Results: Plasma levels of Clusterin were significantly decreased in active LN patients when compared to healthy controls ($p < 0.05$). Plasma Clusterin levels were negatively correlated with CRP, SLEDAI, and 24 hours proteinuria ($p < 0.01$, $p < 0.05$, and $p < 0.05$). In sixteen patients with lupus nephritis, we found that the expression of Clusterin in glomeruli was significantly enhanced in severe LN when compared to mild LN (figure 1, $p < 0.05$).

Abstract SAT0008 – Figure 1. Deposition of Clusterin in LN. * $P < 0.05$.

Conclusions: Decreased Clusterin could involve in the pathogenesis of LN, and the role of renal Clusterin need to be further explored. These findings suggested that Clusterin would be a therapeutic target for lupus nephritis in the future.

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SAT0009 TYROSINE KINASE PATHWAYS IN MONOSODIUM-URATE CRYSTAL-INDUCED INFLAMMATORY RESPONSES

K. Futosi, T. Németh, A. Mócsai. *Department of Physiology, Semmelweis University School of Medicine, Budapest, Hungary*

Background: Deposition of monosodium urate (MSU) crystals in the joints or other tissues is a hallmark in the pathogenesis of gout. The urate crystal-induced inflammation is known to be mediated mainly by neutrophils besides monocytes and macrophages. Although MSU crystal-mediated signal transduction is in the focus of recent investigations, the molecular mechanism is only partially characterised.

Objectives: In this study, we investigated the role of Src family kinases in MSU crystal-induced *in vitro* activation of primary murine and human neutrophils and their *in vivo* significance in experimental model of gout.

Methods: Bone marrow isolated neutrophils from wild type and triple Src family kinases-deficient ($Hck^{-/-}Fgr^{-/-}Lyn^{-/-}$) mice or vehicle and Src-inhibitor treated human neutrophils were stimulated with different concentration of MSU crystals. The superoxide production of the cells was measured by a luminometric assay, while the supernatants of MSU crystal activated cells were analysed by using various enzyme-linked immunosorbent assay (ELISA) kits. The phagocytosis of the urate crystals by neutrophils was followed by videomicroscopy and flow cytometry. Gouty arthritis was induced by injection of MSU crystals into the hind paws of the experimental mice and was assessed by ankle thickness measurements and detection of the synovial cytokine levels by ELISA.

Results: The MSU crystal-induced superoxide release, cytokine production and the crystal-phagocytosis were abrogated in Src family kinases-deficient murine or in Src-inhibitor treated human neutrophils. In contrast to wild type animals, Src family kinases-deficient mice showed significantly decreased paw swelling and neutrophil accumulation at the site of inflammation. In line with this, the synovial levels of interleukin-1 β and CXCL2 were also strongly reduced in Src family kinases-deficient mice compared to wild type animals.

Conclusions: Src family kinases play an indispensable role in MSU crystal-induced superoxide and cytokine production as well as crystal-phagocytosis of neutrophils. The fact, that Src family kinases-deficient mice are partially protected from crystal-induced inflammatory reactions indicate the important role of these kinases in *in vivo* gouty arthritis. These kinases are also play important role in the

development of *in vivo* gouty arthritis as the. Identification of these key players in urate crystal-induced intracellular signalling pathways in neutrophils leads to a better understanding of the pathogenesis of gout and may help to develop novel therapeutic strategies in MSU crystal-associated inflammatory diseases.

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SAT0010 EXCESSIVE FORMATION OF NEUTROPHIL EXTRACELLULAR TRAPS HAVE A DIFFERENT ROLE IN THE PATHOGENESIS OF ANCA-ASSOCIATED VASCULITIS AND SYSTEMIC LUPUS ERYTHEMATOSUS

L.S. van Dam¹, T. Kraaij¹, S.W. Kamberling¹, H.U. Scherer², T.J. Rabelink¹, C. van Kooten¹, Y.O. Teng¹. ¹Nephrology; ²Rheumatology, LUMC, Leiden, Netherlands

Background: Renal involvement in ANCA-associated vasculitis (AAV) and systemic lupus erythematosus (SLE) manifests as autoimmune-mediated glomerulonephritis (AIGN). In AAV, crescentic lesions and a pauci-immune immunofluorescence is typically seen while in SLE endo- and extracapillary proliferative lesions and a full-house immunofluorescence is seen. Although these are clinically divergent autoimmune diseases, neutrophil extracellular traps (NETs) are postulated to be involved in their pathogenesis. NETs are immunogenic, extracellular DNA structures harbouring relevant ANCA- and nuclear auto-antigens. However, it is still unclear how and if NETs can act as a common pathway for both AAV and SLE.

Objectives: To increase our understanding of the potential pathogenic role of NETs in AAV and SLE, the aim of this study was to compare the characteristics of *ex vivo* AAV- and SLE-induced NET formation.

Methods: *Ex vivo* NET formation was quantified by our highly-sensitive NET quantification assay using 3D-confocal microscopy¹ for 82 AAV, 56 SLE patients and 10 healthy controls (HC). Live cell imaging was used to study the morphology and kinetics. Qualitative characteristics of NETs were investigated by immunofluorescence microscopy that detected co-localisation of NET-markers, including citrullinated histon-3 (CitH3) and high mobility group box-1 (HMGB1). Also, the presence of IgG, IgM or IgA autoantibodies on AAV- and SLE-induced NETs was studied. Autoantibodies as trigger of NET formation were investigated by depleting serum from IgG and NET inhibition assays were performed using peptidylarginine deiminase-4 (PAD4) and NADPH inhibitors.

Results: Quantifying *ex vivo* NET formation demonstrated excessive NET formation for both AAV and SLE as compared to HC. AAV-induced NET formation (median [Q1 – Q3]: 20.7 [9.6–74.1]) was significantly higher compared to SLE-induced NET formation (5.6 [2.3–14.3]; $p < 0.0001$). Secondly, live cell imaging revealed lytic NET formation in AAV peaking after 2–4 hours while in SLE non-lytic NET formation with neutrophil clustering occurred within minutes. Thirdly, the presence of CitH3 was significantly higher on AAV-induced NETs, whereas SLE-induced NETs contained significantly more HMGB1. AAV-NETs were triggered independent of IgG, in contrast to IgG dependence of SLE-NETs. Intriguingly, immunofluorescence staining of immunoglobulins revealed a pauci-immune expression on AAV-NETs compared to a full-house expression of IgG, IgM and IgA on SLE-NETs. Both PAD4 and NADPH were involved in AAV- but not in SLE-induced NET formation. To further corroborate on these differences, we found that SLE-NETs were enriched for oxidised mitochondrial DNA as demonstrated by TOMM20 and MitoSOX.

Conclusions: This study demonstrates that excessive NET formation in AAV is intrinsically different to NET formation in SLE. AAV-NETs are characterised by a suicidal lytic PAD4- and NADPH-dependent expulsion of citrullinated NETs, whereas SLE-NETs are characterised by rapidly-induced clusters with HMGB1, enriched for mitochondrial DNA and enhanced immune complex formation altogether supporting a pro-inflammatory role of NETs in the pathophysiology of SLE, including immune-complex mediated, full-house lupus nephritis.

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