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DOI: 10.1136/annrheumdis-2017-eular.3719

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
Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.5706

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.
Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.1608

OP0304 SELECTIVE INHIBITORS OF NUCLEAR EXPORT PREVENT LUPUS PROGRESSION BY TARGETING GERMINAL CENTER FORMATION AND AUTOREACTIVE ANTIBODY SECRETING CELLS

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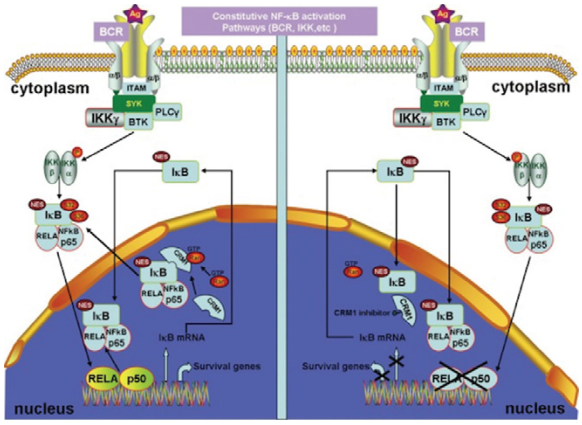
Background: Systemic lupus erythematosus (SLE) is a complex autoimmune disease characterized by simultaneous activation of the innate and adaptive arms of the immune system. The progression of the disease is unpredictable, making its treatment a challenge. Recently the nuclear export protein Exportin 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



cells. It is likely that inhibition of the canonical NFκB pathway underlies KPT-350's inhibitory effect. Together, our findings suggest the potential of SINE compounds to have a significant impact on disease progression in SLE.

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[1] Figure - Zhang KJ and Wang M. Chin J Cancer Res 24(4): 380–393:2012.

Disclosure of Interest: J. Rangel-Moreno Grant/research support from: NIH Small Business Innovation Research grant, S. Cochran Employee of: Karyopharm Therapeutics, S. Tamir Shareholder of: Karyopharm Therapeutics, Employee of: Karyopharm Therapeutics, M. Lee Shareholder of: Karyopharm Therapeutics, Employee of: Karyopharm Therapeutics, S. Shacham Shareholder of: Karyopharm Therapeutics, Employee of: Karyopharm Therapeutics, J. Anolik Grant/research support from: NIH Small Business Innovation Research grant

DOI: 10.1136/annrheumdis-2017-eular.6054

OP0305 TYPE I IFN SYSTEM ACTIVATION IN NEWBORNS EXPOSED TO ANTI-RO/SSA AUTOANTIBODIES IN UTERO

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Background: Overexpression of type I IFN-stimulated genes has been demonstrated in both SLE and SS, and induction of IFNα production in plasmacytoid dendritic cells by immune complexes containing RNA and autoantibodies, including Ro/SSA and La/SSB autoantibodies, has also been shown. During pregnancy, these autoantibodies pass over the placenta to the fetus, but it is not know if an IFN-activation takes place also in the fetus.

Objectives: In the present study, we investigated if the type I IFN system is activated in newborns exposed to anti-Ro/SSA autoantibodies *in utero*.

Methods: Anti-Ro/SSA positive mothers and their babies as well as healthy controls were included in the study. Maternal and cord blood drawn at birth was immediately separated into plasma and PBMC. mRNA expression was analyzed by microarrays, cell surface markers were assessed by flow cytometry and circulating IFNα levels by DELFIA.

Results: We observed increased expression of IFN-regulated genes and elevated plasma IFNα levels not only in anti-Ro/SSA positive women but also in their newborns, with maternal and fetal IFN scores showing a significant positive correlation (r=0.74, p=0.005). Increased expression of MHC class II was observed on $CD14^+$ monocytes of anti-Ro/SSA antibody-exposed babies, suggesting cellular activation. Notably, the IFN score of babies born to mothers receiving immunomodulatory treatment was similar to that of controls.

Conclusions: We demonstrate for the first time that anti-Ro/SSA antibody-exposed babies at risk for neonatal lupus have a pre-activated immune system with an IFN signature, elevated plasma IFNα, and increased MHC class II expression on circulating monocytes. Our data also suggest that maternal immunomodulatory treatment may modulate the IFN activity in the baby.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.6581

OP0306 DOWNREGULATION OF MICRORNAS IN PLASMACYTOID DENDRITIC CELLS IS ASSOCIATED WITH A TYPE I INTERFERON SIGNATURE IN SYSTEMIC LUPUS ERYTHEMATOSUS AND ANTIPHOSPHOLIPID SYNDROME

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Background: The most prominent alteration in the immune system of patients with SLE is a type I interferon (IFN) signature, which we recently also reported in patients with primary APS (PAPS). In SLE and APS, this signature is related to disease activity and vascular disease. Plasmacytoid dendritic cells (pDC) are considered key players in the pathogenesis of SLE and APS as they are major producers of type I IFNs. MicroRNAs (miRNAs) are short non-coding RNAs that modulate gene expression through RNA interference mechanisms and have been implicated in the dysregulation of immune cells in patients with autoimmune diseases. Here we investigated miRNA expression in pDC of patients with SLE and APS in relation to the type I IFN signature.

Objectives: To identify if pDC dysregulation in patients with SLE and APS is associated with alterations of their miRNA expression profile.

Methods: The frequency of circulating pDC was determined by flow cytometry in patients with SLE (n=49), SLE+APS (n=34) and PAPS (n=27) and healthy controls (HC, n=22). RNA was extracted from pDCs isolated from the peripheral blood of patients with SLE (n=20), SLE+APS (n=10), PAPS (n=10) and HC (n=12). pDC miRNA and transcriptome profiles were assessed by RT-qPCR by OpenArray and RNA-sequencing (RNAseq) respectively. Patients were stratified by the presence (IFN-high) or absence (IFN-low) of an IFN signature on the basis of RNAseq. pDC stimulated with TLR7 agonists were analyzed for changes in miRNA expression.

Results: The numbers of circulating pDC were reduced in peripheral blood of patients with SLE, SLE+APS and PAPS (all $p<0.001$) and did not differ among the patient groups. Among 131 expressed miRNAs, 36, 17 and 21 miRNAs were differentially expressed ($p<0.05$) in patients with SLE, SLE+APS and PAPS, respectively, as compared with HC. All but one of these miRNAs were downregulated in the patients versus HC. Only 1 miRNA was differentially expressed when comparing between SLE and SLE+APS patients and between SLE+APS and PAPS patients. No changes in expression of genes related to the biogenesis of miRNAs were observed in the pDC of the patient groups. RNAseq data revealed an IFN signature in pDC, which was strongest in SLE and SLE+APS patients. IFN-high (n=23) patients showed a stronger downregulation of miRNAs as compared with IFN-low (n=17) patients. A total of 9 miRNAs were differentially expressed between IFN-high and IFN-low patients. Pathway enrichment on targets of the top three miRNA ($p<0.001$) distinguishing between IFN-high and –low patients indicated that these miRNAs are potentially regulating pathways relevant for pDC function such as TLR signaling and endocytosis. Activation of pDCs by TLR7 agonists induced a downregulation of miRNAs in pDC, resembling the miRNA expression pattern seen in patients, in particular those with a high type I IFN signature.

Conclusions: Reduced numbers of circulating pDC and downregulation of miRNAs in pDC is shared between SLE, SLE+APS and PAPS patients. Altered miRNA expression in pDC is associated with the presence of a type I IFN signature in SLE and APS. Our data suggest that the reduced expression of a subset of miRNA underlies pDC dysregulation in SLE, SLE+APS and PAPS patients.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.2372

FRIDAY, 16 JUNE 2017

Comorbidities in rheumatoid arthritis

OP0307 TREATMENT OF BAFF TRANSGENIC MICE WITH ANTI-TNF: MONOCLONAL ANTI-TNF ARE ASSOCIATED WITH A HIGHER RISK OF LYMPHOMA THAN ETANERCEPT

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Background: Risk of lymhoma in patients with rheumatoid arthritis (RA) and disease activity is the main risk factor. The impact of treatment, notably of anti-TNF, is unclear: decreasing the risk of lymphoma by controlling activity or alterring anti-tumor immunosurveillance. Anti-TNF are not associated with an increased risk of lymphoma in large epidemiologic studies. However, the risk might vary according to the type or to the dose of anti-TNF.

Objectives: To assess if the risk of lymphoma might differ according to the type of anti-TNF, comparing monoclonal anti-TNF to the soluble receptor. For that, we used BAFF transgenic (Tg) mice as a model of autoimmunity-associated lymphomas. They develop lupus and Sjögren and 3% of them spontaneously developed lymphoma at 12–18 months

Methods: Six months aged BAFF-Tg mice were treated with anti-TNF for 12 months: etanercept (ETA) (n=15, 8 mg/kgx3/week), monoclonal anti-mouse TNF: TN3 19.12 (n=15, 20 mg/kg/week), adalimumab (ADA) (n=12, 20 mg/kg/week) or controls (n=22). Sera were assessed monthly. Crude mortality was compared among the different groups. Histological examination of the spleen was performed. The Fisher's exact test was used to compare the incidence of lymphoma among the groups.

Results: Adjunction of low dose of methotrexate during the 3 first days of treatment prevented immunization in the 3 groups for life. Using L929 cells, a cell line sensitive to TNF induced death, we confirmed that ADA was 8 to 12 times less efficient than ETA to inhibit soluble murine TNF. As expected, the mean level of ETA, TN3 and ADA were 7 μg/ml, 69 μ g/ml and 105 μg/ml, respectively. The