Innate immunity in rheumatic diseases

**SAT0001 MECHANISM AND SIGNIFICANCE OF COMPLEMENT C3 RECEPTOR IN COLLAGEN-INDUCED RHEUMATOID ARTHRITIS MICE MODEL**

L. Dan1, L. Ke2. 1Department of Rheumatology and Immunology, Xi'an No. 5 Hospital, 2Core Research Laboratory, the Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China

**Background:** Rheumatoid arthritis (RA) is a kind of chronic autoimmune disease, mainly manifested as small joint synovitis. Disease progression appears joint swelling, bone and cartilage damage, deformity and action activity. The etiology of RA is still unclear and is generally considered to be an immune-mediated inflammatory disease. The activation pathway and regulation function of the complement system have become the hotspot of the research of RA pathogenesis. Previous studies have found that C3aR knockout mice had lower levels of antibodies to collagen in the Type II collagen-induced rheumatoid arthritis (CIA model), and the molecular mechanism is unclear. The small fragment C3a and large segment iC3b produced by C3 activation are the main effect products, and their biological effects are performed by combining with specific receptors, C3aR and CD11b respectively.

**Objectives:** To investigate the mechanism of C3aR and C3b, the cleavage products of complement C3 in rheumatoid arthritis, binding to their corresponding receptors, the signalling pathway of complement activation and the effect on arthritis conditions.

**Methods:** This study was intended to establish a CIA model on C3aR knockout and CR3 knockout mice (C3aR+/− and CD11b−/−) to investigate the effect of complement C3a-C3aR signalling and iC3b-CR3 signalling on rheumatoid arthritis. Methods using C57BL/6 background transgenic mice (Gifted by King's College London), mice were divided into 3 groups according to the experimental mouse strains: C3aR−/− group, CD11b−/− group and WT control group. The clinical score of the joints in each group was measured after the establishment of the CIA model through collagen induction. Moreover, joint specimens were collected for pathologic grading. Besides, the level of CD4+ T cell, CD8+ T cell, Th17/Treg ratio and the level of IFN-γ/IFN-α of NK cell in mouse spleen were detected by flow cytometry.

**Results:** The clinical score of C3aR−/− group was slightly lower than that of WT group, and the clinical score of CD11b−/− group was significantly higher than that of WT group; 2. Pathological score (12 points): The CIA scores of CD11b−/− group, C3aR−/− group and WT group were 9.35±0.75, 4.81±0.63 and 5.85±0.55 respectively. The CIA scores of CD11b−/− group was significantly higher than that of WT group, which were consistent with clinical score; 3. Through the flow cytometry detection, compared with the WT group, CD4+ T cell, CD8+ T cell and Th17/Treg ratio and the level of IFN-γ/IFN-α of NK cell in mouse spleen were detected by flow cytometry.

**Conclusions:** The CIA model of C3aR−/− group had lower clinical score, pathological score, Th17/Treg ratio and IFN-γ/IFN-α. This study verified the clinical significance of C3a-C3aR signalling and iC3b-CR3 signalling in rheumatoid arthritis.

**Disclosure of Interest:** None declared

**DOI:** 10.1136/annrheumdis-2018-eular.1292

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**SAT0003 SYNOVIAL TISSUE CD1C+ DENDRITIC CELLS IN RHEUMATOID ARTHRITIS EXPRESS HIGH LEVELS OF THE EPIDGNETIC REGULATOR OF INFLAMMATION, MICRONRMA-155 AND INFLAMMATORY CYTOKINES**

A. Elmesmari1, S. Alivernini2, B. Tolusso3, L. Bui4, D. Vaughan1, M.R. Gigante5, F. Federico3, G. Ferraccioli2, E. Gremente2, I.B. McInnes1,4, L. Dan1, L. Ke2, A. Maioli2, M. Kowalczyk3, T. Stolarski1, M. Stolfanski1,4, 1Institute of Infection, Immunity, and Inflammation, University of Glasgow, Glasgow, UK; 2Division of Rheumatology, Fondazione Policlinico Universitario A. Gemelli, Catholic University of the Sacred Heart; 3Institute of Pathology, Fondazione Policlinico Universitario A. Gemelli – Catholic University of the Sacred Heart, Rome, Italy; 4Rheumatoid Arthritis Pathogenesis Centre of Excellence (RACE), Glasgow, Birmingham, Newcastle, UK

**Background:** Dendritic cells (DCs) direct the immune response against pathogens while maintaining self-tolerance by instructing T/B cells in lymphoid organs and peripheral tissues. However, their aberrant activation can lead to chronic inflammation and autoimmunity. Based on the distinct transcriptional, function and distribution, DCs can be broadly categorised as plasmacytoid and myeloid (conventional) DCs. Based on recent single cells sequencing and secretome data, can be divided into CD14+ DCs (DC1), CD14−CD16+ DCs (DC2), CD14−CD16+ CD1C− DCs (DC2a) and CD14−CD16+ CD1C+ CD2a− DCs (DC2b). In addition, populations of CD1c+ CD141+ CD16+ DC (named DC4), that shares some gene expression with DC16+ monocyte and inflammatory DC (inDC). Most to date studies on Rheumatoid Arthritis patients investigated DCs in circulation or in synovial fluid (SF). This provided important insight into epigenetic changes in DC precursors before they enter synovial tissue, e.g. RA blood CD1c+ have deregulated microRNA-34a driven epigenetic control of anti-inflammatory Axl pathway and/or the influence of inflammatory milieu on DCs, respectively. However, neither (peripheral blood) PB or SF are major sites for DC regulated T/B cell activation; instead, DCs control immune responses in the appropriate structures of lymph organs and tissues.

**Objectives:** In this study, we sought to investigate myeloid DCs in synovial tissue with the prospect of better understanding their role in driving autoimmunity in RA.

**Methods:** We developed a flow cytometry sorting strategy to characterise the phenotype of distinct myeloid DC subsets in multiple biological compartments (PB, SF and synovial tissue). Synovial tissue (ST) biopsies (RA n=9; Psoriatic arthritis n=3) were digested with liberase prior the analysis. Peripheral blood DCs (RA n=19, Psoriatic arthritis n=16, healthy donors n=12), and SF DC (n=3) were used as comparators. Synovial tissue, SF and PB DCs were sorted and microRNAs, pro-inflammatory and regulatory cytokine expression analysed by amplified qPCR. In addition, DCs were mapped in synovial tissue in RA (n=8), PsA (n=7) and control non-inflammatory OA (n=5) by immunohistochemy.
SAT0004
INTRA-ARTICULAR INJECTION OF ADIPOSE-DERIVED MESENCHYMAL Stromal CELLS REDUCE EXPERIMENTAL OA PATHOLOGY VIA IL-1β-MEDIATED REALLOCATION AND ENHANCED PHAGOCYTOSIS OF POLYMORPHONUCLEAR LEUCOCYTES
S. van Dalen1, M. van den Bosch1, A. Blom1, V. van der Kraan1, T. Vogl2, T. Casteilla3, P. van Lent1.
1Experimental Rheumatology, Radboud University Medical Center, Nijmegen, Netherlands; 2Institute of Immunology, University of Munster, Munster, Germany; 3STROMAlab, University of Toulouse, Toulouse, France
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-stimulated ASCs (IL-1β-stimulated ASCs, the number of clustered PMNs was significantly increased (2.9-fold increase) when compared to CM of non-stimulated ASCs (NS-CM). After 6 hours co-culturing of 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 hours), 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:

Disclosure of Interest: None declared

SAT0005
INTERLEUKIN-33 AMELIORATES MURINE LUPUS VIA INDUCTION OF REGULATORY T CELLS AND M2 MACROPHAGE POLARISATION
M.Y. Mok1, K. Law2, W.Y. Kong3, Y. Lo4, E. Ng1, C. Luo3, F. Huang4, G. Chan4, K. Chan1, Department of Biomedical Sciences, City University of Hong Kong, Kowloon, Hong Kong
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 that inflammation boosts the protective effect of ASCs.2

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). Kidney extracts of these mice expressed lower mRNA levels of TNFα (median 0.21±0.07 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 77.0±24.6, p<0.001) and IFN-γ (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±5.2% vs 59.3±12.6%, p=0.002). Splenic Treg cells showed predominant mRNA expression of GATA 3 (1.7±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=0.001) 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, FIZZ1 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 FIZZ1 (median 25.0 vs 7.4, p=0.001) but lower 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 0.5±0.5, p=0.008) but lower Rorc (2.6±1.0 vs 3.8±0.8, p=0.008) and Tbx21 (12.6±5.0 vs 29.6±13.7, p<0.001) 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.

Disclosure of Interest: None declared

SAT0006
P2X7 RECEPTOR IN SYSTEMIC LUPUS ERYTHEMATOUS (SLE), EXPLORING A NOVEL PATHOGENETIC PATHWAY IN LUPUS RELATED SEROSITIS
F. Furini1, A. Bottoluzzi1, A.L. Giuliani2, F. Di Virgilio3, M. Govoni4, 1Dept of Medical Sciences, Section of Haematology and Rheumatology, University of Ferrara, Cona (Fe), 2Dept of Morphology, Surgery and Experimental Medicine, Section of Pathology, Oncology and Experimental Medicine, University of Ferrara – ITALY, Ferrara, Italy
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 P2X7 receptor (P2X7R) in SLE pathogenesis. Serositis is 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 P2X7R assembly.

Objectives: To explore the role of innate immune system in SLE evaluating expression and activity of P2X7R and NLRP3, comparing patients with positive and negative history of serositis with healthy control subjects (HC).

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Disclosure of Interest: None declared

SAT0007
DOI: 10.1136/annrheumdis-2018-eular.6478

Disclosure of Interest: None declared