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	99,72(22,4)	100,71(20,8)	100,72(19,2)	0,99	0,29	0,46
BzATP	265,71(22,9)	68,04(56,8)	91,64(7,7)	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(123,8)	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)	7,83(2,02)	0,001	0,001	0,002
RT-PCR P2X7R mean (±SD)	1,24(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)	3,03(1,95)	3,12(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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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

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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.