Introduction Anti-citrullinated protein autoantibodies (ACPA) in RA patients target a wide range of modified proteins and recent findings reveal that monoclonal ACPA are cross-reactive due to recognition of shared consensus citrulline-peptide motifs. Among physiological targets of ACPA, citrullination in neutrophil extracellular trap (NET) products, have been postulated to be important.

Objectives We sought to characterize the anti-nuclear and anti-neutrophil reactivities of different patient-derived monoclonal ACPA.

Methods The study included ten recombinant single B-cell derived RA monoclonal ACPA-IgG with CCP2 reactivity from different cell subsets and compartments (1, 2). They were screened for HEP-2 ANA reactivity, and binding to apoptotic cells or stimulated neutrophils. Binding was compared to CRISPR PAD4 KO cells and neutrophils from PAD2 and PAD4 KO mice. Immunoprecipitation and mass spectrometry were used to identify modified nuclear ACPA targets, and ELISA and Western blot for mAb binding to acetylated epitopes.

Results Four out of ten ACPA clones exhibited strong binding to apoptotic cells, nuclear binding to activated neutrophils, and reactivity to NETs. Three of these were ANA positive. Another NET-reactive ACPA instead displayed a cytoplasmic binding pattern. This cytoplasmic NET-binding was PAD4-dependent, whilst nuclear-mediated NET reactivity was PAD-independent. Using apoptotic cells, acetylated histones were confirmed to be the primary targets of the nuclear reactivity, which could be explained by consensus-motif driven ACPA cross-reactivity. Specifically targeted acetylated histone peptides were identified and the anti-modified protein autoantibody (AMPA) profiles of the ACPA were found to correlate with cell binding.

Conclusions When investigating monoclonal ACPA, our novel data reveal a distinct subset of ACPA with nuclear binding patterns and AMPA activity with acetylated histones (and not citrullinated proteins) in NETs and apoptotic cells.

REFERENCES

1. Titcombe PJ, Wigerblade G, Sippl N, Zhang N, Shmagel AK, *et al.* Pathogenic citrulline-multispecific B cell receptor clades in rheumatoid arthritis. *Arthritis Rheumatol* 2018. doi:10.1002/art.40590
2. Steen J, Forsström B, Sahlström P, Odowd V, Israelsson L, *et al.* Human plasma cell derived monoclonal antibodies to posttranslationally modified proteins recognize amino acid motifs rather than specific proteins. *Arthritis Rheumatol* 2018. doi:10.1002/art.40699

Disclosure of Interest None declared.

P022/O02 DISTINCT MACROPHAGE PHENOTYPE AND BIOENERGETIC PROFILES IN RHEUMATOID ARTHRITIS

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Introduction Synovial macrophages play a key role in RA disease progression, however, the diversity and plasticity of macrophage subsets and their metabolic profile within the joint has yet to be elucidated.

Objectives To phenotype distinct macrophage subsets within the RA joint, and determine the metabolic and inflammatory function of RA macrophages compared to healthy controls (HC).

Methods Synovial-tissue biopsies from RA, PsA and OA, obtained through arthroscopy, were digested to yield a synovial single cell-suspension. Biopsy suspensions and synovial fluid mononuclear cells were analysed using advanced flow-cytometry with the following antibody panel (CD40,CD45,CD64,CD68,CD163,CD206,CD253). CD14+ monocytes were sorted from RA and HC bloods and differentiated/polarized into M1/M2 macrophages. Inflammatory (IL-8,-MCP-1,-IL1b,-CCR5,-IRAK1,-OSM) and metabolic (HIF1a,-PFKFB3,-PKM2,-LDHA,-HK2) markers were measured by RT-PCR, and phagocytosis by OVA luciferase-yellow assays. Glycolysis (ECAR) and oxidative phosphorylation (OCR) were quantified by Seahorse -XFE- technology.

Results RA synovial-tissue and fluid CD68+ macrophages displayed markers typical of both M1(CD40+CD253+) and M2 (CD206+CD163+). A significant increase in frequency of CD68+ and CD64+ macrophages in synovial-tissue compared to fluid was observed (p<0.05), with significant increases in marker expression of CD40,CD163,CD206 (p<0.07). A spectrum of macrophage-subtypes within the inflamed joint was observed, with significant enrichment of a dominant double positive CD206+CD163+ macrophage subtype in the synovial-tissue versus synovial fluid demonstrated (p<0.05). Increased frequency of CD206+CD163+ macrophages and higher expression of activation marker CD40 were demonstrated in RA synovial-tissue compared to PsA and OA. M1 macrophages demonstrate a pro-glycolytic phenotype with significant increases in HIF1a,-HK2,-PKM2,-and PFKB3, compared to M2, effects exacerbated in RA macrophages compared to HC. In parallel, using seahorse-technology RA M1 and M2 macrophages displayed higher ECAR and OCR profiles, in addition to an increased ECAR:OCR ratio compared to HC (p<0.05), evidence that RA macrophages switch to a glycolytic profile. This was paralleled by increased intracellular-cytokines expression of IL-1b,-IL-6 and TNFα and genes expression of IL-8,-IL1b, OSM and MCP-1 (p<0.05). Finally, phagocytic ability of RA M1 was impaired compared to HC.

Conclusions We have identified, for the first time, a dominant macrophages subtype enriched in RA synovial-tissue. Furthermore, RA M1/M2 have distinct metabolic profiles associated with differences in key inflammatory mediator and phagocytosis function.

Disclosure of Interest None declared.

P023 TENOSYNOVITIS AND HLA-SE PREDICT ARTHRITIS ONSET IN ACPA-POSITIVE INDIVIDUALS AT RISK OF DEVELOPING RHEUMATOID ARTHRITIS

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Introduction Anti-citrullinated protein antibodies (ACPA) are predictive markers with pathological effects in rheumatoid