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 fulfill 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 DMRADS (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 cells 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 CD21lowCD38low, 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 CXC5 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, FcgRIIB, CD201, CD23, CD38, CD46, CD68, 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 cell 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


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 spondyloarthritides (JSpA) is a diverse group of related syndromes with shared symptoms and pathogenetic 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 in 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-15, 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 significantly for NLRP3 promoter site (p<0.0220). Expression analysis of selected
miRs showed no significant difference in fold change between jSpA patients and healthy controls.

<table>
<thead>
<tr>
<th>GENES</th>
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<th>Fold Change p</th>
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<td>Controls</td>
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Conclusions: 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 inflammatory cascade 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 new prospects for therapeutic treatment of this complex disease.

REFERENCE:

Disclosure of Interest: None declared


FR00004

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

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Background: Enthesitis 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 (GMCSF), as well as being a haemopoietic growth factor, plays a central role in regulating innate immune and has recently been implicated in the pathogenesis of SpA however has not been studied in ERA.

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

Disclosure of Interest: None declared


FR00005

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

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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 (PPARY) 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 (1.67±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:

Disclosure of Interest: None declared