THE TRANSCRIPTION FACTORS IKZF1 AND IKZF3 MODULATE B CELL ACTIVATION AND DIFFERENTIATION IN SYSTEMIC LUPUS ERYTHEMATOSUS

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Background: The transcription factors IKZF1 (Ikaros) and IKZF3 (Aiolos), essential for the maturation, differentiation and survival of B cells, have been linked to Systemic Lupus Erythematosus (SLE). The cereblon modulator Iberdomide, which induces degradation of IKZF1 and IKZF3, is undergoing clinical trials in SLE. However, the role of IKZF1 and IKZF3 in aberrant plasmablast development and pathogenesis of SLE has not been fully elucidated.

Objectives: To assess the mechanism of IKZF1 and IKZF3 control of gene expression underlying activation and differentiation of B cells in SLE patients.

Methods: CD19+ B cells were isolated from the peripheral blood of patients with SLE recruited at Barts Health NHS Trust (n=25). B cells were cultured for 5 days and stimulated with IL-2, IL-10, IL-15, CD40L and TLR-7 ligand Resiquimod to induce plasmablast differentiation together with Iberdomide (10 nM) or control at either day 0 or 18h prior to harvest at day 4. At day 5, cells were harvested for fluorescence-activated cell sorting (FACS) and measurement of IgG and IgM in supernatants by ELISA. RNA extraction and RNA-sequencing were performed on FACS-sorted CD27 IgD− naïve B cells and CD20**CD27**CD38** plasmablasts and matched baseline B cells.

Results: Iberdomide from day 0 (n=9) significantly reduced the number of CD20**CD27**CD38** plasmablasts (number of sorted CD20**CD27**CD38** plasmablasts measured: SD 4872±6122 in untreated, 3248±8335 in Iberdomide, p=0.03). Accordingly, Iberdomide significantly inhibited the production of IgG and IgM from SLE B cells (p=0.017 and 0.050, respectively). Iberdomide given both on day 0 did not affect the numbers of plasmablasts or the production of IgM and IgG (n=16). However, Iberdomide induced a significant modulation of several genes both in naïve B cells and plasmablasts, as assessed by RNA-seq on sorted cells (400 and 461 differentially modulated genes in naïve B cells and plasmablasts, p adjusted <0.05). Pathway analysis showed that Iberdomide treatment resulted in downregulation of JAK-STAT signalling downstream of IL12 (FDR=7.92E-04), IL12 signalling (FDR=0.0014), and p53 signalling regulation of cell death (FDR=0.0043) and showed a trend to upregulation of RUNX1 signalling and Rho GTPase cycle.

Conclusion: Our work confirms the importance of IKZF1 and IKZF3 in modulating gene expression required for B cell differentiation in SLE, as shown by the ability of Iberdomide to inhibit the differentiation of SLE B cells into plasmablasts. Inhibition of IKZF1 and IKZF3 modulated the expression of several transcriptional programmes in naïve B cells and plasmablasts. While larger scale analysis will be needed to confirm the functional consequences of IKZF1 and IKZF3 modulation in naïve B cells and plasmablasts, our results confirm their relevance in SLE as important regulators of B cell activation and differentiation.

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ASSOCIATION BETWEEN SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) OF THE BAFF GENE AND FATIGUE IN PRIMARY SJÖGREN’S SYNDROME

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Background: Primary Sjögren’s Syndrome (SS) is characterized by B lymphocyte hyperactivity with B cell activating factor (BAFF) acting as an important regulator. Single Nucleotide Polymorphisms (SNPs) of the BAFF gene have been implicated in the pathogenesis of several autoimmune diseases including lupus and SS both of which are characterized by heightened fatigue levels, often compromising quality of life.

Objectives: To explore potential associations between several BAFF gene SNPs and fatigue status experienced by primary SS patients.

Methods: Single nucleotide status was assessed by Functional Assessment of Chronic Illness Therapy–Fatigue (FACT-I T) scale in 178 primary SS patients. Five SNPs rs9514827, rs1041569, rs9514828, rs1224414, rs12583006 of the BAFF gene were tested in DNA extracted from peripheral blood of all patients enrolled in the study using the RFLP-PCR method. A cut-off value of <30 was used, which indicates severe fatigue. Analysis of BAFF SNPs in association with fatigue levels was performed by the online platform SNPStats.

Results: The frequency of T/T genotype of both rs9514828 and rs1224414 was reduced in primary SS patients with severe fatigue compared to those without severe fatigue. Further research is needed to confirm the functional consequences of these SNPs.