Methods: We quantified NETs by measuring dsDNA using ELISA and immunofluorescence. Macrophages were stimulated with BD- and healthy controls (HC)-derived NETs, and IL-8 and TNF- α production were measured by ELISA. NETs-stimulated macrophages were incubated with naive CD4⁺ T cells, and Th1 cell differentiation was examined on day 7 by flow cytometry. Histones H1, H2A, H2B, H3, H4, S100A8 and neutrophil elastase in NETs were analyzed by western blot. Macrophages were stimulated with anti-Histone 4 antibody-treated NETs, and IL-8 production was measured by ELISA.

Results: Circulating NETs (2336 $\pm$ 534 ng/ml vs. 1472 $\pm$ 549 ng/ml, $P=0.0008$) and neutrophil-derived NETs (909.2 $\pm$ 485.2 ng/ml vs. 582.4 $\pm$ 199.2 ng/ml, $P=0.0108$) were significantly higher in BD patients compared with those in HC. BD NETs stimulated macrophages to produce a higher level of IL-8 (17 $\pm$ 4 ng/ml vs. 13 $\pm$ 4 ng/ml, $P=0.0474$) and TNF- α (166 $\pm$ 61 pg/ml vs. 102 $\pm$ 48 pg/ml, $P=0.0132$) than HC NETs. Moreover, BD NETs promoted macrophages to facilitate Th1 differentiation than HC NETs (33 $\pm$ 10% vs. 24 $\pm$ 7%, $P=0.0398$). Western blot analysis revealed more Histone H4 (289076 (144365, 544038) IOD values vs. 42121 (6958, 129625) IOD values, $P=0.0286$), but not Histones H1, H2A, H2B, H3, S100A8 or neutrophil elastase in BD NETs compared to HC NETs. Importantly, neutralizing Histone H4 abrogated the BD NETs-stimulated IL-8 overproduction by macrophages (9.99 $\pm$ 2.07 ng/ml vs. 13.95 $\pm$ 2.91 ng/ml, $P=0.021$).

Conclusion: BD NETs promoted macrophages activation, which might be mediated by a higher level of Histone H4.

References:

- [1] Safi R., Kallas R., Bardawil T., et al. Neutrophils contribute to vasculitis by increased release of neutrophil extracellular traps in Behçet's disease. *J. Dermatol. Sci.* 2018;92:143–150.
- [2] Le Juncour a, Martos r, Loyau s, et al. Critical role of neutrophil extracellular traps (NETs) in patients with Behcet's disease. *Ann Rheum Dis* 2019;78:1274–1282.

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FRI0004

SURFACE AMP DEAMINASE 2 AS A NOVEL REGULATOR MODIFYING THE EXTRACELLULAR ATP-ADENOSINE BALANCE THAT IS DIFFERENTIALLY EXPRESSED IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Background: Adenosine and its nucleotides represent crucial immunomodulators in the extracellular environment. ATP and ADP are released from stressed cells in states of inflammation, whereas adenosine serves as a key anti-inflammatory mediator¹. The ectonucleotidases CD39 and CD73 are responsible for the sequential catabolism of ATP to adenosine via AMP, thereby promoting an anti-inflammatory milieu induced by the "adenosine halo". Great importance has been attributed to these enzymes in the pathogenesis of autoimmune diseases such as rheumatoid arthritis (RA) and as targets in cancer therapy^{2,3}. AMPD2 mediates AMP deamination to IMP, thus constituting an ambiguous mediator both enhancing the degradation of inflammatory ATP and reducing the formation of protective adenosine. Here, we postulate that this pathway is also present on the cell surface of immune cells and modified under inflammatory conditions.

Objectives: Therefore, we analysed surface AMPD2 expression and its modulation on distinct cell lines and primary immune cells.

Methods: Firstly, AMPD2 surface expression was verified by immunoprecipitation from membrane fractions isolated from cell lines (HEK293 and HMEC1) and CD14⁺ monocytes analysed by western blot and mass spectrometry. In addition, surface biotinylation of the aforementioned cells was performed. Also, AMPD2 surface expression was evaluated by flow cytometry, analysing both cell lines (HEK293, HMEC1, THP1, and Jurkat) and primary human immune cells from healthy donors and patients with RA.

Secondly, co-expression of surface AMPD2, CD39 and CD73 on PBMCs was analysed by flow cytometry directly after isolation as well as after a 24h culture period. Moreover, surface expression was assessed after immunostimulation and Golgi transport inhibition.

Results: AMPD2 surface expression was confirmed by western blot and mass spectrometry of (i) precipitated AMPD2 from membrane fractions and (ii) biotinylated surface molecules in HEK293 and HMEC1 as well as CD14⁺ monocytes. Surface expression was reduced after AMPD2 knockdown in HEK293. Flow cytometric analysis further verified AMPD2 surface expression and revealed a significant decrease after Golgi transport inhibition ($p<0.01$). TLR

Figure 1. Surface AMPD2 as a novel regulator of the extracellular ATP-adenosine balance.

stimulation strongly enhanced the surface expression of AMPD2 and CD39 on monocytes ($p<0.05$), whereas dexamethasone at high therapeutic doses inversely affected AMPD2 surface expression on lymphocytes and monocytes ($p<0.01$). Analysis of AMPD2 surface expression on PBMCs from RA patients revealed higher expression levels compared to sex- and age-matched healthy controls ($p<0.05$).

Conclusion: We demonstrate AMPD2 surface expression on immune cells for the first time. Hence, we reveal a novel regulator of the extracellular ATP-adenosine balance that is differentially expressed in RA patients compared to healthy controls. The extracellular conversion of AMP into IMP may constitute a shunt-like mechanism adding to the CD39-CD73 system controlling immunomodulation.

References:

- [1] Regateiro FS, Cobbold SP, Waldmann H. CD73 and adenosine generation in the creation of regulatory microenvironments. *Clinical and experimental immunology* 2013;171(1):1-7. doi: 10.1111/j.1365-2249.2012.04623.x
- [2] Morandi F, Horenstein AL, Rizzo R, et al. The Role of Extracellular Adenosine Generation in the Development of Autoimmune Diseases. *Mediators of inflammation* 2018;2018:7019398. doi: 10.1155/2018/7019398
- [3] Allard B, Longhi MS, Robson SC, et al. The ectonucleotidases CD39 and CD73: Novel checkpoint inhibitor targets. *Immunol Rev* 2017;276(1):121-44. doi: 10.1111/imr.12528

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FRI0005

DIVERSITY OF ANTI-CITRULLINATED PROTEIN ANTIBODY COMPOSITIONS INFLUENCE SYNOVIAL FIBROBLAST REACTIVITY

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Background: Anti-citrullinated protein antibodies (ACPAs) play an important role in rheumatoid arthritis (RA) pathogenesis. We hypothesized that the effect of these antibodies is mediated by their binding to synovial fibroblasts and inducing an increased mobility of fibroblasts¹.

Objectives: In our study, we analyzed and compared fibroblast modulation by ACPA pools obtained from different patients or by a set of monoclonal ACPAs with different fine specificity that were obtained from different tissue sites.

Methods: Synovial fibroblasts were isolated from RA patients synovial tissue biopsies. Individual polyclonal ACPA and control IgGs were purified from sera of four ACPA-positive RA patients by affinity purification on protein G and CCP-2 columns. Monoclonal antibodies were derived from memory B cell isolated from blood2, synovial fluid or bronchoalveolar lavage of RA patients. Whole antibodies and F(ab')₂ fragments were tested in fibroblast migration using IncuCyte live-cell analysis. Blocking experiments were performed with soluble citrullinated proteins in SF migration. Cross-reactivity of the antibodies to citrullinated and acetylated epitopes was tested using PAD inhibitors (CI-amidine and GSK199),

histone acetyltransferases (anacardic acid) and deacetylases (trichostatin A). Binding patterns of monoclonal ACPAs, both whole and F(ab')₂ fragments were analyzed in synovial biopsies obtained from both healthy donors and RA patients.

Results: Three out of four tested individual ACPA were able to promote fibroblast migration. Five out of nine tested monoclonal ACPAs stimulated fibroblast migration. One of these antibodies, clone 1325:01B09 is characterized by cross-reactivity to citrullinated, homocitrullinated and acetylated targets. The effect of 1325:01B09 on fibroblast migration was completely abolished by Cl-amidine or by pre-incubating the antibody with citrullinated fibrinogen or histone but not citrullinated enolase or vimentin. Despite the cross-reactivity to acetylated epitopes, neither anacardic acid nor trichostatin A could modulate the 1325:01B09 effect on fibroblast migration. F(ab')₂ fragments of this antibody stimulated fibroblast migration and labelled podoplanin-positive fibroblasts in inflamed RA synovium similarly to the intact antibody, indicating an Fc-independent effect.

Conclusion: The effect on fibroblast mobility was likely to be mediated by binding to citrullinated epitopes but not through Fc receptors. Detection of fibroblast modulating ACPAs in majority of RA patients indicated that fibroblasts might be key cellular targets in disease pathogenesis, although individual variability might exist in the composition of ACPA cellular targets.

References:

- [1] Sun M, Rethi B, Krishnamurthy A, et al. Anticitrullinated protein antibodies facilitate migration of synovial tissue-derived fibroblasts. *Ann Rheum Dis* 2019;78(12):1621-31. doi: 10.1136/annrheumdis-2018-214967 [published Online First: 2019/09/05]
- [2] Amara K, Lena Israelsson, Ragnhild Stålesen, et al. A Refined Protocol for Identifying Citrulline-specific Monoclonal Antibodies from Single Human B Cells from Rheumatoid Arthritis Patient Material. *Bio-protocol* 2019;9(16)

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FRI0006 ASSESSING PRO-INFLAMMATORY PROPERTIES OF H-FERRITIN BY EX VIVO AND IN VITRO OBSERVATIONS

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Background: The concept of 'hyperferritinemic syndrome' has recently been proposed, suggesting high levels of ferritin as pathogenic pro-inflammatory mediator [1] Ferritin is an intracellular iron storage protein, comprising 24 subunits, heavy (H) and light (L) based on molecular weight. The H/L subunits ratio may be different in tissues, since the ferritin enriched in L subunits (L-ferritin) and the ferritin enriched in H subunits (H-ferritin) may be observed in different tissues, according to pathophysiologic status [1].

Objectives: We aimed to assess the presence of H- and L-ferritin as well as of CD68/H-ferritin and CD68/L-ferritin in bone marrow (BM) biopsies of adult macrophage activation syndrome (MAS) patients. In the same patients, we matched the findings of BM biopsies with sera to identify the main represented subunits of ferritin. Furthermore, we evaluated effects of ferritin, L-ferritin, and H-ferritin on human monocytes, assessing pro- and anti-inflammatory cytokines, and expression of NLRP3 inflammasome. Finally, we checked the ability of monocytes, which were treated with ferritin, to stimulate or not the proliferation of peripheral blood mononuclear cells (PBMCs).

Methods: Immunofluorescence analysis was performed to investigate the tissue presence of L- and H-ferritin in BM biopsies as well as of CD68/H-ferritin and CD68/L-ferritin. Liquid chromatography mass spectrometry (LC-MS/MS) based proteomics was performed to identify L- and H-ferritin in sera proteins. Human monocytes were cultured with M-CSF for 7 days and, after that, treated with ferritin, H-ferritin, and L-ferritin at 10nM, for 120 and 240 minutes. After stimulation, IL-1 β , IL-6, IL-10, IL-12, IFN- γ , TGF- β , TNF, and VEGF were assessed by RT-PCR and, in case of positive finding, evaluated by western blot. NLRP3 inflammasome was also assessed. Finally, the proliferation of PBMCs when co-cultured with ferritin-treated monocytes was tested by a specific proliferation assay.

Results: Immunofluorescence showed an increased H-ferritin expression in the BMs of MAS patients, whereas L-ferritin did not. Conversely, LC-MS/MS identified that the L-ferritin was the dominant form, after stringent probability matching. *In vitro*, H-Ferritin induced a significant increased expression of IL-1 β , IL-6, IL-12, and TNF after 240 minutes. Ferritin also induced a significant increased expression of IL-1 β , IL-6, IL-12, and TNF after 240 minutes. Effects on pro-inflammatory cytokines were more marked with H-ferritin than ferritin. Conversely, no significant effects were retrieved analysing IFN- γ , IL-10, TGF- β , and VEGF after 240 minutes, after ferritin and H-ferritin stimulation. Furthermore, both ferritin and H-ferritin induced a direct effect on NLRP3 inflammasome. Finally, monocytes, which were treated with H-ferritin, stimulated the proliferation of co-cultured PBMCs.

Conclusion: In our work, results showed the presence of H-ferritin and CD68/H-ferritin cells in BM biopsies of MAS patients, by immunofluorescence. Conversely, LC-MS/MS identified L-ferritin in sera proteins of those patients. Furthermore, pro-inflammatory effects of ferritin and, particularly, of H-ferritin on human monocytes were observed *in vitro*, increasing pro-inflammatory cytokines and NLRP3 inflammasome. Finally, H-ferritin-treated monocytes stimulated the proliferation of co-cultured PBMCs.

References:

- [1] Rosario C, et al. *BMC Med* 2013; 11:185.

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FRI0007 MOSPD2: A NOVEL THERAPEUTIC TARGET FOR THE TREATMENT OF RHEUMATOID ARTHRITIS

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Background: Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease with an unknown etiology that affects the joints. B and T lymphocytes are major drivers of arthritis, mediated through their secretion of antibodies against self-antigens and the production of pro-inflammatory cytokines. Nevertheless, innate immune cells play an important role in disease progression, and recruitment of blood monocytes/macrophages was highly correlated with tissue damage in RA patients^{1,2}. Once infiltrated into the joint, monocytes can produce proinflammatory cytokines and chemokines that further escalate the inflammatory process and tissue damage. Consistent with that, genetic and pharmacological intervention targeting CCR2 and CCR5, chemokine receptors which are abundantly expressed on monocytes, was shown to reduce disease severity in multiple preclinical models of RA³. Blocking monocyte migration in RA can be therefore an attractive therapeutic approach, if implied in a manner that can overcome the redundancy of chemokine receptors and ligands.

Motile sperm domain-containing protein 2 (MOSPD2) is a 518 amino acids single-pass membrane protein to which no function was ascribed up until recently. Assessment of MOSPD2 in immune cell subsets showed it is expressed by monocytes and neutrophils. We previously discovered that MOSPD2 regulates human monocyte and neutrophil migration in a chemokine-agnostic manner⁴.

Objectives: Using anti MOSPD2 mAbs, the role of MOSPD2 in mouse monocyte migration in-vitro and RA progression in mice was assessed.

Methods: In-vitro, MCP-1 and SDF-1 chemokines were used to induce migration of mouse splenic monocytes through a trans-well plate in the presence of anti MOSPD2 mAbs. In-vivo, DBA-1 mice were injected with type II collagen on days 0 and 21 to induce arthritis. Mice were then treated twice a week with anti MOSPD2 mAb, anti TNF- α or an isotype control antibody until study end (day 42). Animals were monitored for arthritis score, and upon sacrifice, hind limb joints were examined for pathology.

Results: Anti MOSPD2 mAbs significantly inhibited migration of mouse monocytes in-vitro. Treatment with anti MOSPD2 mAb after disease was already initiated, limited disease progression and significantly ameliorated clinical manifestation at the climax phase of the disease by more than 50% compared with isotype control and anti TNF- α treatment. Pathological examination revealed that treatment with anti MOSPD2 mAb reduced pannus formation and joint inflammation.