assessed in line with the RA MRI scoring system (summed as RAMRIS-inflammation). Both IMB and RAMRIS-inflammation were dichotomised into positive/negative using data from age-matched symptom-free controls as a reference. Cox regression analysed the association of IMB with progression to clinical arthritis; multivariable analyses were used to adjust for RAMRIS-inflammation which is known to associate with progression to clinical arthritis. Analyses were repeated stratified for ACPA-status, since ACPA-positive and ACPA-negative RA are considered separate entities with differences in pathophysiology.

Results: The baseline MRIs showed ≥ 1 IMB in 35% of CSA-patients. Patients with IMB were more likely to also have synovitis (OR 2.5 (95%CI 1.2–4.9)) and tenosynovitis (8.9 (3.4–22.9)) on forefoot MRI, but not osteitis (0.9 (0.5–1.8)). Patients were followed for median 25 months (IQR 19–27). IMB-positive patients developed clinical arthritis more often than IMB-negative patients (HR 3.0 (1.9–4.8)). This association was independent of RAMRIS-inflammation (adjusted HR 2.2 (1.4–3.6)). In stratified analyses, IMB was more frequent in ACPA-positive than in ACPA-negative CSA (68% vs. 30%, $p < 0.001$). Moreover IMB predicted clinical arthritis development in ACPA-positive CSA (HR 2.5 (1.1–5.7)) but not in ACPA-negative CSA patients (1.0 (0.5–2.2)).

Conclusion: One-third of CSA patients have IMB. IMB is frequently present in conjunction with subclinical synovitis and tenosynovitis. It precedes the development of clinical arthritis, and in particular the development of ACPA-positive RA. These results reinforce the notion that not only intra- but also juxta-articular synovial inflammation is involved in the development of RA.

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POS0386

EVOLUTION OF ANTI-MODIFIED PROTEIN ANTIBODY RESPONSES CAN BE DRIVEN BY CONSECUTIVE EXPOSURE TO DIFFERENT POST-TRANSLATIONAL MODIFICATIONS

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Background: Besides anti-citrullinated protein antibodies (ACPA), rheumatoid arthritis patients (RA) often display autoantibody reactivities against other post-translationally modified (PTM) proteins, more specifically carbamylated and acetylated proteins. Immunizing mice with one PTM results in an anti-modified protein antibody (AMPA) response recognizing multiple PTMs. Furthermore, human AMPA, isolated based on their reactivity to one PTM, cross-react with other PTMs at the monoclonal and polyclonal level. However, it is unclear whether the AMPA reactivity profile is “fixed” in time, or whether consecutive exposure to different PTMs can shape the evolving AMPA-response.

Objectives: To investigate the evolution of the AMPA response in mice with controlled exposure to PTMs as well as in AMPA-positive humans.

Methods: Mice were immunized with acetylated (or carbamylated) protein (ovalbumin) twice or cross-immunized with an acetylated and then a carbamylated protein (or vice versa) and their serum was analyzed for AMPA responses with ELISA using a different backbone protein (fibrinogen) bearing the same modifications. Longitudinally collected serum samples of human individuals at risk of RA and with early RA were tested to investigate the evolution of the AMPA responses in humans.

Results: Mice immunized twice with either solely acetylated or solely carbamylated ovalbumin (AcOVA or CaOVA) developed reactivity against both acetylated and carbamylated antigens. Irrespective of the PTM used for the first immunization, a booster immunization with the other PTM resulted in increased titers to the second/booster PTM (Figure 1A), suggesting that immunization with a defined PTM-antigen leads to the generation of anti-PTM memory B cells able to cross-recognize other PTMs. Furthermore, immunizing with CaOVA and boosting with AcOVA (or vice versa) skewed the overall AMPA-response profile towards a relatively higher reactivity against the “booster” PTM (Figure 1B). Human data also illustrated dynamic changes in AMPA reactivity profiles in both individuals at risk of RA and in early RA patients (not shown).

Conclusion: The relationship between different reactivities within the AMPA response is dynamic. The initial exposure to a PTM antigen induces cross-reactive response that can be boosted by the same or other PTMs. The overall reactivity pattern can be skewed by subsequent exposure to other PTMs. These data might explain temporal changes in the reactivity profile of the AMPA response and point to the possibility that the PTM responsible for the initiation of the AMPA response may differ from the PTM predominantly recognized later in time.

Cross-boosting and skewing of AMPA responses in immunized mice

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POS0387

ACPA STATUS CORRELATES WITH DIFFERENTIAL IMMUNE PROFILE OF RHEUMATOID ARTHRITIS PATIENTS

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Background: Rheumatoid arthritis (RA) is a progressive erosive autoimmune disease that affects 1% of the world population. Anti-citrullinated protein autoantibodies (ACPA) are routinely used for the diagnosis of RA, however 20-30% of patients are ACPA negative. ACPA status is a delineator of RA disease endotypes with similar clinical manifestation but potentially different pathophysiology. Elucidating the underlying mechanisms of disease pathogenesis could inform a treat to target approach for both ACPA-positive and ACPA-negative RA patients.

Objectives: To identify peripheral blood and synovial tissue immune population differences that associate with RA disease endotype.

To identify unique RA patient synovial tissue gene signatures and enriched pathways that correlate with ACPA status.

Methods: Detailed high dimensionality flow cytometric analysis with supervised and unsupervised algorithm analysis of ACPApos and ACPAneg RA patient peripheral blood and synovial tissue single cell suspensions. *Ex vivo* peripheral blood and synovial tissue T cell stimulation and cytokine production characterisation. RNAseq analysis with specific pathway enrichment analysis of ACPApos and ACPAneg RA patient synovial tissue biopsies.

Results: Detailed profiling based on high dimensionality flow cytometric analysis of key peripheral blood and synovial tissue immune populations including B cells, T follicular helper (Tfh) cells, T peripheral helper cells (Tph) and CD4 T cell proinflammatory cytokine responses with supervised and unsupervised algorithm analysis revealed unique RA patient peripheral blood B cell and Tfh cell profiles. ACPApos RA patients were characterised by significantly ($*P=0.03$) increased frequency of Tfh (CXCR5⁺CD4⁺) cells and distinct clustering influenced by increased switched (*IgD⁺CD27⁺*) and DN (*IgD⁺CD27⁻*) memory B cells compared to ACPAneg RA patients. Surprisingly synovial tissue B cell subpopulation distribution was similar between ACPAneg and ACPApos RA patients, with significant accumulation of switched and double negative memory B cells, highlighting a key role for specific B cell subsets in both disease endotypes. Interestingly, synovial tissue CD4 T cell

proinflammatory cytokine (TNF- α , IFN- γ , IL-2, GM-CSF, IL-17A, IL-22, IL-4) production was markedly different between ACPAneg and APCapos RA patients with hierarchical clustering and PCA analysis revealing endotype specific cytokine profiles with ACPAneg RA patient synovial T cells showing increased TNF- α (P=0.01) expression. RNAseq analysis of RA patient synovial tissue revealed significant disease endotype specific gene signatures with specific enrichment for B cell receptor signalling and T cell specific pathways in ACPapos compared to ACPAneg RA patients. Additionally, significantly different chemokine receptor expression based on RA patient ACPA status was observed with increased CXCR3 (P<0.001), CCR7 (P=0.002), and CCR2 (P=0.004) but decreased CXCR7 (P=0.007) expression in APCapos compared to ACPAneg RA patient synovial biopsies.

Conclusion: ACPA status associates with unique synovial tissue immune cell and gene profile signatures highlighting differences in the underlying immunological mechanisms involved, therefore reinforcing the need for a treat to target approach for both endotypes of RA.

Figure 1. RNAseq analysis of synovial tissue biopsies revealed specific T cell related pathway enrichment in ACPA positive compared to ACPA negative RA patients (n=50, analysis performed with the *DESeq2* and *pathfinder* pipelines in R).

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POS0388

INTERLEUKIN 40 (IL-40) IS UP-REGULATED IN RHEUMATOID ARTHRITIS (RA) AND ASSOCIATED WITH DISEASE ACTIVITY, LEVELS OF AUTOANTIBODIES AND CHEMOKINES

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Background: Interleukin 40 (IL-40) is newly identified B cell - associated cytokine implicated in humoral immune responses and in B cell development. As B cells play a pivotal role in autoimmunity, we aimed to investigate the function of IL-40 in rheumatoid arthritis (RA).

Objectives: The aim of our study was to determine the function of IL-40 in RA. **Methods:** IL-40 expression in the synovial tissue was determined by immunohistochemistry and immunofluorescence (n=4-5). IL-40 was analysed in the serum/synovial fluid of patients with RA (n=69), systemic lupus erythematosus (SLE; n=69), osteoarthritis (OA; n=44), and in healthy controls (HC; n=25). Given the association of IL-40 with B cells, we analysed the effect of rituximab therapy on the serum IL-40 in 19 patients with RA after 16 and 24 weeks of the therapy. The clinical activity of patients with RA was assessed according to the 28 joint count Disease Activity Score (DAS28). Levels of C-reactive protein (CRP) and autoantibodies were measured by routine laboratory techniques. *In vitro* experiments were performed in RA synovial fibroblasts (n=9). Levels of cytokines and

inflammatory mediators were determined in serum, synovial fluid and supernatants using ELISA or multiplex immunoassay.

Results: IL-40 was overexpressed in RA synovial tissue compared to OA, particularly by synovial fibroblasts and immune cells such as B and T lymphocytes, macrophages and neutrophils. The levels of IL-40 were significantly higher in the synovial fluid of RA patients compared to OA (33.2 (6.6-68.9) vs. 0.7 (0.1-2.4) ng/ml; p<0.0001). In addition, IL-40 was increased in the serum of RA patients compared to SLE, OA or HC (4.8 (1.7-24.9) vs. 1.4 (1.0-1.9), 1.6 (0.6-3.1) or 1.5 (0.7-2.7) ng/ml; p<0.0001 for all) and decreased after 16 (p<0.01) and 24 weeks (p<0.001) in a subgroup of rituximab treated patients with RA. IL-40 levels in RA patients correlated with autoantibodies rheumatoid factor (IgM) and anti-citrullinated protein antibody (ACPA) in the serum (p<0.0001 and p<0.01) as well as in the synovial fluid (p<0.0001 and p<0.001). IL-40 in RA synovial fluid was also significantly associated with DAS28 (p<0.05), synovial fluid leukocyte count (p<0.01), number of swollen joints (p<0.05) and neutrophil attractants IL-8 (p<0.01) and MIP-1 α (p<0.01). RA synovial fibroblasts exposed to recombinant IL-40 increased secretion of IL-8 (p<0.01), MCP-1 (p<0.05) and MMP-13 (p<0.01) compared to unstimulated cells *in vitro* conditions.

Conclusion: Our results show for the first time that IL-40 is elevated in RA and decreases following B-cell depletion therapy. The association of IL-40 with autoantibodies and chemokines may imply its potential involvement in RA development. Moreover, IL-40 up-regulates the secretion of chemokines and MMP-13 by synovial fibroblasts, indicating its role in the regulation of inflammation and tissue destruction in RA.

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POS0389

NEUTROPHIL AND MONOCYTE EXTRACELLULAR TRAPS IN RHEUMATOID ARTHRITIS: POTENTIAL SOURCE OF DIAGNOSTIC BIOMARKERS

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Background: Up-to date quite high rheumatoid arthritis (RA) prevalence has also a trend towards steady increase for several decades. Earliest possible diagnosis and personalized treatment of RA are commonly believed to be principal way to prevent or diminish joint destruction. Emerging biomarkers retrieval has therefore critical importance for improvement of RA treatment outcomes, especially for its biological treatment. One of upcoming sources of new biomarkers is newly discovered phenomenon of neutrophil extracellular traps (NET) and monocyte extracellular traps (MET) formation, specifically in RA.

Objectives: Evaluation of peripheral blood neutrophils and monocytes ability to generate NET and MET spontaneously and after induction *in vitro* in RA.

Methods: The research was carried out in agreement with the WMA Declaration of Helsinki principles. 30 patients with verified RA according to the ACR/EULAR 2010 criteria were included in the study. 30 healthy volunteers were enrolled as a reference group. RA disease activity was assessed using DAS28 score did not exceeded 2.6 in every patient at the inclusion timepoint. Neutrophils and monocytes were isolated with one-step centrifugation procedure using three-layer ficoll-amidotrizoate density gradient with density of upper, intermediate and lower layers 1068 kg/m³, 1080 kg/m³, and 1090 kg/m³, respectively. The cell types in the resulting fractions were identified histochemically, and the extent of cell activation was assessed using common nitro-blue tetrazolium test. Generation of NETs was stimulated by phorbol-12-myristate-13-acetate (PMA). Generation of METs was stimulated using bacterial LPS. The shape and size of NETs and ETs were assessed using fluorescence microscopy with SYBR green.

Results: Mean contamination fraction of neutrophils and monocytes in the reference group did not exceeded 3% and 2%, respectively. Mean purity of neutrophil fraction in RA group was 93.1 $\pm$ 6.1%, and cell viability in every sample was above 95.4 $\pm$ 5.1%. Mean purity of monocyte fraction in RA group was 98.4 $\pm$ 6.4%, cell viability in every sample was above 93.4 $\pm$ 4.8%. Spontaneous NET and MET formation was observed in neutrophils and monocytes isolated from both RA patients and, significantly less, in healthy controls. Neutrophils from ACPA-positive RA patients were found to reveal increased spontaneous and induced NETs formation compared to ACPA-negative RA patients. Monocytes did not demonstrate any difference between these subgroups.

Conclusion: NETs could probably be considered as a candidate source of citrulline autoantigen participating in autoantibody production, whereas METs may play less important role in this phenomenon. NETs and ETs can be considered as potential diagnostic biomarkers of RA. Further studies of NETosis and ETosis in RA patients can promote emerging researches for targeted therapy of RA.

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