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. NET-activated macrophages were incubated with naïve 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 (239 ± 534 ng/ml vs. 1472 ± 549 ng/ml, P = 0.0008) and neutrophil-derived NETs (909 ± 245 ng/ml vs. 582 ± 199 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 ± 4 ng/ml vs. 13 ± 3 ng/ml, P = 0.0447) and TNF-α (166 ± 61 pg/ml vs. 102 ± 48 pg/ml, P = 0.0132) than HC NETs. Moreover, BD NETs promoted macrophages to facilitate Th1 differentiation than HC NETs (33 ± 10% vs. 24 ± 7%, P = 0.0398). Western blot analysis revealed more Histone H4 (289 ± 0.3 ng/ml vs. 544 ± 0.3 ng/ml, 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 ± 2.07 ng/ml vs. 13.95 ± 2.91 ng/ml, P = 0.0221).

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

References:

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**Figure 1.** Surface AMPD2 as a novel regulator of the extracellular ATP-adenosine balance.
Background: The concept of ‘hyperferritinemic syndrome’ has recently been proposed, suggesting high levels of ferritin as pro-inflammatory mediator [1]. Ferritin is an intracellular iron storage protein, comprising 24 subunits. It is released into the serum when iron is not needed by the body and can be used as an inflammatory marker. Ferritin levels are elevated in various inflammatory conditions, including autoimmune diseases such as rheumatoid arthritis (RA). Ferritin is a key mediator in the immune system, participating in innate and adaptive immune responses. Ferritin levels have been associated with disease activity and prognosis in RA patients. The aim of this study was to investigate the role of ferritin and its subforms, ferritin H (H-ferritin) and ferritin L (L-ferritin), in the pathogenesis of RA.

Methods: Blood samples were collected from RA patients and healthy controls. Ferritin levels were measured using an immunoluminometric assay. Ferritin subforms were identified using liquid chromatography mass spectrometry (LC-MS/MS). The effect of ferritin and H-ferritin on PBMCs was assessed by RT-PCR and, in case of positive finding, evaluated by western blot. H-ferritin was shown to stimulate the proliferation of PBMCs, while L-ferritin had no effect.

Results: Immunofluorescence showed an increased H-ferritin expression in the BMs of MA patients, whereas L-ferritin did not. Conversely, LC-MS/MS identified that the H-ferritin was the dominant form, while ferritin was detected in smaller amounts. Ferritin stimulation induced a significant increase in the expression of IL-1β, IL-6, IL-12, and TNF-α after 240 minutes. 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: The concentration of ferritin in the serum of RA patients is elevated, indicating a possible role of ferritin in the pathogenesis of the disease. Ferritin and H-ferritin play a role in the activation of the immune system, contributing to the development of RA.

References:

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