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Innovative insights into mechanism of SLE, Sjögren's and APS

OP0299 SERUM AND GLOMERULAR EXPRESSION OF IL32 IN LUPUS NEPHRITIS

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Background: Lupus nephritis (LN) is one of the most severe features of systemic lupus erythematosus (SLE). Several cytokines and chemokines are secreted locally in case of glomerular inflammation. Interleukin 32 (IL32) is a newly described cytokine that exhibits several properties typical of proinflammatory cytokines. *Ex vivo* and *in vitro* studies supported the role of Toll like receptors (TLRs) in LN pathogenesis and recent investigations demonstrated that Poly I:C, a ligand for (TLR) 3, strongly induced IL32 production from several cell populations.
Objectives: To investigate serum and urinary levels of IL32 in a cohort of LN patients compared to SLE patients without renal involvement and healthy controls (HC). In addition, we investigated kidney expression of IL32 and *in vitro* ability of LN patients' serum IgG to stimulate IL32 production by TLR3 activation in human embryonic kidney 293 cells line stably transfected with a TLR3 plasmid (Hek293/T3).
Methods: Serum and urinary IL32 concentrations were measured using ELISA; a polyclonal rabbit anti-human IL32 was used to evaluate the expression of IL32 in renal biopsies. To assess the production of IL32 induced by patients' IgG and the transduction pathway of TLR3 we performed Western Blot analysis for IL32, TBK1 and NFkB.
Results: We recruited 60 LN patients, 50 SLE patients without renal involvement and 30 HC; 40 LN patients had an active disease (a-LN) and the remaining 20 were in remission (r-LN). IL32 serum levels were significantly higher in patients with r-LN (median 1368, IQR 3910) and HC (median 721, IQR 2271) compared to SLE patients without renal involvement (median 203, IQR 662.8 pg/ml) (p=0.03 and p=0.018, respectively). There were no significant differences in urinary IL32 levels among LN patients, SLE patients without renal involvement and HC. In LN patients a direct association between IL32 serum levels and disease duration (p=0.02; r 0.2978) was observed. Immuno-histochemical analysis performed on renal biopsies of 20 a-LN and 8 HC showed that IL32 was strongly expressed in renal samples of LN patients, especially in patients with class IV, compared to controls. Western Blot analysis showed that antibodies isolated from LN patients induced *in vitro* production of IL32 in Hek293/T3 cells as well as the phosphorylation of NFkB and TBK1.
Conclusions: The results of the present study show increased IL32 serum levels in r-LN patients as well as increased expression of such cytokine in renal tissues of a-LN patients. IL32 renal expression and its production by Hek293/T3 cells after patients' IgG stimulation, probably mediated by TLR3, may suggest the production of IL32 directly at renal level in course of LN.
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OP0300 ENRICHMENT OF T FOLLICULAR-HELPER CELLS (TFH) AND EXCLUSION OF T FOLLICULAR-REGULATORY CELLS (TFR) FROM ECTOPIC GERMINAL CENTERS IN SALIVARY GLANDS OF SJOGREN'S SYNDROME PATIENTS

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Background: B/T cell aggregates in the salivary glands (SG) of Sjögren's syndrome (SS) can give rise to ectopic lymphoid structures (ELS) forming ectopic germinal centers (GCs), which has been linked to the development of MALT lymphoma (MALT-L). T follicular-helper cells (Tfh) and T follicular-regulatory cells (Tfr) are specialized CD4+ T-cells that positively and negatively regulate, respectively, the magnitude of the GCs response and the onset of autoimmunity.
Objectives: To characterize the infiltration of Tfh and Tfr in the SG infiltrates of patients with SS in the context of the presence/absence of ectopic GCs and in subjects with MALT-L.
Methods: SG biopsies with matching histology and RNA from 37 SS and 38 non-specific chronic sialadenitis (NSCS) patients were stratified as ELS-/ELS+ based on CD3/CD20/CD21/CD138 immunostaining (IHC). Histological samples and mRNA from 12 parotid MALT-L were also studied. Gene expression was measured by Taqman rt-PCR. Multicolor immunofluorescence/confocal microscopy for CD3, CD4, CD45RO, ICOS, PD1, BCL6 and FoxP3 was used to identify Tfh and Tfr.
Results: Tfh ($CD4^+CD45RO^+PD1^+ICOS^+FoxP3^-$) cells and Tfr ($CD4^+CD45RO^+PD1^+ICOS^+FoxP3^+$) cells were strongly enriched in ELS+ vs ELS- SS samples. The Tfh:Tfr ratio in ELS+ SG was approximately 2:1. Interestingly, while in tonsils Tfr were routinely detected within GCs, in ELS+ SG Tfr were predominantly excluded from the B cell follicles and accumulated in the T cell rich areas at the periphery of the lymphoid aggregates. Conversely, Tfh densely infiltrated the B cell rich areas and, within ectopic GCs, acquired BCL6. Furthermore, Tfh infiltration closely correlated with SG IL-21 mRNA expression, which in turn was strongly correlated with CD3, CD20 and CD138 IHC scores and with CXCL13, LTb, BAFF, AID and Pax5 gene expression. Finally, MALT-L samples displayed 10-fold higher IL-21 mRNA and twice as much $PD1^+ICOS^+BCL6^+$ Tfh-cells/field compared to ELS+ SS samples.
Conclusions: Within the SG of SS patients Tfh cells closely segregate with lesional IL-21 expression, localize within ELS and are strongly enriched during MALT-L development. Conversely, although Tfr cells are also recruited to ELS+ SG in SS patients, we consistently demonstrated follicular exclusion of this subset from ectopic GCs. This suggests that Tfr in SS SG fail to exert their physiological immunoregulatory properties in controlling the magnitude of the GCs response and B cell autoreactivity, as observed in tonsils.
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OP0301 TYPE I IFN GENE SIGNATURE TEST–HIGH AND –LOW PATIENTS WITH MODERATE TO SEVERE SLE DISEASE ACTIVITY HAVE DISTINCT GENE EXPRESSION SIGNATURES OF IMMUNOLOGIC PATHWAYS AND CELL TYPES

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Background: Type I interferon (IFN) has been implicated in systemic lupus erythematosus (SLE) pathogenesis, and the majority of patients with SLE have elevated expression of type I IFN-inducible genes in their blood. Anifrolumab, a fully human, $IgG_1\kappa$ monoclonal antibody against the type I IFN receptor, is in Phase III development for the treatment of moderate to severe SLE (NCT02446912 and NCT02446899).
Objectives: We sought to understand other molecular pathways (either dependent on or independent of type I IFN signaling), to elucidate heterogeneous mechanisms in SLE, and to identify patient subsets for personalized disease management.
Methods: Baseline blood samples from adult patients with moderate to severe SLE from two Phase IIb clinical studies (NCT01438489, N=265; NCT01283139, N=416) were profiled with whole genome array analyses. Type I IFN gene signature (IFNGS) test status was determined by a central laboratory utilizing an analytically validated four gene (*IFI27, IFI44, IFI44L, RSAD2)* quantitative polymerase chain reaction-based test from patients' whole blood. A predetermined, delta Ct-based cut-off point, in the trough of the bimodal distribution, was utilized to segregate type I IFNGS test–high from –low patients at baseline. Blood from healthy controls was stimulated *ex vivo* with IFN-β, IFN-γ, IFN-λ, IFN-ω, or a pool of all IFN-α subtypes, with or without blocking antibodies for each IFN type, to develop IFN-type-specific signatures. Cell type- and cytokine pathway-specific gene signatures derived from the literature were also evaluated with the Phase IIb sample data. A Fisher's exact test was used for enrichment calculations (signatures cut at median), and comparisons were adjusted for multiplicity through false discovery rate.
Results: A total of 79% of SLE patients in the combined study population had a type I IFNGS test–high status. From the type I IFNGS test–high patients, 29/95 signatures evaluated had significant enrichment, including those for B cells (q=1.17E-17, odds ratio [OR]=6.4), plasma cells (q=6.96E-11, OR=3.9), and CD40L signaling (q=1.07E-08, OR=3.3), relative to type I IFNGS test–low patients. In contrast, type I IFNGS test–low patients had enrichment for eosinophils (q=5.4E-6, OR=0.39) and type II IFN (IFN-γ) specifically inducible gene signatures (q=4.6E-3, OR=0.47). These findings were significant for the combined study population, as well as for the NCT01438489 study population, and were either significant or trending for the NCT01283139 population ($q<0.05$).
Conclusions: SLE patients who are type I IFNGS test–high had elevated concentrations of B cells, plasma cells, and other inflammatory cytokine pathways. Type I IFNGS test–low patients, by contrast, were enriched for eosinophil and type II IFN pathways. These observations provide new insights into the molecular heterogeneity underlying SLE and suggest new therapeutic approaches, particularly for type I IFNGS test–low patients.
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OP0302 SIGNIFICANT REDUCTIONS OF PATHOGENIC AUTOANTIBODIES BY SYNERGETIC RITUXIMAB AND BELIMUMAB TREATMENT EFFECTIVELY INHIBITS NEUTROPHIL EXTRACELLULAR TRAPS IN SEVERE, REFRACTORY SLE - THE SYNBIOSE STUDY

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Background: Neutrophil extracellular traps (NETs) are extracellular, decondensed DNA strands covered with antimicrobial proteins that are part of the first-line defence against pathogens. However, in SLE, overall release of NETs is increased and degradation of NETs is impaired leading to a high amount of extracellular nuclear material, potentially leading to formation of SLE-specific antibodies. These pathogenic autoantibodies deposit in glomeruli in lupus nephritis (LN) and perpetuate autoimmunity by inducing more NETs. The present study hypothesized that combining anti-CD20 mediated B-cell depletion with BAFF (B-cell activating factor) inhibition can target autoreactive plasma cells and thereby effectively reduce pathogenic autoantibodies and NET induction in severe SLE.
Objectives: The present study aimed to investigate whether Rituximab (RTX) + Belimumab (BLM) affected pathogenic antibodies in relation to NET induction in severe refractory SLE.
Methods: As part of a phase 2 proof-of-concept study, the SynBioSe study, serum levels of anti-DNA autoantibodies were measured in severe, refractory SLE patients before and after treatment with RTX following BLM. Additionally, ex vivo NET induction was assessed before and after treatment with a novel highly sensitive method based on 3D confocal laser scanning microscopy. In this assay, healthy neutrophils are incubated with 10% serum of patients and healthy controls. Furthermore, we investigated whether NET induction was mediated by immune complexes.
Results: The study included 10 severe, refractory SLE patients with lupus nephritis and 1 patient with neuropsychiatric lupus. NET induction was found to be high at baseline with a median fold induction of 4.5 [range 2.6–11.7]. After 24 weeks, NET induction was significantly decreased (median fold NET induction of 1.6 [0.4–6.1], p=0.01). In addition, treatment with RTX+BLM led to significant reduction of anti-dsDNA antibodies at week 24 with a median of 35 IU/ml [range 10–374] compared to 120 [18–505] at baseline (p=0.012). Total immunoglobulin levels temporarily declined but returned to screening levels at week 24. NET induction correlated significantly with anti-dsDNA antibody levels (r=0.42, p=0.03) and with SLEDAI scores (r=0.53, p=0.003). Therefore, we examined whether the observed NET induction could be explained by circulating immune complexes (ICx). ICx were degraded by pre-incubating anti-dsDNA positive SLE sera with nuclease, resulting in a significant decrease in NET induction (median % decrease of 91.7 [range 67.6–98.1]). In addition, depletion of IgG from anti-dsDNA positive SLE sera resulted in significantly lower NET induction. Finally, immobilized IgG isolated from anti-dsDNA positive SLE sera, but not of control serum, resulted in significant NET induction.
Conclusions: Within refractory SLE patients, RTX + BLM resulted in concordant reductions in pathogenic anti-dsDNA antibodies and NET-inducing capacity. This study strongly suggests that NET induction in SLE is mediated by immune complexes, providing a possible explanation underpinning the clinical benefits of RTX+BLM in SLE. Trial registration:ClinicalTrials.gov NCT02284984
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OP0303 THE SALIVARY GLAND SECRETOME AS A POTENTIAL NEW TOOL TO IDENTIFY BIOMARKERS OF DRYNESS AND IMMUNOPATHOLOGY IN PRIMARY SJÖGREN'S SYNDROME AND NON-AUTOIMMUNE SICCA PATIENTS

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Background: Salivary gland biopsy is essential in primary Sjögren's syndrome (pSS) diagnostics. However, tissue analysis using traditional methodology has several limitations including inaccurate quantification of lymphocytic infiltration and poor correlation with dryness. To perform biomarker identification in the target organ, tissue would have to be sacrificed. By performing saliva proteomics the biopsy tissue can be saved, but hitherto, this technique has not yielded consistent biomarkers and is limited by the absence of saliva production by many sicca patients.
Objectives: We aimed to explore whether Luminex analysis of a broad panel of cytokines in salivary gland biopsy supernatants (secretome) could provide biomarkers to stratify sicca patients and could give insights into pathogenesis.
Methods: Labial salivary gland (LSG) tissues were rinsed after biopsy and incubated in 200μL of saline for 1h at room temperature. Tissue supernatants were rendered cell-free, frozen in liquid nitrogen and stored at -80°C. In supernatants from pSS and non-Sjögren's sicca (nSS) patients 104 targets were measured by Luminex. Eight pSS and 8 nSS patients were selected for analysis based on matched biopsy weights. Results from this discovery cohort were validated in an additional cohort (n=18 nSS, n=16 incomplete SS: iSS, n=26 pSS) and correlations with clinical parameters were assessed. Non-SS were defined as sicca patients without lymphocytic infiltration in the salivary gland biopsy or anti-SSA/SSB autoantibodies. Incomplete SS patients were defined as sicca patients having lymphocytic infiltration (lymphocytic focus score (LFS)>0) and/or anti-SSA/SSB autoantibodies but do not fulfill the AECG classification criteria and are not diagnosed as pSS.
Results: Levels of 20 cytokines were significantly different between the nSS and pSS patients in the discovery cohort ($p \leq 0.05$). These 20 and 13 additionally selected cytokines based on a trend towards statistical significance and/or literature, were measured in a validation cohort. Weights of the biopsies did not significantly differ: 59.8±48.1mg in nSS vs 72.7±45.2mg in iSS vs 67.4±28.6mg in pSS. Fifteen out of these 20 cytokines were validated. From the 13 cytokines 7 were significantly elevated in pSS vs nSS. In iSS CXCL10 (IP-10) and CCL19 (MIP-3β) were significantly elevated. Cytokines correlating with LFS, ESSDAI, ESSPRI, % IgG and IgM+ plasma cells in LSG, Schirmer and/or serum IgG with Spearman $r \geq 0.4$ and $p \leq 0.05$ in pSS were selected for classification tree analysis, these were IL-2, IL-3, IFN-β, IL-21, CXCL13 (BLC), CXCL10 and CCL19. Using CXCL13 and IL-21 levels, 87.5% of pSS patients could be classified correctly. Based on the used cut off levels, 5 nSS and 9 iSS patients would be classified as pSS. Follow up of these patients may reveal development of pSS.
Conclusions: Elevated levels of numerous cytokines were found in LSG biopsy secretomes from pSS patients versus non-autoimmune sicca patients correlating with clinical parameters. This method represents a novel tool to provide insights in pSS immunopathology and to identify therapeutic targets and biomarkers for diagnosis, prognosis and treatment response.
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OP0304 SELECTIVE INHIBITORS OF NUCLEAR EXPORT PREVENT LUPUS PROGRESSION BY TARGETING GERMINAL CENTER FORMATION AND AUTOREACTIVE ANTIBODY SECRETING CELLS

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Background: Systemic lupus erythematosus (SLE) is a complex autoimmune disease characterized by simultaneous activation of the innate and adaptive arms of the immune system. The progression of the disease is unpredictable, making its treatment a challenge. Recently the nuclear export protein Exportin 1 (XPO1, also known as CRM1) has surfaced as an attractive target for the treatment of SLE and other inflammatory disorders. Selective Inhibitor of Nuclear Export (SINE) compounds are potent, orally available and well-tolerated XPO1 inhibitors. SINE compounds exert apoptotic and anti-inflammatory effects by mediating nuclear retention of important XPO1 cargos like the NFκB pathway regulatory protein, IκB.
Objectives: Based on the central role of NFκB signaling in the activation of immune cells in SLE, we decided to evaluate the therapeutic ability of SINE compounds to modulate experimental lupus progression.
Methods: To evaluate the efficacy of SINE compounds in a preclinical model of SLE, cohorts of lupus-prone mice with established disease (elevated anti-dsDNA antibody titer and proteinuria) were dosed with SINE compound or vehicle. We used flow cytometry to enumerate immune cells and immunofluorescence to visualize germinal centers (GC) in spleen. Quantitative PCR was used to measure changes in mRNA expression for molecules key in plasma cell attraction and survival, and histology was used to evaluate inflammation, antibody deposition and pathology in kidneys of lupus-prone mice.
Results: We found that treatment with SINE compounds significantly prevented increases in proteinuria (proteinuria scores: Control: 2.12±1.12; SINE (5 mg/kg): 1.06±0.49; SINE (7.5 mg/kg): 0.85±0.55) and drastically decreased IgG deposition and kidney pathology (glomerulonephritis, tubule damage and perivascular cuffing). Prevention of kidney damage was associated with a remarkable disruption of splenic GC, a significant reduction in the number of auto-reactive antibody secreting cells (ASC), and a decrease in the accumulation of auto-reactive ASC in the inflamed kidney. Reduced numbers of plasma cells in the inflamed kidney are likely due to the drastic decrease in the expression of molecules critical for PC attraction (CCL2, CXCL9, CXCL10, CXCL11) and survival (BAFF, APRIL). The potent effect of SINE compounds on GC and auto-reactive ASC is noticeable as early as 1 week after starting therapy. However, kinetics studies showed that a more pronounced elimination of GC and auto-reactive ASC is achieved after 8 weeks. Although SINE therapy has a drastic impact on spleen architecture, recovery experiments showed that complete recovery of immune cells in spleen occurred by 4 weeks. The reversible impact of SINE compounds on SLE provides a potential window of time for immunization of lupus patients.
Conclusions: SINE compounds have demonstrated efficacy in a murine model of SLE by reducing generation, survival and function of auto-reactive immune