Poster Presentations

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Innate immunity in rheumatic diseases

FRI0001

NEUTROPHILS IN GRANULOMATOSIS WITH POLYANGIITIS DISPLAY FEATURES OF PYROPTOSIS

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Background: Granulomatosis with polyangiitis (GPA) is characterized by extravascular necrotizing granulomatous inflammation and systemic ANCA – associated (AAV) vasculitis with neutrophils as a key player in the pathogenesis (1). We and others have shown that neutrophil-related cell death mechanisms contribute to chronic inflammatory processes in AAV (2, 3). Recently, another form of inflammatory cell death primarily described in monocytes called pyroptosis was also discovered in neutrophils (4). A cardinal feature of pyroptosis is the activation of the NLRP3 inflammasome, a sensor of different pathogen- and damage-associated molecular patterns (PAMP, DAMP), following caspase-1-mediated processing and secretion of IL-1beta (5).

Objectives: The aim of this study was to investigate, if neutrophils from GPA patients express pyroptosis-related components NLRP3, active caspase 1 and cleaved IL-1beta.

Methods: Polymorphonuclear leukocytes (PMN) were isolated from peripheral blood of GPA patients and healthy controls (HC) (n = 10 each). Expression of NLRP3, inactive/active caspase 1 and active IL-1beta was determined by western blot. In addition, peripheral blood mononuclear cells (PBMC) were isolated from GPA and HC. mRNA expression of *nlrp3* and *il1b* was determined by qPCR. To exclude false-positive results by contamination with monocytes we performed flow cytometry analysis of whole blood samples with markers CD3, CD14, CD15, CD66b and NLRP3.

Results: PMN from GPA patients showed markedly increased expression of NLRP3, active caspase 1 and active IL-1beta compared to HC. In contrast, there was no difference between GPA and HC on the mRNA level of neither *nlrp3* nor *il1b* in PBMC. In addition, we confirmed by flow cytometry increased expression of NLRP3 in PMN from GPA, but not in monocytes.

Conclusion: Here we provide evidence, that neutrophils from GPA undergo pyroptosis, demonstrated by increased NLRP3, active caspase 1 expression as well as IL-1beta processing. Neutrophils are present in high numbers at the site of granulomatous lesions of inflamed tissue in GPA and IL-1beta is increased in GPA sera (2). Therefore, neutrophils represent a potential source of IL-1beta in GPA. Given the fact that GPA-associated features such as massive release of necrosis-related DAMP or microbial agents such as *Staphylococcus aureus* (6) can activate the NLRP3-inflammasome, we identified here a potential relevant mechanism of neutrophils contributing to chronic inflammation of GPA.

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FRI0002

S100A11 (CALGIZZARIN) IS RELEASED DURING NEUTROPHIL EXTRACELLULAR TRAPS FORMATION AND STIMULATES RELEASE OF IL-6 AND TNF IN RHEUMATOID ARTHRITIS

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Background: S100A11 protein, a member of S100 family, has been associated with several autoimmune inflammatory conditions such as rheumatoid arthritis (RA). Although the pathogenesis of autoimmune diseases is not fully understood, the formation of neutrophil extracellular traps (NETs) seems to play a certain role. Recent data indicate that S100A8/A9 is released via NETosis and can further augment inflammatory responses.

Objectives: The aim of our study was to examine the association of S100A11 with NETs in RA.

Methods: To assess the expression of S100A11 by neutrophils of RA synovial tissue (n=8), immunofluorescence staining of S100A11 and myeloperoxidase (MPO) was performed. The levels of S100A11 and MPO in RA synovial fluid (n=23) were measured by ELISAs (RayBiotech and Abcam), and the activity of peptidyl arginine deiminases (PADs) was measured by an in-house immunoassay. NETosis was induced by adding phorbol 12-myristate 13-acetate (PMA) to neutrophils from RA patients (n=7). Release of NETs was visualised by immunocytochemistry (n=7) and the presence of S100A11 in supernatants was analysed by ELISA (RayBiotech). Neutrophils purified from healthy donors (n=5) were stimulated by S100A11 and the release of cytokines TNF and IL-6 was measured by ELISA (RayBiotech).

Results: S100A11 was expressed by synovial tissue neutrophils of the RA patients (n=8). The levels of S100A11 in the synovial fluid of RA patients (n=23) correlated with the levels of a NETosis marker MPO (r=0.562, p=0.005) and with PADs activity (r=0.690, p<0.001), which affects NETs immunogenicity. Neutrophils treated with LPS (n=7) did not up-regulate the secretion of S100A11 compared to unstimulated controls (0.23±0.05 vs. 0.29±0.07 ng/ml; p=ns). However, the release of S100A11 was markedly up-regulated in PMA-stimulated neutrophils undergoing NETosis compared to untreated controls (1.16±0.17 vs. 0.29±0.07 ng/ml; p<0.001). Moreover, diphenyleneiodonium treatment abolished PMA-induced S100A11 secretion. By immunofluorescence staining (n=8) we demonstrated that neutrophils activated by PMA release NETs containing S100A11 protein. In addition, extracellular S100A11 augmented the inflammatory response of neutrophils from healthy donors (n=5) via IL-6 and TNF in comparison with unstimulated cells (0.39±0.11 vs. 0.05±0.01 pg/ml; p<0.05 and 0.31±0.06 vs. 0.09±0.03 pg/ml; p<0.05).

Conclusion: Here we show for the first time that release of S100A11 by neutrophils is dependent on NETosis. Moreover, extracellular S100A11 augments the inflammatory response by inducing TNF and IL-6 secretion in neutrophils.

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FRI0003

ELEVATED HISTONE H4 IN NEUTROPHIL EXTRACELLULAR TRAPS PROMOTES MACROPHAGE ACTIVATION IN BEHÇET'S DISEASE

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Background: Neutrophil-released neutrophil extracellular traps (NETs) are upregulated and promote autoinflammation and thrombosis in Behçet's disease (BD), a multi-system inflammatory disease with unknown etiology^{1,2}. However, whether NETs promote macrophage activation in BD remains unclear.

Objectives: To investigate the potential role of NETs in promoting aberrant macrophage activation in BD.