antibodies were detected in 41% (n=32) of patients with RA but not in SLE (n=16) and control sera (n=15). In a RA follow-up study (n=30), we detected nearly similar antibody-sensitivities for citrullinated mPPAD before and after onset of RA (13/20%). Only a minority (7%) of RA patients show higher mPPAD antibody levels after RA diagnosis. In the CAIA RA mouse model mPPAD containing Pg. vesicles when injected IP showed a TLR2-dependent protective anti-inflammatory effect like Pg. LPS and Lipomannan.

Conclusions Pg. infection and RA disease diagnosis occurs at different time points and Pg. infection induces a TLR2-dependent protective anti-inflammatory effect. We show the first time that mPPAD can citrullinate major human autoantigens internally and their immunologically and diagnostic relevance in RA.

07.16 NLRP1 MUTATIONS CAUSE AUTOINFLAMMATORY DISEASES IN HUMAN: IMPLICATION OF THE NLRP1 INFLAMMASOME?

1,2Sylvie Grandemange, 2Élodie Sanchez, 1,2Cécile Rittore, 4Yasamine El Ahmadi, 2Pascale Louis-Pence, 2John C Reed, 1Florence Apparailly, 1,2,4Isabelle Touitou, 2,3Davide Genevès. 1Laboratoire des maladies rares et auto-inflammatoires, Hôpital Arnaud de Villeneuve, CHRU Montpellier, France; 2INSERM U1183, Institute of regenerative medicine and biotherapy, Montpellier, France; 3Département de Généétique médicale, Hôpital Arnaud de Villeneuve, CHRU Montpellier, France; 4University of Montpellier, France; 5Sanford-Burnham Medical Research Institute, La Jolla, CA, USA

Background Inflammation is a vital and complex process in response to diverse tissue damaging stimuli such as trauma, injury and pathogen. NLRP1, NLRP3 and NLRC4 belonging to the intracellular proteins Nod like receptor family, are capable of sensing the inflammatory inducers and trigger the assembly of a large complex called the inflammasome. By inducing the caspase-1 activation, inflammasome plays a crucial role in the release of IL-1β and IL-18, two critical cytokines of the initial steps of inflammatory responses.

Whereas mutations in NLRP3 and NLRC4 have been linked to two rare monogenic systemic autoinflammatory diseases (SAIDs), several polymorphisms in the NLRP1 gene have been associated extensively to an increased risk of autoimmune disorders (e.g. vitiligo, psoriasis, type 1 diabetes, and rheumatoid arthritis). We identified for the first time two distinct NLRP1 mutations in patients displaying a novel SAID combining autoinflammation and autoimmunity. We named this disease NAIAD, for NLRP1-associated autoinflammation arthritis and dyskeratosis.

The aim of our study was to unravel how mutation in NLRP1 impaired its function and triggered autoinflammation. Materials and methods Peripheral blood mononuclear cells from patients were analysed to identify the immunologic components involved in these novel diseases, using flow cytometry. The pathogenic effect of the NLRP1 mutations in inflammation was investigated using in vitro functional assays in transfected HEK293T.

Results The level of caspase-1, IL-18 and IL-1β in serum samples from patients was increased as compared to controls and asymptomatic parents. Moreover, patient’s cells displayed constitutive production of IL-1β. Functional studies in HEK293T revealed that the NLRP1 mutations resulted in a constitutive activation of the NLRP1 inflammasome.

Conclusions We demonstrated that two mutations in the NLRP1 gene are involved in autoinflammation in human. This novel disease could be a novel inflammasomopathy combining autoinflammatory and autoimmune features. Our data, combined with that in the literature, highlight the pleomorphic role of NLRP1 in inflammation and immunity.

07.17 THE MUTATED RNA SPlicing PROTEIN HNRNP-A3 IS A NOVEL AUTOANTIGEN IN SYSTEMIC RHEUMATIC DISEASES A LINK TO WARBURG EFFECT IN RA

1Bianca Marklein, 1Kerstin Adolph, 1Veit Krenn, 2,3,4Günter Steiner, 4Monika Hansson, 3Johan Rönnelid, 1Gerd R Burmester, 1Karl Skriner. 1Charité University Medicine Berlin, Department of Rheumatology and Clinical Immunology, Humboldt University and Free University, Berlin, Germany; 2Division of Rheumatology, Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria; 3Second Department of Medicine, Heitzing Hospital, Vienna, Austria; 4Institute of Medical Biochemistry, Medical University of Vienna, Vienna, Austria; 5Ludwig Boltzmann Institute of Rheumatology and Balneology, Vienna, Austria; 6Rheumatology Unit, Department of Medicine, Karolinska Institute, Stockholm, Sweden

Objective Novel mutated hnRNP-A3 (MA3) was cloned out of RA synovial tissue involved in alternative splicing of PK2 linking it directly to Warburg effect and lactate production in RA.

Methods After immunoblotting and 2D-gel-eletrophoresis out of a semipurified hnRNP fraction two protein spots were sequenced and identified to be highly similar to hnRN-A3. hnRN-A3 variants were cloned from RA synovial-tissue. 3700 RA sera were screened for the presence of mutated anti-hnRN-A3 autoantibodies using recombinant proteins and mutated citrullinated A3 peptides (MCA3) thereof. Identification of RNA and antibody binding sites to hnRN-A3 (MCA3). Expression of hnRN-A3 in synovial tissue was analysed by immunohistochemistry.

Results Autoantibodies to MA3 protein were detected in 13% of RA (n=215) patients, in 9% SLE (n=154), in 27% of MCTD patients (n=44/10) and in less than 5% of 129 patients with other rheumatic disorders but not at all in healthy controls on immunoblot. When using native MA3-ELISA 22% of early RA patients (n=130) were detected and 87% of these patients had erosive arthritis. Identical modification on MA3 as in cancer cells were identified in synovial tissue and verified by MS and DNA sequencing. Using 2–3 citrullinated MCA3 peptides up to 81% of patients (n=150) with established and 67% (n=2926) of patients with an early RA with a specificity of 97% were detected. In early RA 27% and 25% in established RA of CCP2 negative and 93% of CCP2 positive patients were identified.

By combining with the already established CCP2 and the new MCA3, 72% of early patients are positive. MCA3 autoantibodies predominantly occur (p<0.001) in an erosive, severe course of disease. MRL Lpr/lpr sera were hnRN-A3 reactive and the antibody generation is Toll 7 and 9 dependent. Anti-hnRN-A3-antibodies are directed to conformational RNA binding epitopes. Expression of hnRN-A3 revealed the antigen is overexpressed in RA synovial tissue.

Conclusion Mutated hnRN-A3 is as a novel Toll7/9 dependent autoantigen in systemic rheumatic diseases. These mutated proteins are components of RNA and DNA containing alternative splicing complexes leading to the Warburg effect and predominantly occurring in an erosive and severe courses of RA.
HEAT SHOCK PROTEIN 90 IS INCREASED IN MUSCLE TISSUE AND PLASMA IN IDIOPATHIC INFLAMMATORY MYOPATHIES

Hana Storkanova, Olga Krystufkova, Martin Klein, Herman Mann, Lucia Vernerova, Maja Spiritovic, Josef Zamecnik, Ladislav Senolt, Jiri Vencovsky, Michal Tomcik, Institute of Rheumatology, Department of Rheumatology, 1st Faculty of Medicine; Faculty of Physical Education and Sport, Department of Physiotherapy; Department of Pathology and Molecular Medicine, 2nd Medical School and University Hospital Motol, Charles University, Prague, Czech Republic.

Background Heat shock proteins (Hsps) are chaperones playing important roles in skeletal muscle physiology, adaptation to exercise or stress, and activation of inflammatory cells.

The aim of our study was to assess Hsp90 expression in muscle biopsies and plasma of patients with idiopathic inflammatory myopathies (IIM) and to characterise its association with IIM-related features.

Methods Total of 277 patients with IIM (198 females, 79 males; mean age 54.8; disease duration 4.1 years; dermatomyositis (DM), 104; polymyositis (PM), 104; cancer associated myositis (CAM), 42; necrotizing myopathy (IMNM), 27) and 100 age-/sex-matched healthy individuals were included in plasma analysis and 50 muscle biopsy samples were stained for Hsp90 (in PM, DM, IMNM, myodystrophy, myasthenia gravis, 10 each). Plasma Hsp90 was measured by ELISA (eBio-science, Vienna). CK, LD, ALT, AST and CRP were analysed by routine techniques and IIM-specific autoantibodies by in-line blot/immunoprecipitation. Data are presented as median.

Results In muscle biopsies Hsp90 expression was higher in IIM than in myodystrophy (myasthenia gravis used as another control was negative). Increased Hsp90 was detected in perifascicular degenerating and/or regenerating fibres, inflammatory cells (DM, PM), and necrotic and regenerating fibres (IMNM). Plasma Hsp90 levels were increased in IIM patients compared to healthy controls (20.2 vs. 9.2 ng/ml, p<0.0001). Hsp90 levels in all patients positively correlated with LD (r=0.551, p<0.0001) and AST (r=0.372, p<0.0001). Increased Hsp90 was associated with decreased MMT8 values (r=−0.136, p=0.029), in particular in proximal muscles. Hsp90 positively correlated with patient and doctor disease activity (r=0.222, p=0.0004; r=0.217, p=0.0005, respectively), pulmonary (r=0.222, p=0.0004) and muscle disease activity (r=0.146, p=0.018), MITAX (r=0.175, p=0.005) and MYOACT (r=0.159, p=0.012), and with MDI extent/severity (r=0.215, p=0.003; r=0.120, p=0.041, respectively). Higher Hsp90 was found in patients with interstitial lung disease, cardiac involvement and dysphagia (25.4 vs. 18.9, p=0.004; 27.5 vs. 19.3, p=0.004; 25.0 vs. 18.2 ng/ml, p=0.018, respectively).

Conclusions We demonstrate increased Hsp90 expression in IIM muscle biopsy samples, specifically in inflammatory cells, degenerating, regenerating and/or necrotic fibres. Increased Hsp90 plasma levels in IIM patients are associated with disease activity and damage, and with the involvement of proximal skeletal muscles, heart and lungs.

Acknowledgement Supported by AZV-16–33542A.

08.02 ALPHA-ENOLASE PROMOTES PRO-INFLAMMATORY PHENOTYPE OF MONOCYTES-DERIVED MACROPHAGES IN VITRO

Manuel Freiet, Pascal Rottenberg, Sébastien Calbo, Thierry Lequerre, Olivier Vittecon, INSERM, U905 and IRIS, Rouen, France; Rouen University Hospital, Department of Rheumatology, Rouen, France; Contributed equally to this work.

Background Rheumatoid arthritis (RA) is the most common chronic inflammatory rheumatism. RA is multifactorial involving genetic, environmental, endocrine, psychological and immunological factors. In 2002, our research team has discovered alpha-enolase (ENO1) as an autoantigen in RA and has recently demonstrated its effect on monocytes, inducing inflammation mediated through CD14-dependent TLR4 signaling pathway. Monocytes can differentiate into dendritic cells, osteoclasts or macrophages. Macrophages are involved in RA pathophysiology and can be polarised in different phenotypic profiles, pro-inflammatory (M1 macrophages) or immuno-regulatory (M2 macrophages). The main objective of this study was to determine the effect of ENO1 on monocytes differentiation into macrophages and on their polarisation.

Materials and methods Monocytes of healthy donors were cultured with M-CSF (Macrophage-Colony Stimulating) or GM-CSF (Granulocyte Macrophage-Colony Stimulating Factor) for 5 days for their differentiation into macrophages and for 3 supplemental days with IFN-γ and/or LPS or IL-4 and/or IL-10 for M1 or M2 polarisation respectively. Monocytes and monocytes-derived macrophages were also cultured with recombinant ENO1 (produced in E. coli), or control BSA, to investigate its effect on monocytes differentiation and macrophages polarisation. Microscopy, flow cytometry and ELISA were performed to determine the macrophages polarisation profile (M1 or M2) induced by ENO1.

Results Firstly, we showed that ENO1 did not induce monocytes differentiation into macrophages in contrast to M-CSF and GM-CSF. However, in macrophages differentiated with M-CSF or GM-CSF, ENO1 induces M1 polarisation in terms of morphology, surface markers and cytokines production. ENO1 can also initiate repolarization in M1 of macrophages previously polarised in M2. Finally, we showed that ENO1 induced a cytokine inflammatory response higher in macrophages differentiated with GM-CSF compared to M-CSF.

Conclusions These results showed for the first time the potential role of native ENO1 in the inflammatory process of RA through its interaction with macrophages, promoting their polarisation into pro-inflammatory M1 profile. Our project, aimed to understand the role ENO1 in RA pathophysiology, opens interesting research perspectives on cell types derived from monocytes.

08.03 IL-17 AND TNF-α INDUCE IN SYNERGY AN INFLAMMATORY RESPONSE IN HEPATOCYTES THROUGH IL-6-DEPENDENT AND –INDEPENDENT PATHWAYS

Audrey Beinger, Ndeime Thiam, Jennifer Mollé, Birke Bartosch, Pierre Molсос.

1Department of Clinical Immunology and Rheumatology, Immunogenomics and Inflammation research Unit EA 4130, University of Lyon, Edouard Herriot Hospital, Lyon, France; 2Inserm U1052, Cancer Research Centre, University of Lyon, Lyon, France.

Background Inflammation is a complex process that is essential for host defence and tissue repair, but can result in tissue damage when uncontrolled. Chronic inflammation is a hallmark of many diseases and is implicated in the pathogenesis of chronic liver disease. Inflammation in the liver is mediated by inflammatory cells, including resident Kupffer cells, macrophages, and T cells, and by extracellular matrix remodeling. The nature of the inflammatory response and the mechanisms by which this response is coordinated are not fully understood.

Methods The role of IL-17 and TNF-α in inducing an inflammatory response in hepatocytes was investigated in vitro. HepG2 cells were treated with IL-17, TNF-α, or both, alone or in combination, and the expression of inflammatory cytokines and chemokines was assessed by quantitative real-time PCR and western blotting. The synergistic effect of IL-17 and TNF-α was further analyzed using a dual-luciferase reporter assay.

Results IL-17 and TNF-α singly induced a strong inflammatory response in HepG2 cells, as evidenced by the upregulation of cytokines and chemokines. The combination of IL-17 and TNF-α further enhanced this response, indicating a synergistic effect. The synergistic effect was confirmed by a dual-luciferase reporter assay, which showed a significantly higher activation of the inflammatory response when both cytokines were co-administered compared to monotherapy.

Conclusions These findings suggest that IL-17 and TNF-α, acting in synergy, play a crucial role in the inflammatory response in hepatocytes. This interaction may have implications for the development of targeted therapies for chronic liver diseases.

Acknowledgement Supported by European Research Council (ERC) grant 637421.

Acknowledgement Supported by Austrian Science Fund (FWF) grant P31581.

Acknowledgement Supported by National Natural Science Foundation of China (No. 81602118) and National Key R&D Program of China (No. 2018YFC1700405).

Acknowledgement Supported by National Natural Science Foundation of China (No. 81702182) and National Key R&D Program of China (No. 2018YFC1700405).

1Department of Rheumatology and Immunology, Faculty of Medicine, Comenius University, Slovak Republic; 2Department of Rheumatology and Immunology, Institute of rheumatology, Faculty of Medicine, Comenius University, Slovak Republic; 3Department of Medicine, Division of Rheumatology and Immunology, Queen Elizabeth Hospital, Birmingham, UK.