have an increased expression of mTNF linked to activity of the diseases. RA patients have an impaired maturation of monocytes to M2 macrophages. This might suggest that RA monocytes have a propensity for preferential maturation towards a pro-inflammatory M1 phenotype thus contributing to synovial inflammation. Deeper characterisation of M1/M2 and effect of the different types of anti-TNF on this differentiation process are on-going and will be presented.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.7142

AB0112 PATHOGENETIC MECHANISMS IN EARLY RHEUMATOID ARTHRITIS: POSSIBLE CORRELATION BETWEEN TH17 AND TREG CELLS AND GUT MICROBIOTA STRUCTURE: A PILOT STUDY

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Background: In Rheumatoid Arthritis (RA) pathogenesis T helper17 (Th17) and T regulatory cells (Treg) are largely represented.¹ Recent studies highlighted the role of intestinal mucosa environment in modulation of T cells function. The composition of gut microbiota influences the Th17/Treg cells balance and the host immune response,² so the exposure to deranged intestinal microbiota may be crucial in RA.

Objectives: The aim of the study was to compare Th17 and Treg cells and gut microbiota composition in patients with early RA (ERA) and in a control group (CG) at baseline and after treatment.

Methods: Currently, 10 ERA patients and 10 subjects belonging to the CG have been enrolled. All ERA patients were evaluated before (T0) and after 3 months (T1) of treatment with methotrexate (MTX) and glucocorticosteroids (GCS). Blood and faecal samples were collected. After PBMC isolation, staining with conjugated mAbs targeting specific surface and intracellular antigens (CD4 and CD25, IL-17 and FoxP3 respectively) have been used in order to distinguish Th17 and Treg cells. The composition of the faecal microbiota has been analysed by Next Generation Sequences on Illumina MiSeq platform, through 16S rDNA V3-V4 targeted sequencing.

Results: At T0, the percentage of Th17 cells was higher in patients than in the CG (p<0.0001) while Treg cells were higher in the CG (p=0.013). At T1, the total number of CD4⁺ and Th17 cells was decreased (p=0.007, p=0.027) while the frequency of Treg cells increased (p=0.028). A normalisation of Treg cells, with frequencies comparable to CG, was present after treatment. Regarding gut microbiota, at phylum level no difference between patients at T0 and the CG were present but we observed a tendency to decrease in the frequency of Actinobacteria after therapy. Furthermore, the relative abundance of Actinobacteria correlated positively with the circulating levels of Th17 (p=0.012, r=0.59) and with the Th17/Treg at T0 (p=0.010, r=0.6), while Nitrospirae correlated positively with Treg (p=0.028, r=0.68) at T1. A significant increase of the relative abundance in the Lachnospiraceae family in patients at T1 compared with T0 (p=0.042) and CG (p=0.043) were noticed.

Conclusions: Our results highlight the presence of an imbalance between Th17 and Treg cells in patients with ERA. In agreement with literature,³ MTX and GCS

influence on Th17 decreasing and Treg cells increasing in patients with ERA, so we can hypothesise that part of the clinical response is own to the improvement in T cells balance. Previous data⁴ reported that Actinobacteria are strongly correlated with the production of IL-17 and a reduction of Nitrospirae has been associated to increased inflammatory responses and to gut permeability in mice.⁵ Lachnospiraceae family play an important role in the maintenance of intestinal homeostasis.⁶ The correlation between gut microbiota composition and Th17/Treg axis observed in our patients may suggest the involvement of some bacteria family in Th17/Treg cells balance in the lamina propria of RA patients treated with MTX, even in the early phases of the disease.

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Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.6654

AB0113

EFFICACY OF TREATMENT WITH PROBIOTICS IN THE INFLAMMATORY ACTIVITY OF PATIENTS WITH RHEUMATOID ARTHRITIS. SYSTEMATIC REVIEW OF THE LITERATURE

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Objectives: To study the effectiveness of the use of probiotics in the control of inflammatory activity of patients with rheumatoid arthritis and analyse its effect on their metabolic profile.

Methods: A bibliographic search was carried out in Medline and Embase. The search strategy included the terms MeSH and the free text of "lactobacillus", "bacillus", "probiotics" and "rheumatoid arthritis." The search strategies were carried out by two authors, which were included according to the type of studies: meta-analysis, systematic reviews and clinical trials, depending on the type of participant: adults with RA who have received probiotics, the main outcome measures: changes in the Disease Activity Score (DAS28), Simplified Disease Activity Index (SDAI) and Clinical Disease Activity Index (CDAI), as well as each of the parameters that constitute them: C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), visual analogue scale of the doctor and the patient (EVA), number of painful joints (NAD) and inflamed (NAI) and functional status by Health Assessment Questionnaire (HAQ) Secondary variables: number of adverse events and parameters of metabolic activity. The quality of the evidence was analysed following the guidelines of the Scottish Network of Intercollegiate Guidelines (SIGN).

Results: After the selection of 34 articles, 9 articles were finally included. All were randomised, double-blind, placebo-controlled clinical trials (RCTs) with a level of evidence between 1+ and 1++ and a recommendation grade of A and B. Seven CDs showed improvement in arthritis measurements. In Peltonen et al. observed a high rate of improvement in the experimental group than in the control group (3.1 vs 2, p=0.027). Mandel et al. they described improvement of the EVA in the experimental group (p=0.046). Zamani et al. they described an improvement in DAS28 (-0.3±0.4 versus -0.1±0.4, p=0.01). Vaghef-Mehrabany et al defined this improvement (p<0.01). Pineda et al. showed an improvement in HAQ at 3 months in the experimental group (from 0.97 to 0.80, p=0.02), although not in ACR20 (p=0.33). Allipour et al. found improvements in CRP between the two groups (mean [95% CI]=2.03 [0.54–3.51], p=0.009); NAD: (mean [95% CI]=0.72 [0.25, 1.19], p=0.003); NAI: (mean [95% CI]=0.351 [0.13, 0.58], p=0.003); EVA: (mean [95% CI]=16.71 [8.91, 24.50] p<0.001; DAS-28: (average [CI] 95%)=0.31 [0.02, 0.61], p=0.039) and in cytokine levels, Hatakka et al observed no significant improvement in the experimental group in HAQ, NAD and NAI, and Nenonen et al. Did not observe differences in DAS28. In the last, EC of Vaghef-Mehrabany of 2017 metabolic measures were evaluated without finding significant improvements If an improvement in insulin resistance was observed as measured by the HOMA B index in the study by Zamani et al.

Conclusions: Treatment with probiotics seems to be effective in controlling the inflammatory activity of rheumatoid arthritis.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.4676

AB0114

MODULATION OF ENDOTHELIAL FUNCTION BY PROINFLAMMATORY CYTOKINES INVOLVED IN RHEUMATOID ARTHRITIS. FOCUS ON IL-17A, IL-20, IL-23 AND IL-9

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Background: Rheumatoid Arthritis (RA) is the most common inflammatory rheumatic disease, characterised by high circulating of pro-inflammatory cytokines. RA is associated with an increased cardiovascular risk secondary to the accelerated atherogenesis which is the consequence of endothelial dysfunction (ED)¹. In addition to the well-known cytokines (TNF- α , IL-1 β and IL-6), emerging data identified new cytokines such as IL-17A, IL-20, IL-23 and IL-9 as putative key-players of the pathogenesis of RA. To date, whether these cytokines might contribute to RA-associated endothelial dysfunction is not known.

Objectives: This study investigated the effect of IL-17A, IL-20, IL-23 and IL-9 on endothelium-dependent relaxation in response to acetylcholine (ACh) in rat aortic rings.

Methods: Experiments were conducted on thoracic aortic rings from male Lewis rats (11 week old), incubated for 1 hour or 24 hour at 37°C with 2 concentrations of each cytokine (IL-17A: 250 pg/ml and 10 ng/ml; IL-20: 500 pg/ml and 5 ng/ml; IL-23: 80 pg/ml and 10 ng/ml; and IL-9: 300 pg/ml and 10 ng/ml). Incubation with 10 ng/ml TNF- α was used as a positive control and with vehicle as negative control. At the end of the incubation period, endothelial function was studied by assessing concentration-response curves to ACh (10⁻¹¹–10⁻⁴ mol/L) after phenylephrine (PE, 10⁻⁵ mol/L) or KCl (30 mmol/L)-induced contractions.

Results: As described in the literature², a 24h- but not 1h-incubation with TNF- α reduced ACh-induced relaxation. The same result was obtained with IL-17A (10 ng/mL). By contrast, IL-20 did not change ACh-induced relaxation whatever the concentration and the incubation time. Impairment in vascular relaxation was observed after exposure to IL-9 (10 ng/mL), both after 1h- and more severely after 24h-incubation. As regards IL-23, an effect was observed only after 1 hour incubation and with high concentration.

Conclusions: Our data demonstrated that IL-17A, IL-23 and IL-9 but not IL-20 induced endothelial dysfunction, with different kinetics profiles. Among the cytokines evaluated, IL-9 exhibited the most important effect thus revealing a new putative role of this pleiotropic cytokine in RA-associated cardiovascular risk. Further studies are needed to confirm these data on animal models of diseases.

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Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.3099

AB0115

PROPHYLACTIC AND THERAPEUTIC ACTIVITY OF ALKALINE PHOSPHATASE IN ARTHRITIC RATS: SINGLE AGENT ACTIVITY AND IN COMBINATION WITH METHOTREXATE

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Background: Alkaline phosphatase (AP) is a gate-keeper of innate immune system responses by detoxifying (dephosphorylating) inflammation triggering moieties (ITMs) released from endogenous and external sources¹ and maintaining physiological barriers.

Objectives: We examined whether AP's broad mechanism of action may serve as a safe therapeutic, either as single agent or combined with methotrexate (MTX), in rheumatoid arthritis (RA).