Results: Myeloid DCs are scarce in control non-inflammatory OA synovial tissues and their number increased substantially in PsA and RA tissues. Phenotyping data revealed that all myeloid DC subsets can be present in inflamed RA and PsA synovium. However, CD1c$^+$ DC populations (DC2/DC3) were the most abundant in RA synovial tissues and the gene expression analysis of CD1c$^+$ sorted from RA synovial biopsies showed an increase in the expression of epigenetic regulator of inflammatory response miR-155 and IL-6, TNF and IL-23 as compared to circulating cells.
Conclusions: CD1c$^+$DCs from RA synovial tissues had epigenetically regulated activated phenotype (miR-155 and miR-34a^2) that through the production of cytokines could maintain tissue activation of autoreactive Th1 and Th17 cells and contribute to inflammation.

REFERENCES:

[1] Villani AC, et al. Science 2017. doi:10.1126/science.aah4573
[2] Kurowska-Stolarska M, et al. Nat Commun 2017. doi:10.1038/ncomms15877

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SAT0004 INTRA-ARTICULAR INJECTION OF ADIPOSE-DERIVED MESENCHYMAL STROMAL CELLS REDUCE EXPERIMENTAL OA PATHOLOGY VIA IL-1B-MEDIATED REALLOCATION AND ENHANCED PHAGOCYTOSIS OF POLYMORPHONUCLEAR LEUCOCYTES

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Background: Injection of adipose-derived mesenchymal stromal cells (ASCs) into knee joints after induction of experimental inflammatory osteoarthritis (CiOA) reduces development of joint pathology.[1] This protection is only achieved when ASCs are applied in early CiOA, which is characterised by synovitis and high levels of S100A8/A9 and IL-1β, suggesting that inflammation boosts the protective effect of ASCs.[2]
Objectives: To examine the role of synovitis in ASC-mediated amelioration of CiOA pathology.
Methods: CiOA was induced by intra-articular collagenase injection. Knee joint sections were stained with haematoxylin/eosin and immunolocalization of polymorphonuclear leucocytes (PMNs) and ASCs was performed using antibodies for NIMP-R14 and CD271, respectively. Chemokine expression induced by IL-1β or S100A8/A9 was assessed with qPCR and Luminex. Migration of PMNs through transwell membranes towards ASC-conditioned medium (CM) was examined using flow cytometry. ASC-PMN co-cultures were analysed microscopically and with Luminex. Phagocytic capacity of PMNs was measured with labelled zymosan particles.
Results: Intra-articular injection of saline in knee joints of day 7 CiOA induced a flare already after 6 hours, characterised by particularly PMNs scattered throughout the synovium. Although ASC injection resulted in comparable numbers of PMNs, these cells however, were clustered around ASCs. IL-1β-stimulation of ASCs *in vitro* strongly increased expression of PMN-attracting chemokines KC, CXCL5, and CXCL7, whereas S100A8/A9 did not. Migration of PMNs towards CM of IL-1β-stimulated ASCs (IL-1β-CM) was significantly enhanced (2.9-fold increase) when compared to CM of non-stimulated ASCs (NS-CM). After 6 hours co-culturing PMNs with IL-1β-stimulated ASCs, the number of clustered PMNs per ASC was significantly increased. Interestingly, association of PMNs with ASCs significantly diminished the release of KC protein by ASCs (69% lower after 24 hour), and also strongly reduced the release of S100A8/A9 protein by the PMNs. Moreover, phagocytic capacity of PMNs was strongly enhanced after priming with CM of IL-1β-stimulated ASCs.
Conclusions: Local application of ASCs in inflamed CiOA knee joints results in attraction and clustering of PMNs with ASCs in the synovium, which is likely mediated by IL-1β-induced up-regulation of chemokine release by ASCs. This results in lowered S100A8/A9 levels and enhanced phagocytic capacity of PMNs, enabling the clearance of debris to attenuate synovitis and promote tissue repair.

REFERENCES:

[1] ter Huurne M, et al. Antiinflammatory and chondroprotective effects of intraarticular injection of adipose-derived stem cells in experimental osteoarthritis. Arthritis Rheum 2012. Nov;64(11):3604–13.
[2] Schelbergen RF, et al. Treatment efficacy of adipose-derived stem cells in experimental osteoarthritis is driven by high synovial activation and reflected by S100A8/A9 serum levels. Osteoarthritis Cartilage 2014 Aug;22 (8):1158–66.

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SAT0005 INTERLEUKIN-33 AMELIORATES MURINE LUPUS VIA INDUCTION OF REGULATORY T CELLS AND M2 MACROPHAGE POLARISATION

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Background: The levels of IL-33, a Th2 promoting cytokine, and the soluble form of its receptor ST2 were reported to be elevated in serum of patients with active systemic lupus erythematosus (SLE), suggesting a role of the IL-33/ST2 axis in the pathogenesis of SLE.
Objectives: This study aims to examine the effect of IL-33 in disease severity of murine lupus.
Methods: IL-33 was injected intraperitoneally 3 times per week to pre-diseased MRL/lpr mice aged 12 weeks for 6 weeks. Control group was given 1% BSA injection. Urine protein was monitored weekly by albustix and protein assay. Immunophenotyping of splenocytes was examined by flow cytometry. Splenic CD11b +monocytic cells were isolated by microbeads for mRNA examination.
Results: IL-33-treated mice (n=9) developed significantly less proteinuria compared to BSA-treated group (n=9). Kidney histology of the IL-33-treated group showed remarkably less mesangial deposit, diffuse proliferative glomerular changes and crescents, and had significantly lower renal composite score compared to controls (median 2.0 vs 9.9, p<0.001). Kidneys of these mice expressed lower mRNA levels of TNF-α (32.1±14.7 vs 77.0±27.8, p<0.001), IL-6 (median 0.6 vs 4.7, p=0.003), IL-1β (31.1±10.1 vs 77.8±24.6, p<0.001) and iNOS (p=0.006). Immunophenotyping of splenocytes showed significantly increased CD4 +CD25+regulatory T (Treg) cells (4.0%±1.2% vs 2.2±0.2%, p<0.001) that expressed remarkably higher Foxp3 (76.0%±5.0% vs 59.3±12.6%, p=0.002). Splenic extracts showed predominant Gata3 (0.37±0.20 vs 0.12±0.09, p=0.01) and Foxp3 (0.42±0.16 vs 0.17±0.11, p=0.002) mRNA in IL-33-treated mice. These Treg cells expressed high cell surface ST2 (8.9%±2.7% vs 4.5±2.0%, p=0.008). There was significant expansion of splenic CD11b+population in IL-33-treated mice (17.8±10.5 vs 8.8±3.0, p=0.01) that expressed significantly higher CD206 (5.2%±0.9% vs 2.9±0.9%, p=0.002). Isolated splenic CD11b+cells expressed significantly higher mRNA of Arg1, FIZZI and Ym-1 and IL-10 (all p=0.01) with reduced expression of iNOS (p=0.02). Kidney extracts of IL-33 treated mice also had elevated mRNA levels of M2 markers including Arg1 (median 199.8 vs 36.1, p=0.004) and FIZZI (median 25.0 vs 2.7, p<0.001) and reduced MCP-1 (12.7±6.5 vs 35.1±12.0, p<0.001). There was also significantly higher levels of mRNA of Foxp3 (median 43.0 vs 20.8, p=0.006) and Gata 3 (1.7 ±0.5 vs 0.9±0.5, p=0.008) but lower Rorc (2.6±1.0 vs 3.8±0.8, p=0.008) and Tbx21 (12.6±6.0 vs 29.6±13.7, p=0.003) in the kidneys.
Conclusions: Exogenous IL-33 led to significantly less proteinuria and renal inflammation. These mice had significantly higher splenic Treg cells with prominent Foxp3 expression. Isolated CD11b+cells from spleen and kidney extracts demonstrated mRNA levels of M2 macrophage polarisation.
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SAT0006 P2X7 RECEPTOR IN SYSTEMIC LUPUS ERYTHEMATOSUS (SLE). EXPLORING A NOVEL PATHOGENETIC PATHWAY IN LUPUS RELATED SEROSITIS

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Background: Recent studies have focused attention on the involvement of innate immunity and in particular on the activation of NLRP3 inflammasome by purinergic signalling mediated by P2 × 7 receptor (P2 × 7R), in SLE pathogenesis.[1] Serositis are typical SLE manifestations often associated with increased inflammatory indices and promptly responding to colchicine whose action could be mediated by its effect on microtubules during P2 × 7R assembly.
Objectives: To explore the role of innate immune system in SLE evaluating expression and activity of P2 × 7R and NLRP3, comparing patients with positive and negative history of serositis with healthy control subjects (HC).

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 NLP3 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 i*n 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 an LN) respect to HC (expecially comparing LN vs HC) while IL-6 levels appeared to be higher in SLE patients (expecially 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
Ongoings immunosoppressive 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 ongoings (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	SLE	LS	LN	p (HC vs SLE)	p (LS vs LN)	p (HC vs LS)	p (HC vs LN)
IL-1β plasma levels pg/ml; mean (±SD)	2,3±0,08	2,5±0,2	2,3±0,11	2,8±0,8	0,56	0,26	0,97	0,38
IL6 plasma levels pg/ml; mean (±SD)	0,32 ± 0,09	0,53± 0,45	0,78±0,56	0,39±0,29	0,41	0,08	0,14	0,83
Basal Ca2+ (Fura2) nM mean (±SD)	106±17,3	105,3± 11,4	95,6±16,7	116,4±15,2	0,88	0,35	0,59	0,71
ΔCa2+ (Fura2) nM mean (±SD)	128,25±52,08	59,7±28,3	58,3±7,3	61,3±29,7	**0,002**	0,84	**0,01**	**0,02**
Surnatant's dosage of IL-1β produced by PMBC (500 000 cells) pg/ml; mean (±SD)								
RPMI	48,5±19,5	81,9±18,1	41,1±19,5	21,4±8,7	0,08	0,06	0,46	**0,01**
LPS	93,8±29,3	50,5±24,3	79±12,1	31,2±10,7	**0,01**	**0,002**	0,16	**0,004**
LPS+BzATP	967,2±224	1026,7±509,8	1003.4±672.6	1046.7±376.5	0,96	0,39	0,46	0,46
BzATP	265,7±159,9	68,0±56,8	91,6±78,7	47,8±15,9	**0,001**	0,17	**0,043**	**0,045**
Surnatant's dosage of IL6 produced by PMBC (500 000 cells) pg/ml; mean (±SD)								
RPMI	2,7 ± 0,3	133,3±165,9	59,8±121,3	255,7±176,0	0,22	0,11	0,46	0,07
LPS	79,1± 46,6	299,1±108,1	251,0±123,8	368,1±88,5	**0,02**	0,29	**0,03**	**0,049**
LPS+BzATP	69,12±47,4	264,6±114,3	193,4±98,1	359,6±33,5	**0,03**	**0,04**	0,07	**0,049**
BzATP	2,84±0.18	115, /±164,4	7.83±0.02	259,/±169,1	0,28	**0,04**	0,39	0,052
RT-PCR P2X7R mean (±SD)	1,3± 0,5	0,82±0,38	0,72± 0,24	0,93±0,5	0,12	0,46	0,1	0,36
RT-PCR NLP3 mean (±SD)	2,2±1,11	3,03±1,95	3,12±1,61	2,94±2,5	0,45	0,6	0,36	0,71

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 pathway[2]). 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.

REFERENCES:

[1] Francesco Di Virgilio, et al. Purinergic signalling in autoimmunity: A role for the P2X7R in systemic lupus erythematosus? Biomedical journal 2016;39:326–338.
[2] The impact of agonists and antagonists of TLR3 and TLR9 on concentrations of IL-6, IL10 and sIL-2R in culture supernatants of peripheral blood mononuclear cells derived from patients with systemic lupus erythematosus. Agnieszka Klonowska-Szymczyk et al; Postepy Hig Med Dosw (online), 2017; 71: 867–875.

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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, parelled 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 transitionary subtype of tissuespecific 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 transitionary 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.