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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

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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

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FRI0002

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

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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

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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

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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