FRIDAY, 15 JUNE 2018

Basic science in paediatric rheumatology

FRI0001

EXTENDED OLIGOARTICULAR AND POLYARTICULAR JUVENILE IDIOPATHIC ARTHRITIS PATIENTS HAVE A SIMILAR B CELL PHENOTYPE WHEN COMPARED TO ESTABLISHED RHEUMATOID ARTHRITIS

R.A. Moura¹, A. Brito¹, S. Oliveira¹, R.L. Teixeira^{1,2}, V.C. Romão^{1,2}, V. Teixeira^{1,2}, R. Campanilho-Marques^{1,2}, F. Oliveira-Ramos^{1,2}, J.E. Fonseca^{1,2}. ¹Instituto de Medicina Molecular João Lobo Antunes; ²Rheumatology Department, Centro Hospitalar de Lisboa Norte, EPE, Hospital de Santa Maria, Lisbon Academic Medical Centre, Lisbon, Portugal

Background: Our group has recently described that the majority of polyarticular juvenile idiopathic arthritis (pJIA) and a large fraction of extended oligoarticular JIA (oJIA) patients fulfil classification criteria for rheumatoid arthritis (RA) in adulthood. B cells play several important roles in RA pathogenesis, but it is still unclear if the pattern of B cell involvement in pJIA and extended oJIA follows what has been described for adults with RA.

Objectives: The main goal of this study was to characterise peripheral blood B cell phenotype and cellular activation in pJIA and extended oJIA patients when compared to established RA.

Methods: Blood samples were collected from JIA patients (n=10; mean age 10±4 years), established RA patients treated with synthetic DMARDs (n=10; mean age 72±7 years) and two corresponding groups of age- and sex-matched healthy donors. B cell phenotype was characterised by flow cytometry and B cell apoptosis was assessed after 48 hour of *in vitro* cell culture.

Results: JIA patients recruited in this study were either classified as extended oJIA (n=6) or pJIA (n=4). Seven JIA patients (4 extended oJIA and 3 pJIA) were treated with methotrexate and three patients (2 extended oJIA and 1 pJIA) were untreated. We found that JIA patients had similar CD19 +B cell levels in circulation when compared to controls, but significantly higher CD19 +B cell frequencies in comparison to established RA. In addition, increased frequencies of transitional (IgD +CD38++) and naïve (IgD +CD27+) B cell subpopulations were observed in JIA patients when compared to RA. However, established RA patients had significantly higher levels of CD21^{low}CD38^{low}, post-switch (IgD-CD27+) and IgD-CD27-memory B cell subsets when compared not only to controls, but also to JIA patients. No significant differences were detected in pre-switch (IgD +CD27+) memory and plasmablasts (IgD-CD38++) levels in JIA patients when compared to both controls and RA. Furthermore, the frequency of CD5 +B cells, CD5 median fluorescence intensity (MFI), CD40 MFI and CXCR5 MFI B cell expression levels were significantly increased in JIA patients when compared to established RA, but not to controls. No significant differences were observed between JIA and established RA patients in BAFF-R, FcγRIIB, CD21, CD23, CD38, CD86, CD95, HLA-DR, TLR9 and RANKL expression on B cells. After 48 hour of *in vitro* cell culture a significantly higher B cell death was found in JIA in comparison to RA patients.

Conclusions: The increased frequencies of transitional, naïve and CD5 +B cells in circulation and reduced levels of memory B cell subpopulations in JIA patients when compared to established RA are probably related to an immature immune system present in children when compared to adults. Nevertheless, the similarity in B cell phenotype found between extended oJIA, pJIA and established RA patients suggests an early B cell involvement in the pathogenesis of these two categories of JIA.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.5963

FRI0002

ANALYSIS OF B CELLS AND T CELLS SUBPOPULATIONS AND COLLAGEN SPECIFIC T CELL REPERTOIRE IN JUVENILE IDIOPATHIC ARTHRITIS PATIENTS

G. Di Sante^{1,2}, M.C. Caparelli³, B. Tolusso², C. Di Mario², M. Valentini¹, F. Ria¹, G. Ferraccioli², R. Cimaz³, E. Gremese². ¹Institute of General Pathology, Università Cattolica del Sacro Cuore – Fondazione Policlinico Universitario A. Gemelli; ²U.O. C. of Rheumatology, Fondazione Policlinico Universitario A. Gemelli – Università Cattolica del Sacro Cuore, Rome; ³Institute of Pediatric Rheumatology, Azienda Ospedaliera A. Meyer, Florence, Italy

Background: The cause of the breach in immune tolerance in the arthritic joint is not fully understood; many associations between subsets of JIA and HLA and non-HLA molecules have been described.¹ An important role is played by T cell population, that is driven also by its specific T cell receptor (TCR) repertoire; it has been previously observed that synovial T cells exhibit oligoclonal TCR repertoires.²

Objectives: To examine frequency and distribution of human Collagen_{261–273}-specific T cells and the phenotypes of B and T cells subpopulations and the role of DR alleles in JIA, in order to find new biomarker for management of JIA.

Methods: HLA genotyping and CDR3 TRBV-TRBJ spectratyping (TCR repertoire Immunoscope analysis)³ were performed on Peripheral blood mononuclear cells (PBMCs) on a total of 40 Juvenile Idiopathic arthritis (JIA) patients (and in 2 cases also in samples of Synovial fluids) and 6 Healthy Controls. The enrolled patients were mainly affected by polyarticular arthritis (26 out 40) and were free of CS (38 out 40) and cDMARDs (24 out 40) treatments. The mean disease duration was 37 months. All the patients were ACPA and RF negative and the mean ESR and PCR values were respectively 39.5±32.4 mm/h and 17.5±37.4 mg/L. B cells and T cells subpopulations were analysed by flow cytometer assays.

Results: In our cohort 4 patients were DR4+ (10%) and 8 were DR1+ (20%). In the entire cohort no differences were found in terms of B cells subpopulations, but dividing the cohort on the basis of the age of disease onset it was possible to identify a upregulation of Switched B cells compartment in younger patients more than the JIA with an exordium after 12 years, confirming the data recently published.⁴ We checked for the presence of collagen specific TRBV25-TRBJ2.2 T cells, whom the expansion were significantly associated with disease activity and modulated by therapy in RA patients, as described in our previous work.³ Our preliminary results in a so small cohort of patients indicate that the same expansion in JIA patients seems to associate with JADAS and DR4/DR1 positivity, independently from any conventional and biological treatment. Moreover our T cells subpopulation analysis allowed to find interesting correlation between Tregs and switched memory, Tregs and double negative (IgD^{neg}/CD27^{neg}) B cells (r=0.476, p=0.04) and Tregs and DR4/1 positivity (0.432 p=0.03) and between CD27^{high}/CD38^{high} cells and IL17a producing cells (r=0.414, p=0.04); these correlations are more significant in JIA patients with a disease onset at a young age (age <6 years).

Conclusions: These preliminary results suggest that the analysis of collagen specific T cells repertoire, T and B cells subpopulations and HLA-DR haplotype can provide useful information to characterise peculiar details of each JIA patient

REFERENCES:

- [1] Ravelli, Martini. Lancet 2007.
- [2] Wedderburn LR, et al. Int Immunol 1999.
- [3] Di Sante, et al. Ebiomedicine 2015.
- [4] Marasco E, et al. Arth Rheum 2018.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.5415

FRI0003

EPIGENETIC ALTERATIONS LEADING TO SPECIFIC EXPRESSION PATTERNS OF IMMUNE RESPONSE REGULATING GENE MIGHT BE RESPONSIBLE FOR DISTINCTIVE MICROBIOTA COMPOSITION AND DISEASE DEVELOPMENT IN JUVENILE SPONDYLOARTHRITIS PATIENTS

L. Lamot^{1,2}, K. Gotovac Jercic³, A. Blazekovic³, T. Catela Ivkovic⁴, M. Vidovic¹, M. Lamot¹, S. Kapitanovic⁴, F. Borovecki³, M. Harjacek^{1,2}. ¹Department of Pediatrics, Clinical Hospital Center Sestre Milosrdnice; ²Department of Pediatrics; ³Department of Functional Genomics, University of Zagreb School of Medicine; ⁴Laboratory for Personalized Medicine, Rudjer Boskovic Institute, Zagreb, Croatia

Background: Juvenile spondyloarthritis (jSpA) is a diverse group of related syndromes with shared symptoms and pathogenic mechanisms in which both extrinsic environmental factors and intrinsic genetic background perpetuate inflammatory response through immune system alterations. Recently obtained gene signatures in jSpA patients revealed TLR4 and CXCR4 gene had increased, while NLRP3 and PTPN12 had decreased expression.¹ Although gene expression is regulated by various mechanisms, the increasing numbers of studies is showing the importance of epigenetic mechanisms in this fundamental biological process.

Objectives: To investigate the possible mechanistic role of DNA promoter region methylation and several non-coding micro RNA (miR-150, miR-146a, miR-181a, miR-223) in jSpA patients regarding the expression of genes with previously observed alterations.

Methods: The expression of specific microRNAs was analysed in 8 jSpA patients and 5 matched controls using RT-PCR with predeveloped microRNA assays. Methylated DNA Immunoprecipitation (MeDIP) was performed in 19 patients and 7 controls. Enrichment in MeDIP fraction was determined by qRT-PCR using the AriaMx.

Results: The difference in fold enrichment of immunoprecipitated DNA was significant only for NLRP3 promotor site (p=0.0220). Expression analysis of selected

miRs showed no significant difference in fold change between jSpA patients and healthy controls.

Table 1. Results of DNA promoter methylation and previously performed gene expression analysis in juvenile spondyloarthritis patients¹

GENES	Fold Enrichment of Immunoprecipitated DNA		Fold Change of Expression ¹	
	Controls (n=7)	p	Fold Change	p
Patients (n=19)				
TLR4	0213	0153	0,5759	+3,91 <0,01
NLRP3	11,24	5412	0,0220	-5,00 <0,01
CXCR4	0303	0170	0,2658	+1,87 <0,01
PTPN12	0338	0202	0,3768	-2,63 <0,01

Conclusions: Our study indicated epigenetic modifications are probably responsible for some of the expression alterations in jSpA patients in the initial phase of the disease. Since NLRP3 has a crucial role in inflammasome assembly and inflammasomes have been shown to shape microbiota, it is reasonable to assume dysbiosis in jSpA patients can at least partially be explained by reduced NLRP3 expression due to hypermethylation, stressing for the first time the epigenetic contribution to jSpA pathophysiology. While it is still not clear if these epigenetic alterations are caused by genetic mutations in epigenetic factors or exposure to certain environmental factors that mediate the occurrence of aberrant epigenetic profiles, the discovery of DNA methylation-based signature of the NLRP3 gene could have important implications in addressing extrinsic and intrinsic contribution to jSpA pathophysiology, whereas the possibility of reverting epigenetic modifications opens whole new prospects for therapeutic treatment of this complex disease.

REFERENCE:

- [1] Lamot L, Borovecki F, Tambic Bukovac L, Vidovic M, Perica M, Gotovac K, et al. Aberrant expression of shared master-key genes contributes to the immunopathogenesis in patients with juvenile spondyloarthritis. *PLoS ONE* 2014;9(12):e115416.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.2326

FRI0004

GRANULOCYTE MACROPHAGE COLONY STIMULATING FACTOR IS SECRETED AT HIGHER LEVELS FROM STIMULATED MONOCYTE-DERIVED MACROPHAGES FROM PATIENTS WITH ENTHERSITIS RELATED ARTHRITIS AND IS SIGNIFICANTLY ENHANCED BY THE UNFOLDED PROTEIN RESPONSE

C. Fisher^{1,2}, D. Eleftheriou¹, D. Sen^{1,2}, Y. Ioannou¹. ¹Arthritis Research UK Centre for Adolescent Rheumatology, University College London; ²National Institute for Health Research University College London Hospitals Biomedical Research Centre, University College London and University College London Hospital, London, UK

Background: Entersitis related arthritis (ERA) is a subtype of juvenile idiopathic arthritis exhibiting many similarities to the adult spondyloarthropathies (SpA). The innate immune system and intracellular stress responses, including the unfolded protein response (UPR), have been implicated in the pathogenesis of SpA. Granulocyte macrophage colony stimulating factor (GM-CSF), as well as being a haemopoietic growth factor, plays a central role in regulating innate immunity and has recently been implicated in the pathogenesis of SpA¹ but has not been studied in ERA.

Objectives: To compare levels of GM-CSF produced by monocyte-derived macrophages (MDMs) from patients with ERA and healthy controls and to observe the effect of inducing the UPR on those levels.

Methods: Peripheral blood monocytes were isolated from 39 patients with ERA (68% HLA B27 positive, 84% male, median age 16 years 4 months, median disease duration 3 years 10 months) and 21 age and gender-matched healthy controls and differentiated *in vitro* with macrophage-colony stimulating factor. Cells were treated with interferon gamma for 24 hours to upregulate HLA B, washed and then stimulated with lipopolysaccharide (LPS) alone (50 ng/mL) or LPS and tunicamycin (TM) (5 µg/mL), an inducer of the unfolded protein response. GM-CSF was measured from the cell culture supernatants after 24 hours culture by luminex assay.

Results: Levels of GM-CSF at baseline were similar in patients and healthy controls [median 121.3 pg/mL (IQR 96.6–194.0 pg/mL) vs 157.1 pg/mL (124.2–203.3 pg/mL), *p*=0.1]. However, with LPS stimulation, MDMs from patients secreted significantly higher levels of GM-CSF [median 1853 pg/mL (IQR 1206–3061 pg/mL) vs 1342 pg/mL (IQR 713.3–1797 pg/mL), *p*=0.0057]. On stimulation with TM in addition to LPS, GM-CSF production was further enhanced in both patients and healthy controls [median 9027 pg/mL (IQR 4746–13961 pg/mL) vs

3834 pg/mL (IQR 1603–9158 pg/mL)] and remained significantly higher in patients (*p*=0.0096). To investigate the effect of the UPR, fold change in GM-CSF was calculated for each sample between MDMs stimulated with LPS alone and MDMs stimulated with both LPS and TM. Median fold change in patients was 3.95 (IQR 1.54–5.47) and 2.36 (IQR 0.49–4.69) in healthy controls. Interestingly, MDMs from patients who were HLA B27 positive exhibited significantly higher median fold change in GM-CSF with UPR induction compared to HLA B27 negative patients [4.14 (IQR 2.22–6.10) vs 1.33 (IQR 0.36–3.91), *p*=0.0098]. No associations were seen with different treatment regimes in the patient group.

Conclusions: MDMs from patients with ERA produce significantly higher levels of GM-CSF after stimulation compared to healthy controls and this is further enhanced by the UPR, especially in HLA B27 positive patients. These results potentially implicate GM-CSF in the pathogenesis of ERA and thus further support the concept of GM-CSF as a novel target for treatment in certain subgroups of patients.

REFERENCE:

- [1] Al-Mossawi MH, Chen L, Fang H, Ridley A, de Wit J, Yager N, et al. Unique transcriptome signatures and GM-CSF expression in lymphocytes from patients with spondyloarthritis. *Nat Commun* 2017 Nov 15;8(1):1510.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.5457

FRI0005

THE RELATIONSHIP BETWEEN JUVENILE SYSTEMIC LUPUS ERYTHEMATOSUS AND THE TRANSCRIPTION FACTORS NF-KAPPAB AND PPAR-GAMMA

S. Sahin¹, S. Dumus², A. Adrovic¹, K. Barut¹, R. Gelisgen², H. Uzun², O. Kasapcopur¹. ¹Pediatric Rheumatology; ²Department of Biochemistry, Istanbul University, Cerrahpasa Medical School, Istanbul, Turkey

Background: Systemic lupus erythematosus (SLE) is an autoimmune disease characterised by high-levels of autoantibodies mainly targeting nuclear antigens and loss of self-tolerance. Peroxisome-proliferator activated receptor gamma (PPARγ) and nuclear factor-kappa beta (NF-κB) are transcription factors, which, within normal levels, have shown to be crucial in immunomodulation namely, activation and development of normal lymphocytes, negative and positive selection of T and B cells. High-levels of NF-κB has inflammatory properties such as release of autoreactive T cells. On the contrary, PPARγ has anti-inflammatory effects, which has been demonstrated to be effective when used early in prevention of disease in murine models of systemic lupus erythematosus.

Objectives: Herein, we investigated whether NF-κB and PPARγ could exert opposite effects in the immune response and the possible implications in immunomodulation of juvenile systemic lupus erythematosus.

Methods: Serum NF-κB and PPARγ levels were measured in 42 juvenile systemic lupus erythematosus. In addition, 19 juvenile systemic sclerosis and 25 age-matched healthy children were selected for patient control and healthy control, respectively. We have also assessed the relation of these transcription factors with disease activity and anti-ds DNA.

Results: The control group did not differ from the juvenile SLE and juvenile systemic sclerosis patients for age (*p*>0.05). According to our study, serum NF-κB levels of juvenile SLE and juvenile systemic sclerosis patients were significantly higher (1.87±1.0 and 2.17±1.0 versus 1.25±0.7), while serum PPARγ levels were significantly lower than that of healthy controls (1.58±0.6 and 1.52±0.5 versus 2.03±0.9). The difference was not significant between juvenile systemic lupus erythematosus and juvenile systemic sclerosis. In patients with juvenile systemic sclerosis serum NF-κB levels negatively correlated with serum PPARγ levels (*R*=−0.49; *p*=0.032); however, this relationship was not observed in juvenile SLE patients and healthy controls.

Conclusions: Increased serum NF-κB levels represent upregulated signalling cascades, so it is associated with increased levels of pro-inflammatory cytokines. Since juvenile systemic sclerosis and juvenile systemic lupus erythematosus are autoimmune diseases, patients had high levels of NF-κB and low levels of PPAR than controls, as expected. Previous studies revealed that PPARγ activation inhibits NF-κB transcriptional activity. Correlation results in juvenile systemic sclerosis cohort are compatible with this finding, however not in juvenile systemic lupus erythematosus patients. This could be due to the limited number of patients. Further studies with large number of patients are needed to better elucidate the implication of these transcription factors in therapeutic pathways.

REFERENCES:

- [1] Zubair A, Frieri M. NF-κB and systemic lupus erythematosus: examining the link. *J Nephrol* 2013;26(6):953–9.
[2] Hayden MS, Ghosh S. NF-κB, the first quarter-century: Remarkable progress and outstanding questions. *Genes Dev* 2012;26(3):203–234.