Methods: We studied SLE patients with (LS) or without (LN) history of serosites and HC matched for sex and age. Demographic, clinical, therapeutic and clinimetric data were retrospectively collected. After isolation of peripheral blood mononuclear cells (PBMCs) from whole blood by centrifugation on Ficoll gradient, P2 × 7R and NLRP3 expression were evaluated by RT-PCR analysis while activity was analysed after stimulation with BzATP (P2 × 7R agonist), by measuring intracellular calcium changes using Fura2-AM fluorescent probe. Finally *in vitro* IL-1β and IL6 production by short term cultured PBMCs (in RPMI and after stimulation with either LPS or BzATP or BzATP + LPS), and IL-1β and IL6 plasma levels were evaluated by ELISA.

Results: 30 HC and 31 patients were enrolled: 13 (40,6%) LS vs 18 (59,4%) LN. 30 were female. No significant differences about demographic, disease activity and serological features were found between LS and LN and almost all patients were taking low dose steroids and immunomodulatory therapy (table 1). No significant difference in plasmatic levels of IL-1β and IL-6 were found between SLE and HC also considering separately the LS group. RT-PCR analysis of PBMC from SLE subjects showed reduced P2 × 7 and slightly augmented NLRP3 expression. *In vitro* IL-1β release was diminished in SLE patient (both LS and LN) respect to HC (especially comparing LN vs HC) while IL-6 levels appeared to be higher in SLE patients (especially LN) (table 2).

Abstract SAT0006 – Table 1. Clinical features of SLE patients (LS vs LN)

	LS (13)	LN (18)	P
Age (95% CI)	43 (35,8-50)	40 (35,8-44,2)	0,68
Disease duration (months) (95% CI)	141,5 (82,9-200,2)	107,1 (62,7-151,5)	0,31
SLEDAI 2K (95% CI)	5,2 (2,0-8,3)	3,9 (1,4-6,8)	0,34
ECLAM (95% CI)	1,3 (-0,2-2,6)	1,6 (0,4-2,8)	0,68
SLICC (95% CI)	0,8 (0,3-1,4)	0,5 (0,05-0,8)	0,19
ANTI DNA positivity (n%)	13 (100%)	15 (83,3%)	0,49
Low levels of Complement C3 and C4 (n%)	10 (76,9%)	6 (33,3%)	0,03
VES mm/h (95% CI)	15 (12-18)	18 (11-24)	0,37
RCP mg/dl (95% CI)	0,77 (0,13-1,14)	0,47 (0,38-0,56)	0,24
Ongoing immunosuppressive drugs n(%)			
Hydroxychloroquine	10 (76,9%)	14 (77,8%)	1
Azathioprine	2 (11,1%)	2 (15,4%)	1
Mycophenolate mofetil	1 (7,7%)	3 (16,7%)	0,62
Cyclosporine	0	1 (5,6%)	1
Rituximab	0	1 (5,6%)	1,0
Belimumab	1 (7,7%)	0	0,42
Methotrexate	2 (20%)	1 (6,3%)	0,54
Steroids ongoing (n%, dosage)	12 (92,3%)	13 (72,2%)	0,35
Cumulative steroid gr (95% CI)	25,4 (12,6-38,2)	14,1 (6,2-22)	0,09

Abstract SAT0006 – Table 2. activity of P2X7 in HC vs SLE

	HC	LS	LN	p (HC vs LS)	p (LS vs LN)	p (HC vs LN)
IL-1β plasma levels pg/ml, mean (SD)	2,5(0,20)	2,5(0,2)	2,5(0,1)	0,96	0,25	0,97
IL-6 plasma levels pg/ml, mean (SD)	0,32 ± 0,09	0,53 ± 0,45	0,78(0,56)	0,39(0,39)	0,41	0,08
Basal Ca2+ (Fura2) nM mean (SD)	106(17,3)	105,81 ± 11,4	95,61(6,7)	0,08	0,35	0,59
ΔCa2+ (Fura2) nM mean (SD)	128,25(12,08)	18,74(8,3)	61,36(2,7)	0,002	0,84	0,01
Sumatant's dosage of IL-1β produced by PBMC (500 000 cells) pg/ml, mean (SD)						
RPMI	48,56(5,5)	91,94(18,1)	41,14(9,5)	0,08	0,06	0,01
LPS	91,89(29,3)	90,54(24,3)	79(12,1)	0,81	0,02	0,005
LPS+BzATP	99,22(22,4)	100,74(20,8)	100,74(20,8)	0,96	0,29	0,46
BzATP	265,73(29,9)	68,04(56,8)	91,64(78,7)	0,001	0,17	0,001
Sumatant's dosage of IL-6 produced by PBMC (500 000 cells) pg/ml, mean (SD)						
RPMI	2,7 ± 0,3	133,34(165,9)	158,81(21,3)	0,22	0,11	0,46
LPS	79,14 ± 46,6	299,34(108,1)	251,06(23,8)	0,02	0,29	0,005
LPS+BzATP	68,12(47,4)	264,64(114,3)	159,49(8,1)	0,03	0,01	0,07
BzATP	2,84(0,18)	121,23(14,4)	7,89(2,02)	0,001	0,001	0,002
RT-PCR P2X7R mean (SD)	1,34(0,3)	0,82(0,38)	0,72(0,24)	0,59(0,5)	0,12	0,46
RT-PCR NLRP3 mean (SD)	2,21(1,1)	0,01(1,95)	1,12(1,61)	2,94(2,5)	0,45	0,36

Conclusions: P2 × 7R expression and activity appeared to be reduced in SLE compared to HC as demonstrated by evaluation of intracellular calcium changes and IL-1β levels produced by PBMC. Conversely, IL-6 was released at higher levels from PBMC of patients compared to HC, thus suggesting that production of this cytokine could be mediated by another pathway (possibly TLR pathway²). Other P2 × 7 dependent responses, such as pyroptosis or T and B lymphocyte functions, need to be investigated. Overall, these preliminary results suggest that P2 × 7R and inflammasome activation do not seem to be the main pathogenetic pathway in SLE patients with history of serositis.

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Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.7068

SAT0007

DISTINCT MACROPHAGE PHENOTYPE AND BIOENERGETIC PROFILES IN RHEUMATOID ARTHRITIS

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Background: Diversity of macrophage subsets within the joint remains unknown. The concept of macrophage polarisation into M1 inflammatory macrophages and M2 tissue-resolving macrophages, paralleled by changes in the bioenergetic cell profile, has received much attention. Hence, we aimed to examine the phenotype of macrophages within the inflamed RA joint, along with the metabolic and inflammatory capacity of RA monocyte-derived macrophages compared to healthy individuals.

Objectives: To examine the phenotype, metabolic and inflammatory profile of pro-inflammatory and anti-inflammatory macrophages within the inflamed RA joint.

Methods: Blood obtained from healthy and RA donors, CD14 + cells sorted and differentiated into macrophages for 8 days. Macrophages were polarised to either M1 (LPS and IFNγ) or M2 (IL-4). Markers of polarisation, metabolism and inflammation were quantified by Real Time-PCR. Seahorse technology measured the major energy-using pathway, oxidative phosphorylation (OCR). Finally, synovial tissue (ST) was digested to yield a single cell suspension, this was then stained using a panel of fluorochrome antibodies (CD45, CD40, CD68, CD64, CD163, CD206, CD253), and subsequently analysed using FlowJo software.

Results: M1 macrophages were confirmed by increased expression of KLF6 while M2 macrophages expressed high TGM2, PPARG and STAB1. M1 cells had significantly higher expression of pro-glycolytic genes (HIF1α, HK2, LDHA, PFKFB3 and PKM2), which were deficient in M2 macrophages and higher compared to healthy control. This was paralleled by higher pro-inflammatory cytokines levels (IL-8, OSM, MCP-1, RANTES IRAK-1, CCR5 and SOCS3) in M1 vs M2 macrophages, with RA derived macrophages showing higher expressions of pro-inflammatory mediators compared to healthy control. G6PD, PHD3 and PDK1/2 were significantly decreased in M1 yet increased in M2 macrophages, and along with this seahorse technology demonstrated that M2 macrophages have higher baseline OCR. Finally, ST analysis determined approximately 40% of CD45 + cells are positive for the pan-macrophage marker CD68. Interestingly, the classical paradigm of M1 and M2 macrophages is not found. Instead, a spectrum of macrophages were identified with approximately 57% of ST macrophages expressing M2 markers (CD206, CD163) with the M1 activation marker CD40.

Conclusions: This study demonstrated distinct metabolic profiles in M1/M2 RA macrophages; their opposing roles in perpetuating and resolving inflammation, respectively. We have identified, for the first time, a transitional subtype of tissue-specific macrophages, suggesting that these cells remain plastic and function according to their microenvironment.

This study demonstrated distinct metabolic profiles in M1/M2 RA macrophages; their opposing roles in perpetuating and resolving inflammation, respectively. We have identified, for the first time, a dominant transitional subtype of tissue specific macrophages with at least six other phenotypically distinct subtypes also present, suggesting that these cells remain plastic and function according to their microenvironment.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.2195

SAT0008

DYSREGULATION OF THE CIRCULATING AND RENAL CLUSTERIN IN LUPUS NEPHRITIS

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Background: Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease with lupus nephritis (LN) as one of the main causes of poor prognosis. Although the complement system participates in the development of LN, the exact mechanism is not clear. The present study was to investigate the pathogenesis of LN by detecting the levels of Clusterin (CLU), one of complement regulatory proteins in the plasma and renal specimens of patients with LN.

Objectives: To determine the circulating and renal Clusterin levels and its clinical associations in patients with LN.

Methods: Plasma Clusterin levels were examined by enzyme-linked immunosorbent assay in 66 SLE including 50 LN patients and 40 healthy controls. Immunohistochemical method was used to detect the expression of Clusterin in renal biopsy tissues of 16 LN patients. Severity of LN was assessed according to the abbreviated version of the International Society of Nephrology/Renal Pathology Society classification.

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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Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.3942

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

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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.

Acknowledgements: MTA-SE "Lendület" Inflammation Physiology Research Group of the Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary and F.K. was a recipient of János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.5172

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

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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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Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.2429