**Scientific Abstracts**

**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 Immunossays 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.

**Conclusions:** 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.

**REFERENCES:**


**Disclosure of Interest:** None declared


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**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 Th17 related 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=0.001) while Treg cells were higher in the CG (p=0.019). 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 observed but we presented 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) was 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 owed to the improvement in T cells balance. Previous data reported that Actinobacteria are strongly correlated with the production of IL-17 and a reduction of Nitrosipepe has been associated to increased inflammatory responses and to gut permeability in mice. Lachnospiraceae family play an important role in the maintenance of intestinal homeostasis. 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


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 inflammatory activity of patients with rheumatoid arthritis and analyse its effect on 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 (10–14–10–4 mol/L) after phe- nylephrine (PE, 10–5 mol/L) or KCl (30 mmol/L) -induced contractions.

Results: As described in the literature, 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 cyto- kines 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


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 mo- lecules (ITMs) released from endogenous and external sources and maintaining physiological bariers.

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).

Disclosure of Interest: None declared


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 rheu- matic disease, characterised by high circulating of pro-inflammatory cytokines. RA is associated with an increased cardiovascular risk secondary to the acceler- ated atherogenesis which is the consequence of endothelial dysfunction (ED) 1. In addition to the well-known cytokines (TNF-α, IL-1β) and IL-6, emerging data iden- tiﬁed new cytokines such as IL-17A, 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 (10–14–10–4 mol/L) after phe- nylephrine (PE, 10–5 mol/L) or KCl (30 mmol/L) -induced contractions.

Results: As described in the literature, 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 cyto- kines 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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