SLE, Sjön's and APS - aetiology, pathogenesis and animal models

POS0807 A TRANSCRIPTIONAL PROFILE OF FATIGUE IN PRECLINICAL AUTOIMMUNITY WHICH IS PRESERVED IN SLE AND SJOGREN'S SYNDROME

Keywords: Systemic lupus erythematosus, -omics, Sjögren syndrome

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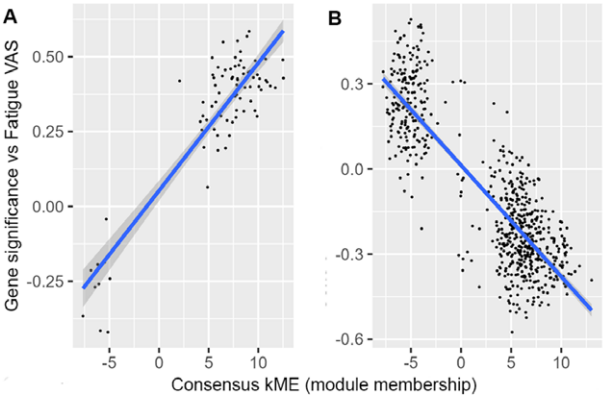
Background: Fatigue is a prevalent and debilitating symptom in autoimmune connective tissue diseases (CTDs). Its immunological mechanisms are poorly understood. Although targeted therapies can improve fatigue, the effect is modest and variable. This is likely because, in established disease, immunological drivers of fatigue may be obscured by multifactorial effects from glucocorticoids, accrued damage and comorbidity. However, fatigue frequently pre-dates formal diagnosis or end-organ manifestations, suggesting immunological mechanisms may be better interrogated in the preclinical phase. SLE and primary Sjogren's syndrome (pSS) are preceded by a preclinical phase of anti-nuclear antibody (ANA) positivity with no or non-specific symptoms years before clinically manifest inflammation. Although only a minority ultimately develop overt CTDs, ANA-positive individuals demonstrate complex immune dysregulation including increased interferon (IFN) pathway activation [1]. We hypothesise that immune disturbances underlying fatigue in SLE and pSS are i) established during the earlier ANA-positive preclinical phase; ii) contribute to the symptom burden among ANA-positive individuals, and (iii) are differentially modulated in established disease states.
Objectives: To investigate unique and conserved peripheral blood immune cell transcriptional signatures associated with symptomatic fatigue in ANA-positive preclinical patients and established SLE and pSS.
Methods: Bulk RNASeq was performed on peripheral blood mononuclear cells isolated from 35 ANA-positive preclinical individuals demonstrating ≤1 clinical criterion for classifiable CTD, symptom duration <12 months and naive of therapy, of whom 15 later progressed to SLE/pSS and 20 did not progress. Disease controls with SLE (n=18), pSS (n=10) were also analysed. Weighted gene co-expression network analysis was used to identify gene expression modules associated with fatigue in preclinical subjects. Consensus networks were constructed for Preclinical:SLE and Preclinical:pSS to identify fatigue-associated modular signatures which are retained in established CTDs. Gene ontology enrichment was evaluated using g:profiler.
Results: Within the preclinical transcriptomic network 5 module eigengenes showed significant and specific association with patient fatigue VAS. A type-I IFN signature, centred upon canonical ISGs, *MX1*, *IRF7* and *IFIT5*, was positively correlated with fatigue score (R=0.48, p=0.003) and conserved across preclinical, SLE and pSS networks, with subtly different modular organisation. One further module, enriched for tRNA modification was significantly correlated with fatigue in preclinical subjects (R=0.41, p=0.01) but with no counterpart preserved in either SLE or pSS networks. The modular signature with strongest negative association with fatigue (R= -0.35, p=0.004) in preclinical subjects was enriched for mitogen-activated protein kinase (MAPK) cascades, heat shock and protein folding chaperone activity, and included transcription factors *JUN* and *ATF3*. This signature was retained in pSS patients, but did not persist in the SLE consensus network.
Conclusion: We describe novel modular transcriptomic signatures associated with fatigue in the preclinical phase of autoimmunity which demonstrate differential persistence and activity in SLE and pSS. These pathways may help identify individuals with immune-mediated fatigue most amenable to therapy, and could provide insights into new therapeutic targets for fatigue across a range of autoimmune diseases.



Scatterplot of Gene Significance for fatigue vs. Module Membership (MM) for genes located in two preclinical transcriptomic modules. One module with significant positive association with fatigue (A) is conserved across preclinical, SLE and pSS networks. Another module (B) negatively associated with fatigue was conserved in preclinical and pSS networks but not in SLE.

Figure 1.

REFERENCE:
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POS0808 MICA/B-DEPENDENT ACTIVATION OF CYTOTOXIC NATURAL KILLER CELLS BY INFLAMMATORY CDC2 CONTRIBUTE TO PRIMARY SJÖGREN´S SYNDROME PATHOLOGY

Keywords: Animal models, Innate immunity, Sjögren syndrome

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Background: Primary Sjögren´s syndrome (pSS) is an example of an inflammatory autoimmune disorder largely mediated by IFN responses, leading to damage of exocrine glands that has been linked to autoreactive adaptive immune cells, such as Th17, CD8+ T cell and B cell [1, 2, 3]. However, the potential contribution of different innate immune cells such as Natural Killer (NK) cells and dendritic cells (DC) to pSS pathology remains understudied.
Objectives: We identify the molecular mechanisms regulating pathogenic crosstalk between NK and DC in pSS using samples from pSS patients and an experimental *in vivo* SS model.
Methods: Phenotypical analysis of myeloid and NK cell subsets in PBMCs from 47 pSS patients and 56 healthy donors (HD) was performed by flow cytometry. Histological analysis of SG from n=7 pSS was performed by confocal microscopy. Cytotoxic function of NK cells was assessed by culture with a K562-GFP target cell line. Transcriptional analysis of sorted Mo, CD1c+ and CD141+ cDC from four pSS patients and four HD was performed by RNA-seq. Co-culture between sorted NK cells, Mo and CD1c+ or CD141+ cDCs was used for test functional interactions. Regulation of ligands for NK cell receptors on cDC was analyzed by FACS after stimulation with poly I:C in the absence or the presence of specific siRNAs. Finally, altered DC and NK cell phenotypes and interactions with Th17 and B cells were analyzed in a murine Sjögren-mouse model induced by poly I:C intraperitoneal injections [4] in which we used a depleting anti-NK1.1 antibody or an isotype control each 4 days.
Results: Here, we identified an enriched transitional CD16^{+} CD56^{hi} NK cell subset in pSS individuals associated with higher NK cell cytotoxic function *in vitro*. In addition, elevated proportions of inflammatory CD64^{+} cDC2 exhibiting increased levels of MICa/b (p= <0,01), the ligand for the activating receptor NKG2D, were observed in the blood of these patients. Circulating cDC2 from pSS were capable of efficiently inducing activation of cytotoxic NK cells *ex vivo* and were found near CD56^{+} NK cells in salivary glands (SG) from pSS patients. Interestingly, cDC2 from pSS were characterized by preferential transcriptional activation of IFN signatures associated to the RIG-I/DDX60 pathway and its target genes. These sensors regulate the expression of MICa/b ligands on cDC2. Finally, increased proportions of CD64^{hi} cDC2 (p=<0,0001) expressing RAE-1 (p= <0,01), a murine activating NKG2D ligand, and transitional NKG2D^{+} CD11b^{+} CD27^{+} NK cells (p=<0,001) were present *in vivo* in the SG of an *in vivo* model of

pSS. Remarkably, depletion of NK cells during the inflammation onset prevented subsequent induction of IL-17^{+} CD4 (p= <0,01) and memory IgD^{-} IgM^{-} CD38^{hi} B cells (p=<0,0001) in the SG.

Conclusion: Thus, our study provides novel innate immune cellular and molecular mechanisms contributing to pSS pathology and identifies new potential therapy targets.

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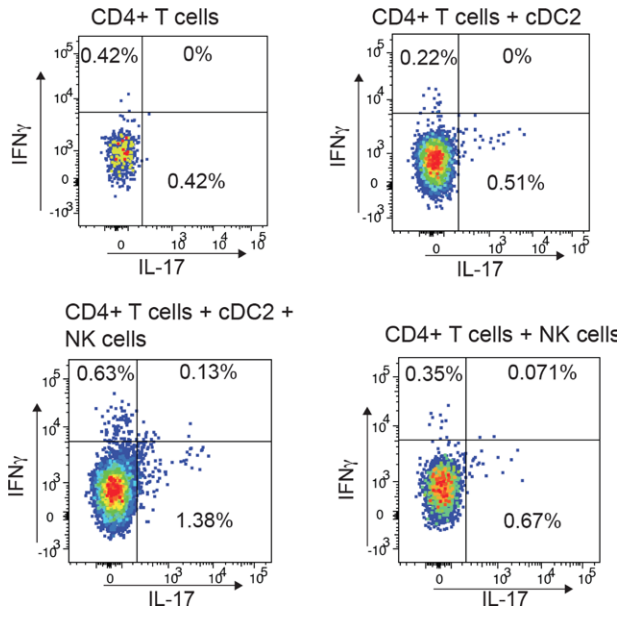

Figure 1.

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POS0809 HARNESSING CELL ENERGY METABOLISM TO SUPPRESS SALIVARY GLAND INFLAMMATION IN SJÖGREN SYNDROME

Keywords: Sjögren syndrome

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Background: SG epithelial cells (SGEC) play a key role in sustaining inflammation in Sjögren Syndrome (SS), which is indeed termed an 'autoimmune epithelitis'. However, the mechanisms responsible for the inflammatory activation of SGEC remain largely undetermined. Our line of research indicates that SGECs in SS exhibit profound changes in cell energy metabolism as indicated by aberrant expression of autophagy [1]. Inhibitions of autophagic process results in a down regulation of SGECs activation [1], thus indicating a crucial role of SGEC energy metabolism in the induction of autoimmune epithelitis.

Objectives: Aim of this study is to characterize metabolic changes occurring in SS SGECs and dissect the link between these changes and their acquired pro-inflammatory function.

Methods: SGECs were isolated from minor SG biopsies deriving from patients with SS and sicca. Intracellular metabolomic analysis was performed on direct ex vivo isolated primary SGECs. As read out of functional activation of SS SGECs, supernatants from SGECs coltures were collected to perform ELISA test in order to evaluate the expression of the pro-inflammatory mediator IL-6.

Results: Principal component analysis (PCA) of high-throughput metabolomics analysis of sicca (n=7) and SS (n=7) SGECs revealed a separation along the component 1 axis (46.6% of variance) indicating profound differences in the intracellular metabolome (**Figure 1a**). Unsupervised clustering analysis of metabolites revealed profound metabolic differences between SS and (n=7) sicca (n=7) SGECs (**Figure 1b**). Analysis of selected metabolites confirmed a shift towards increased glycolysis and TCA cycle activation in SS SGECs (**Figure 1c**). Supernatant concentrations of IL-6 were higher in SS (n=21) compared to sicca (n=14) SGECs (**Figure 1d**).

Conclusion: SGECs from SS patients display altered cell energy metabolism with evidence of increased glycolysis and activated TCA cycle. A metabolic driven pro-inflammatory status of SS SGECs seems confirmed by increased basal expression of IL-6. Validation of our metabolomic results, along with transcriptomic and epigenetic studies, is currently ongoing in SGECs from SS and sicca to dissect the link between changes in cell energy metabolism and their acquired pro-inflammatory phenotype.

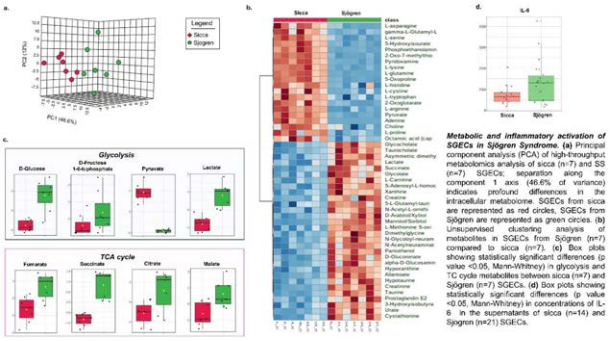

Figure 1.

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Disclosure of Interests: None Declared.

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POS0810 T FOLLICULAR HELPER CELLS IN BLOOD MIRROR SALIVARY GLAND-INFILTRATING T CELLS IN PRIMARY SJÖGREN'S SYNDROME

Keywords: Sjögren syndrome, Adaptive immunity

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Background: Although clonal expansion of autoreactive T cells have been identified in the peripheral blood and salivary gland of primary Sjögren's syndrome (pSS) (1), the relationship between peripheral immune environments and inflammatory organs remains still unclear.

Objectives: Here, we examined which T cell subsets in blood share the same T cell receptor (TCR) αβ with T cells infiltrated at labial salivary gland (LSG) in patients with pSS, and evaluated mechanisms of their differentiation.

Methods:

1) TCR repertoires of each effector memory T cell subset (Th1, Th17, Tfh1, Tfh2, Tfh17) in blood, and LSG-infiltrating T cells obtained from the same pSS patient were analyzed by TCR sequence (*n*=1).
2) The proportion of each T cell subset in blood was compared between patients with pSS (*n*=30) and healthy controls (HC) by flow cytometry (*n*=20).