IgG autoantibody levels ($P < 0.05$) towards another antigen, dual specificity mitogen-activated protein kinase kinase 6 (MAP2K6), were also observed in ACPA-seronegative subjects compared to ACPA-seropositive and controls. In contrast, we found significantly higher IgG autoantibody levels ($P < 0.05$) in ACPA-seropositive individuals compared to ACPA-seronegative and controls towards two antigens, anosmin-1 (ANOS-1) and muscle related coiled-coil protein (MURC). ANOS-1 shows also significantly higher IgG reactivity frequency in ACPA-seropositive individuals compared to ACPA-seronegative and controls (22%, 9% and 6% respectively; $P < 0.05$). Interestingly, three out of the four antigens discovered to be associated with the ACPA status in early RA are highly expressed in lungs and heart, two of the main extraarticular sites affected in RA. No significant differences were observed at IgA levels for any of the antigens analyzed.

Table 1. Scheme of the different phases of the study, the features within each phase and the results. The reactivity to four antigens allows to distinguish ACPA-seronegative (ACPA-), ACPA seropositive (ACPA+) and controls.

Phases	Untargeted discovery	Targeted discovery	Targeted validation
Number of samples	80 ACPA- 80 ACPA+	80 ACPA- 80 ACPA+	358 ACPA-372 Controls 386 ACPA+
Antigen array platform	Planar arrays	Suspension bead array 1	Suspension bead array 2
Number of antigens	2660	62	27
Number of candidate biomarkers	62	27	4 (TSPYL4, MAP2K6, ANOS1, MURC)

Conclusion: Upon further validation in other early RA sample cohorts, our data suggest the measurement of these four autoantibodies may be useful for the early diagnosis of ACPA-seronegative RA and give insight into the pathogenesis of the different RA subsets.

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POS0393

FIBRIN DEPOSITION IS AN ACTIVE TRIGGER OF CARTILAGE DEGENERATION IN RHEUMATOID ARTHRITIS

T. Hügler¹, S. Nasi¹, D. Ehrichou¹, P. Omoumi², A. So¹, N. Busso¹. ¹Lausanne University Hospital, CHUV, Department of Rheumatology, Lausanne, Switzerland; ²Lausanne University Hospital, CHUV, Radiology, Lausanne, Switzerland

Background: Fibrin(ogen) maintains inflammation in various disorders but has never been linked to cartilage damage in rheumatoid arthritis (RA) or other forms of inflammatory arthritis.

Objectives: To investigate the role of fibrin deposition on cartilage integrity in arthritis.

Methods: Fibrin deposition on knee cartilage was analyzed by immunohistochemistry in RA patients and in murine adjuvant-induced arthritis (AIA). In chondrocytes, fibrinogen expression (*Fga*, *Fgb*, *Fg*) and procoagulant activity were evaluated by qRT-PCR and turbidimetry respectively. Fibrin-induced catabolic genes were assessed by qRT-PCR in chondrocytes. Fibrin-mediated chondro-synovial adhesion (CSA) with subsequent cartilage tears was studied in co-cultures of human RA cartilage with autologous synovial cells, in the AIA model, and by MRI. The link between fibrin and calcification was examined in human RA cartilage stained for calcific deposits and *in vitro* in fibrinogen-stimulated chondrocytes.

Results: Fibrin deposition on cartilage correlated with the severity of cartilage damage in human RA explants and in AIA wildtype (WT) mice, while fibrinogen deficient (*Fg*^{-/-}) mice were protected. Accordingly, fibrin upregulated catabolic enzymes (*Adamts5* and *Mmp13*) in chondrocytes. Secondly, CSA was present in fibrin-rich and damaged cartilage in AIA WT but not in *Fg*^{-/-} mice. In line, autologous human synovial cells, cultured on RA cartilage explants, adhered exclusively to fibrin-positive degraded areas. Gadolinium-enhanced MRI of human joints showed contrast-enhancement along cartilage surface in RA patients but not in controls. Finally, fibrin co-localized with calcification in human RA cartilage and triggered chondrocyte mineralization inducing pro-calcification genes (*Anx5*, *Pit1*, *Pc1*) and cytokine (IL-6). Although at a much lesser extent, we observed similar fibrin-mediated mechanisms in osteoarthritis (OA).

Conclusion: Fibrin deposition directly impacts on cartilage integrity via induction of catabolism, mechanical stress, and calcification. Potentially, fibrin is a key factor of cartilage damage occurring in RA as a secondary consequence of inflammation.

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POS0394

NAD+ BOOSTERS REESTABLISH THE ALTERED NAD+ METABOLISM OF LEUKOCYTES FROM RHEUMATOID ARTHRITIS PATIENTS IMPROVING THEIR OXIDATIVE, APOPTOTIC AND INFLAMMATORY STATUS

C. Perez-Sanchez¹, L. M. Sánchez-Mendoza², M. D. C. Abalos-Aguilera¹, N. Barbarroja Puerto¹, M. Luque-Tévar¹, A. M. Patiño-Trives¹, I. Arias de la Rosa¹, J. A. Moreno², M. I. Burón², J. A. González-Reyes², E. Collantes Estevez¹, A. Escudero Contreras³, C. Lopez-Pedraza¹, J. M. Villalba². ¹IMIBIC, Rheumatology Service, IMIBIC/Reina Sofia Hospital/University of Cordoba, Cordoba, Spain, Córdoba, Spain; ²IMIBIC, Department of Cell Biology, Physiology and Immunology, University of Cordoba, Campus de Excelencia Internacional Agroalimentario, ceiA3, Córdoba, Spain, Córdoba, Spain; ³IMIBIC, Rheumatology Service, IMIBIC/Reina Sofia Hospital/University of Cordoba, Cordoba, Spain, Cordoba, Spain

Background: NAD⁺ is an important cofactor and second messenger for multiple cellular processes that exhibits antioxidant, anti-apoptotic and anti-inflammatory properties. Pre-clinical studies in animal models of Rheumatoid Arthritis (RA) have demonstrated the therapeutic potential of NAD⁺ boosters in the control of the disease activity. However, to date no studies have been set up to evaluate the NAD⁺ metabolism and the therapeutic effects of NAD⁺ boosters in RA patients.

Objectives: 1- To study the NAD⁺ metabolism in RA patients and its association with key clinical features. 2- To evaluate the effect of anti-TNF therapy in the NAD⁺ metabolism.

3- To analyze the beneficial effects of NAD⁺ boosters in leukocytes from active RA patients.

Methods: Plasma and PBMCs were purified from 100 RA patients and 50 healthy donors (HDs). Moreover, an additional cohort of 50 RA patients treated with Anti-TNF therapy was analyzed before and after 6 months of treatment. NAD⁺ levels were determined by using the NAD/NADH-Glo Assay. NAD⁺-consuming genes expression were analyzed by RT-PCR. In parallel, PBMCs from eight HDs and eight active RA patients were treated *ex vivo* with 1 mM of NAD⁺ boosters including nicotinamide (NAM), nicotinamide riboside (NR), and nicotinamide mononucleotide (NMN). After 24 hours, intracellular reactive oxygen species (ROS) levels (DFCHDA) and the percentage of apoptotic PBMCs (annexin V/PI) were assessed by flow cytometry. Lastly, a panel of key pro-inflammatory genes were evaluated by RT-PCR.

Results: NAD⁺ and NADH levels were significantly reduced in plasma and PBMCs of RA patients compared with HDs and directly related to disease activity (DAS28, CDAI, SDAI). Accordingly, the expression levels of genes involved in the consumption of NAD⁺ such as SIRT-1, CD38 and PARP-1 were found up-regulated in PBMCs from RA patients. Anti-TNF therapy for 6 months restored the altered NAD⁺ levels towards those showed by HDs. Furthermore, the clinical response promoted by Anti-TNF therapy (changes in DAS28) correlated with changes in NAD⁺ levels. The *in vitro* treatments of PBMCs isolated from active RA patients with NAD⁺ boosters significantly increased the NAD⁺ levels and promoted a deep reduction of intracellular ROS levels, the percentage of apoptotic cells and the expression levels of key inflammatory mediators, such as IL-6, IL-8, IL-1β, TNF-α, CCL2, IL-23, and STAT-3.

Conclusion: 1. NAD⁺ metabolism is altered and associated with the disease activity of RA patients, involving both, reduced NAD⁺ levels and increased expression of NAD⁺-consuming genes. 2. Anti-TNF therapy restored NAD⁺ levels, which were directly linked to the clinical effectiveness. 3. NAD⁺ boosters reduced the oxidative, apoptotic and inflammatory profiles of RA leukocytes through the parallel increase of intracellular NAD⁺ levels. Thus, NAD⁺ boosters might be considered novel therapeutic tools for RA patients.

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POS0395

ANTI-ACETYLATED PROTEIN ANTIBODIES IN RHEUMATOID ARTHRITIS (RA): CLUES FOR THE STARTING POINT OF AUTOANTIBODY RESPONSES IN RA

T. J. van Wesemael¹, A. L. Dorjée¹, T. Huizinga¹, A. van der Helm - van Mil¹, R. Toes¹, D. van der Woude¹. ¹Leiden University Medical Center, Rheumatology, Leiden, Netherlands

Background: Rheumatoid arthritis (RA) is characterized by autoantibodies such as rheumatoid factor (RF) and anti-modified protein autoantibodies (AMPAs) like anti-citrullinated protein antibodies (ACPA) and anti-carbamylated protein

antibodies (anti-CarP). Recently, another AMPA: anti-acetylated protein antibodies (AAPA) have been found in RA patients [1]. The prevalence of AAPA antibodies and their isotypes have yet to be determined. Since isotype profiles reflect the breadth of an immune response, the prevalence of AAPA isotypes in arthritis patients with and without RA can help to understand the relevance of this autoantibody response in RA.

Objectives: To describe the prevalence of AAPA isotypes in arthritis patients with and without RA.

Methods: In 650 RA patients fulfilling the 1987 RA criteria and 555 non-RA arthritis patients from the Leiden Early Arthritis Cohort, baseline serum samples were screened by ELISA for IgG, IgM and IgA to an acetylated- and control peptide that was based upon the CCP-2 backbone. The cutoff for positivity was based on 80 controls (mean + 2SD). A sample was considered positive if it was above the cutoff and was 0.1 optical density higher on the acetylated peptide than on the control peptide.

Results: AAPA IgG was found in 36% of RA patients versus 6.7% of non-RA arthritis patients (figure 1a). Within RA patients, AAPA IgG antibodies were mostly present in the ACPA-(CCP-2) positive group (64% in ACPA-positive, compared to 5% in ACPA-negative). Levels of AAPA IgG and IgA were higher in RA patients than in healthy controls and non-RA arthritis patients (figure 1b), however, surprisingly, no difference in levels was found for IgM.

When isotype profiles in AAPA- positive arthritis patients were compared, patients

Table 1. Anti-acetylated protein antibody (AAPA) isotype overlap in AAPA positive patients.

AAPA isotype	RA patients (n=310) n (%)	Non-RA arthritis patients (n=106) n (%)
IgG+IgM-IgA-	115 (37.1)	28 (5.1)
IgG-IgM+IgA-	52 (16.8)	48 (8.7)
IgG-IgM-IgA+	14 (4.5)	13 (2.3)
IgG+IgM+IgA-	24 (7.7)	3 (0.5)
IgG+IgM-IgA+	37 (11.9)	4 (0.7)
IgG-IgM+IgA+	9 (2.9)	8 (1.4)
IgG+IgM+IgA+	59 (19.0)	2 (0.4)

AAPA: anti-acetylated protein antibodies, RA: rheumatoid arthritis

with RA were more often positive for two or more isotypes than patients without RA, and thus displayed considerably more overlap in AAPA isotypes compared to non-RA patients (table 1). Intriguingly, IgM AAPA was the most prevalent isotype in non-RA patients, versus IgG in RA patients.

Conclusion: AAPA are detected in one third of RA patients, and mainly in the ACPA-positive subgroup. The predominance of IgM AAPA in non-RA arthritis patients and healthy controls suggests that healthy persons can develop AAPA IgM without the development of RA. These results also suggest that in healthy individuals, AAPA responses can occur, but do not mature past the IgM-stage, while in RA patients, the AAPA-response does mature and might form a "starting point" for development of other AMPA leading to the concurrent present of several AMPA in disease.

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Figure 1. Prevalence and levels of anti-acetylated protein antibodies (AAPA) in rheumatoid arthritis (RA) and non-RA arthritis patients.

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POS0396

THE LEVEL OF PERIPHERAL REGULATORY T CELLS IS ASSOCIATED WITH THE CHANGES OF INTESTINAL MICROBIOTA IN PATIENTS WITH RHEUMATOID ARTHRITIS

B. Wu¹, J. An¹, T. Ding¹, H. Xue¹, X. F. Li¹, C. Wang¹. ¹The Second Hospital of Shanxi Medical University, The Department of Rheumatology, Taiyuan, China

Background: Rheumatoid arthritis (RA) is a systemic autoimmunity inflammation disease characterized with chronic aggressive arthritis and the presence

of abnormal antibodies. Several observations showed that the breakdown of immune tolerance caused by many complex interactions was involved in the development of RA^[1]. However, the pathogenesis of RA remained unclear. It has been confirmed that the imbalance of Th17 and Treg cells play a crucial role in destroying immune tolerance^[2]. Besides, researches showed that intestinal microbiota can influence host immunity by acting on the immune cells to play pro-inflammatory or anti-inflammatory effect, and in turn immune system can also regulate the microbiota^[3, 4]. Thus, a frontier point of view in the field of rheumatism, immune microecology, was proposed, which is a novel concept for the breakdown of immune tolerance. Studies have confirmed that there was an imbalance of intestinal microbiota in patients with RA^[4]. But the relationship between the CD4+T subsets cells and intestinal microbiota in RA is unknown.

Objectives: We detected and compared the absolute number of CD4+T cells subsets in the peripheral blood and the proportion or abundance of intestinal microbiota in patients with RA and healthy adults, and then analyzed the relationship between them to explore the role of CD4+T cells subsets and intestinal microbiota in the pathogenesis of RA.

Methods: We collected the sample of stool and blood from 15 patients with RA hospitalized at the Second Hospital of Shanxi Medical University and 8 age and gender-matched healthy controls(HC). The absolute number of CD4+T cells subsets including Th1, Th2, Th17 and Treg cells were detected by flow cytometry. The 16S rRNA in the stool specimens were sequenced by the Roche/45 high-throughput sequencing platform. We analyzed whether there was correlation between CD4+T subsets cells and intestinal microbiota.

Results: Patients with RA had a higher level of *Christensenellaceae* and a lower level of *Pseudomonadaceae* as compared with those of HCs at the family level (p<0.05). And at the genus level, the patients with RA had higher levels of *Ruminococcus torques*, *Christensenellaceae R-7*, *Ruminiclostridium 9* and *Ruminococcus 1* compared with those of HCs (p<0.05) (Figure 1). And the *Ruminococcus torques* at the genus level was negative correlated with the absolute number of Treg cells (p<0.001) (Figure 2).

Conclusion: The results here suggested that there were different proportion or abundance of intestinal microbiota between the patients with RA and HCs. And the changes of intestinal microbiota such as *Ruminococcus torques* were associated with Treg cells, further indicating that the imbalance of intestinal microbiota in RA can destroy the immune tolerance. The above results uncovered that the intestinal microbiota had immunomodulatory function, which may be the upstream mechanism participated in the pathogenesis of RA.

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Figure 1. At the family level (a-b) and the genus level(c-f), the relative abundance of intestinal microbiota in patients with RA and HCs were different. Data were expressed as median (Q1, Q3) and analyzed by Wilcoxon test. (***) P < 0.001, ** P < 0.01 and * P < 0.05).