Figure 3. The proinflammatory cytokine levels of TNF-α, IL-17, and IL-6 in the serum samples from the treated and control group. (means±SD; n=7 per group; * P<0.05, ** P<0.01, *** P<0.001).

Conclusion: Leflunomide may prevent and improve salivary gland hypofunction and inhibit immune activation in NOD mice, providing a theoretical basis for evaluating leflunomide in the treatment of Sjogren’s syndrome.

References:

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THU0230

HSA-MIR-513C-3P OVEREXPRESSION DECREASES XBP-1S CORRELATING WITH INCREASED INFLAMMATION AND AUTOANTIBODIES IN SALIVARY GLANDS FROM SJÖGREN’S SYNDROME PATIENTS

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Background: Endoplasmic reticulum (ER) stress and the Unfolded Protein Response (UPR) are linked to inflammation in a variety of human pathologies including autoimmune diseases. Salivary glands (SG) from Sjögren’s syndrome (SS) patients have high levels of IFN-γ among other cytokines, which trigger or exacerbate protein unfolding or misfolding, inducing ER stress. In this study, we focused on the IRE1α/CHOP-XBP1 pathway of the UPR, whose transcription factor is XBP-1s, which induces genes linked to regulation of the α-XBP-1 pathway of the UPR, whose transcription factor is XBP-1s, which induces genes linked to regulation of the α-catabolic pathways. The XBP-1 mRNA is regulated by DNA methylation and miRNAs, whereas XBP-1s mRNA levels are determined by qRT-PCR.

Objectives: Due to hsa-miR-513c-3p overexpression has been reported in SG from SS patients [2] and that XBP-1s is a predicted target of hsa-miR-513c-3p, the aim of this study was to evaluate whether miRNA levels of XBP-1s is modulated by hsa-miR-513c-3p and XBP-1s.

Methods: SG biopsies from 16 SS-patients with low and high focus score were obtained. The miRNA expression was determined by a miRNA microarray containing 358 miRNAs. The XBP-1s expression was validated by qRT-PCR.

Results: By Taqman assays we validated the overexpression of hsa-miR-513c-3p in SG from 8 SS patients with low (p=0.03) and 8 SS patients with high (p=0.003) focus score, compared with SG from 5 controls. In the same samples, a decrease of XBP-1s transcript levels was observed in SG from SS-patients with low (p=0.002) and high (p=0.026) focus score. XBP1s transcript levels were negatively correlated with hsa-miR-513c-3p (r=-0.47, p=0.014), Rs (r=-0.73, p=0.0009), ANA (r=-0.7, p=0.0033) and focus score (r=-0.72, p=0.0011). Stimulation of 3D-acini with 1ng/mL IFN-γ increased the hsa-miR-513c-3p levels (p=0.014) and decrease the XBP-1s transcript levels (p=0.027). A negative correlation was found between hsa-miR-513c-3p and XBP-1s transcript levels in 3D-acini stimulated with IFN-γ (r=-0.87, p=0.0001). The XBP-1s transcript levels were decreased in HSG cells transfected with hsa-miR-513c-3p mimic and increased in HSG cells transfected with the miRNA inhibitor.

Conclusion: IFN-γ-induced upregulation of hsa-miR-513c-3p is consistent with the presence of STAT3-binding elements in its promoter region. Our findings suggest that the combined action of miRNAs and DNA methylation modulated by IFN-γ could explain the altered expression of XBP-1s, a key transcription factor involved in cellular proteostasis, affecting secretory function in LSG from SS-patients. Our results confirm previous correlations found between XBP-1s protein levels and clinical parameters of SS-patients, suggesting an association of XBP-1s with inflammation and impaired SG function.

References:

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THU0229

IGG ANTIBODIES AGAINST PHOSPHORYLCHOLINE ARE NEGATIVELY ASSOCIATED WITH DISEASE ACTIVITY, DISEASE DAMAGE, CARDIOVASCULAR DISEASE AND ATHEROSCLEROSIS IN SLE: POTENTIAL UNDERLYING MECHANISMS.


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Background: Phosphorylcholine (PC) is an important component in cellular membranes and in lipoproteins that is exposed and recognized by the immune system, when cells undergo apoptosis or lipoproteins like LDL undergo oxidation. PC is also exposed in some microorganisms including nematodes and bacteria (non-self). We reported that IgM anti-PC is associated with protection in atherosclerosis, SLE, RA and other chronic inflammatory conditions. We also reported potential underlying protective mechanisms: 1: increase in clearance of human dead cells, 2: inhibition of uptake of oxLDL in macrophages, 3: inhibition of cell death, 4: anti-atherosclerotic, 5: decrease in mortality of T regulatory cells in SLE-patients’ T cells from a low level and also in plaque T cells. We generated in-house fully human IgG1 anti-PC clones for experimental studies to study anti-PC properties in humans. In contrast to mice, anti-PC are not germ-line encoded with a dominant clone.

Objectives: We here study IgG1 and IgG2 anti-PC, with focus on atherosclerosis and SLE and properties of fully human IgG1 clones, in relation to SLE.

Methods: We determined anti-PC by ELISA in 116 SLE-patients and 110 age- and sex-matched controls. For functional studies, we used three in-house generated, fully human monoclonal IgG1 anti-PC (A01, D05, E01). Apoptosis was induced in Jurkat T-cells and pre-incubated with A01, D05, E01 or isotype control IgG1 and effects on effecocytosis by human macrophages studied. Anti-PC peptide/protein characterization was determined using a proteomics de novo sequencing approach.

Results: IgG1 but not IgG2 anti-PC levels were higher among SLE patients (p=0.02). IgG1 anti-PC was negatively associated with SLICC and SLEDAI (OR: 2.978 CI: 0.876-10.998; OR: 5.108 CI 1.3 20.067 respectively) and negatively associated with CVD, atherosclerotic plaques and echoluent (potentially vulnerable) plaques but the association for the two former was not significant after controlling for confounders. D05 had maximum effect on macrophage effecocytosis efficiency, followed by A01 and E01. The monoclonal antibodies showed differential binding specificity to PC and PC associated neo-epitopes. Peptide analysis showed difference in the CDR3 region of the three anti-PC IgG1 clones which are crucial for recognition of PC on apoptotic cell surface and other neo-epitopes.

Conclusion: Anti-PC IgG1 is negatively associated with disease activity, and disease damage in SLE, but the negative association with CVD is also dependent on confounding risk factors. One potential underlying mechanism could be increased clearance of dead cells.

References:


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**THU0232**

**INTERFERON SIGNATURE IN LUPUS KIDNEY IS CORRELATED WITH REMISSION WITHIN 56 WEEKS**

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**Background:** Activation of the type I interferon (IFN) pathway has been implicated in the initiation of systemic lupus erythematosus (SLE) and most SLE patients show increased expression of IFN-regulated genes in peripheral blood mononuclear cells or whole blood. However, the IFN signature in lupus kidney is not well examined especially at single cell resolution.

**Objectives:** To clarify the significance of the IFN signature in lupus kidney at single cell resolution.

**Methods:** 18 lupus kidney (LN) and 34 transplanted kidney (KTx) samples were included in the study. Residual frozen kidney biopsies were collected after clinical diagnosis. The tissue from one donor was split into two. One portion was used for total RNA-Seq (tRNA-Seq) by SMARTer Stranded Total RNA-Seq Kit v2 - Pico Input Mammalian (Takara/Clontech). The rest was used for single nucleus RNA-Seq (snRNA-Seq) using Chromium Single Cell 3′ Reagent Kits v3 (10x Genomics) (7 LN and 17 KTx). For the tRNA-Seq, the sequence reads were aligned to Ensemble genome annotation (Ens93) by STAR and the aligned reads were counted by htseq, IFN score of tRNA-Seq was calculated using the reported method [1] per each module (M1.2, M3.4 and M5.12). For the snRNA-Seq, the sequenced reads were processed on the standard pipeline of CellRanger (10x Genomics) and the data was visualized using Seurat. IFN score of snRNA-Seq was computed by the method reported by Arazi et al. [2].

**Results:** 11 LN had clinical remission and 7 LN showed non remitted disease within 56 weeks after the surgery. There were no statistical significance co-variants such as age, gender and WHO class in pathology, IFN score of M1.2, M3.4 and M5.12 were significantly increased in LN with remission within 56 weeks (median 0.773 vs 0.659, 0.595 vs 0.243 and 0.415 vs 0.100: p-value 0.03, 0.01 and 0.02 [Wilcoxon rank-test]) in tRNA-Seq. In the snRNA-Seq, the lupus kidney with low IFN score showed restricted IFN signature in the endothelial cells mainly, which can be detected even in the controls, but those with high IFN score indicated broadly spread IFN signature among all of the cell types.

**Conclusion:** LN with high IFN score in kidney tissue is correlated with remission within 56 weeks. LN with low IFN score showed IFN signature restricted to endothelial cells but those with a higher IFN score revealed broadly affected cell types with IFN signature. These results suggest that the IFN signature of LN may start from endothelial cells and then spread to the whole kidney.

**References:**


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