THU0229 DNA HYPOMETHYLATION AND DECREASED HYDROXYMETHYLATION IS ASSOCIATED WITH DECREASED ANTIOXIDANT RESPONSE IN SYSTEMIC LUPUS ERYTHEMATOUS PATIENTS

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Background: Systemic lupus erythematosus (SLE) shows increased DNA demethylation. An intermediate step to DNA demethylation is the DNA hydroxymethylation, where 5-mC is oxidized into 5-hmC. Hydroxymethylation is not completely understood and it may be related to oxidative stress in SLE patient.

Objectives: To analyze the association between the hydroxymethylation and demethylation, with the antioxidant response and SLE pathophysiology.

Methods: We analyzed in 142 SLE patients and 34 healthy controls the serum concentration of glutathione (GSH) and glutathione disulphide (GSSG) by UPLC-MS/MS, superoxide dismutase (SOD) and total antioxidant capacity (TAC) by colorimetric methods. 5-mC and 5-hmC levels were measured by colorimetric methods. Complete blood-test was made and clinical data by personal interview was collected. Biostatistical analysis with R (3.3.2.) was performed.

Results: There is a correlation between the methylation and hydroxymethylation rate ($P<0.001$), and both were lower in patients than in controls ($P=0.024$; $P<0.001$). GSH and GSSG values were lower in patients ($P=0.033$ y $P=0.003$), but GSH/GSSG ratio was not statistically different in both groups. SOD levels were higher in patients ($P=0.001$), but TAC did not show significant differences. Higher demethylation is associated to lower TAC values in patients and healthy controls ($P=0.005$; $P=0.01$). In patients, decreased SOD values are associated with higher demethylation and lower hydroxymethylation rates ($P<0.001$; $P=0.007$). SOD and TAC levels are increased in SLE patients with higher demethylation and lower hydroxymethylation ($P=0.001$; $P<0.001$). We did not observe any association between 5-mC or 5-hmC levels and GSH, GSSG or GSH/GSSG ratio. Higher demethylation is associated to vascular symptoms (defined by RELESSER study) and lupus anticoagulant (AL) positivity ($P=0.041$; $P=0.015$), and lower hydroxymethylation to mucocutaneous damage (defined by RELESSER study) and AL positivity ($P=0.015$; $P=0.009$). Lower levels of GSH and GSSG were associated to increased accumulated damage assessed by SLICC ($P=0.01$; $P=0.005$), and lower SOD values with longer disease duration ($P=0.001$).

Conclusions: We observed higher demethylation and lower hydroxymethylation in SLE patients than in controls, related to increased SOD activity. Moreover higher demethylation leads to lower TAC levels. These epigenetic disorders are related to antioxidant response disruptions in SLE patients, probably because of the chronic inflammatory condition. Our results suggest that epigenetic processes are involved in SLE pathophysiology.

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THU0230 HIF1A (RS11549465) AND AKNA (RS10817595) GENE POLYMORPHISMS ARE ASSOCIATED WITH PRIMARY SJÖGREN'S SYNDROME

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Background: HIF-1 α is the main transcriptional regulator of cellular and developmental response to hypoxia, and also participates in the integration of the T cell receptor and cytokine receptor-mediated signals of CD4⁺ helper cells. Another transcription factor also involved in the immune responses is the AT-hook transcription factor (AKNA).

Objectives: To evaluate the allele and genotype frequencies of polymorphic sites of HIF1A and AKNA genes in primary Sjögren's syndrome (pSS).

Methods: We included 110 patients with pSS and 141 ethnically matched healthy controls. Three HIF1A gene polymorphisms (Pro582Ser, Ala588Thr, and C191T) and two AKNA genes polymorphism (-1372C>A, and Pro624Leu) were genotyped using TaqMan probes in a Real-Time PCR instrument. Associations between pSS and genotypes, alleles and inheritance models of the SNPs of interest were evaluated by logistic regression adjusted by age and gender.

Results: Most of the patients were females (95.4%), median age 55 years and median disease duration of 7.8 years. The C/T genotype and the T allele of the HIF1A Pro582Ser polymorphism protected against pSS (OR=0.22; 95% CI=0.09–0.52; $P<0.01$; and OR=0.26; 95% CI=0.12–0.58; $P<0.01$; respectively). Whereas under a recessive model adjusted by age and gender, the AKNA

-1372C>A polymorphism A/A genotype was associated with an increased risk of pSS (OR=2.60; 95% CI=1.11–6.12; $P=0.03$). Parotid enlargement was associated with the HIF1A gene polymorphism (rs11549465), conferring protection for the C/T genotype vs. C/C (OR=0.22; 95% CI 0.05–0.90, $P=0.03$). The allele T of the same polymorphism was also associated with parotid enlargement (OR=0.24, 95% CI=0.05–0.95; $P=0.04$). We did not find any association with any other clinical or serological feature.

Conclusions: We identified HIF1A Pro582Ser T allele and C/T genotype as well as AKNA -1372C>A polymorphism A/A genotype as genetic factors associated with pSS. Further studies in other populations are needed to validate our findings and to shed some light on their functional implications.

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THU0231 IL-17 POLARIZATION OF MAIT CELLS DERIVE FROM THE ACTIVATION OF TWO DIFFERENT PATHWAYS

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Background: Primary Sjögren Syndrome (pSS) is a chronic inflammatory disorder affecting exocrine glands. Both IL-23 and the downstream cytokines IL-17 and IL-22 are recognised as key players in the disease. Therefore, the identification of the cellular sources and inducers of IL-17 is crucial in the understanding of the drivers of inflammation in pSS. Mucosal-associated invariant T (MAIT) cells recognize riboflavin derivatives presented by the MHC class I-like molecule MR1.

Objectives: Recently, MAIT cells have been implicated in the pathogenesis of autoimmune disorders and found expanded in salivary glands of pSS patients. Their expression of IL7R and IL23R, makes them potential contributors to the pathogenesis of pSS.

Methods: Mononuclear cells from 16 patients with pSS and 14 subjects with non Sjogren Syndrome sicca syndrome (nSS) were isolated from blood and salivary glands. We assessed the phenotype and cytokine expression of MAIT cells by surface and intracellular flow cytometry analysis. The function of MAIT cells was assessed upon *in vitro* stimulation with recombinant IL-7, IL-23 or their specific ligand lumazine (a Vitamin B2 precursor previously reported to be recognized by and specifically activate MAIT cells).

Results: MAIT cells were reduced in frequency in peripheral blood but not in minor salivary glands of patients with pSS, compared with nSS. *In vitro* stimulation of MAIT cells from pSS patients induced cytokine production which was dependent on priming with IL-7, IL-23 or specific antigen (lumazine) stimulation. Particularly, IL-7 and IL-23 guarantee IL-17 polarization of MAIT cells by two different pathways via the activation of STAT3 or ROR γ respectively.

Conclusions: This study confirms a potential role for MAIT cells in pSS and, for the first time, links IL-7 and IL-23 to the IL-17 polarization of MAIT cells in these patients.

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THU0232 INHIBITING THE ACTIVATION OF COMPLEMENT C5 BY MESENCHYMAL STEM CELLS IN THE TREATMENT OF LUPUS NEPHRITIS

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Background: Excess activation of the complement system is involved in the development and progression of systemic lupus erythematosus (SLE). Allogeneic umbilical cord mesenchymal stem cells (UC-MSC) transplantation has achieved good clinical efficacy for refractory SLE, however, the exact mechanism is unclear. Our previous work found that deposits of C5a and C5b-9 were significantly enhanced in severe lupus nephritis (LN), which suggested that antagonists of C5 might contribute to the treatment of LN, in other words, effective therapy for LN may be involved in inhibiting the activation of C5.

Objectives: To observe the clinical effect of UC-MSC on SLE model mice (B6.lpr), and explore the mechanism of MSC inhibiting the activation of complement C5 in lupus mice.

Methods: 26-week-old female B6.lpr mice were randomly allocated in four groups, which were given the following treatments, CTX (200mg/kg), UC-MSC (1×10^6), C5aRa (W-54011, 1mg/kg), and an equal volume of 0.5%DMSO. 24 hours urine and peripheral blood were collected periodically. All mice were sacrificed at 40 weeks of age. Urine protein to creatinine ratio was used to estimate daily urine protein excretion. Plasma creatinine was measured by a biochemical analyzer. Plasma levels of C3, C5a, soluble C5b-9, and anti-dsDNA

antibody were determined by ELISA. Histopathological evaluation of renal lesions was undertaken by HE, PAS, PASM and Masson staining under light microscopy. Podocyte foot processes were assayed by the transmission electron microscopy. Depositions of C5a, C5b-9, and MBL were detected by immunohistochemistry assay. Immunofluorescence was utilized to detect the expression of C5aR1, IgG, IgM, IgA, C3, C1q, and properdin in the glomeruli.

Results: At the end point, proteinuria of mice in the former three groups was significantly reduced when compared with the control mice, and renal function was also improved in both MSCT and CTX groups. Plasma level of C3 was significantly elevated in mice of MSCT and C5aRA groups. Furthermore, mice in MSCT group appeared a remarkably decreased C5a in the circulation. Compared to control mice, no significant difference was found in plasma levels of anti-dsDNA and SC5b-9, although there were decline trends in other three groups. Pathological analysis showed that the proliferation of glomerular cells and foot process fusion were significantly inhibited in MSCT treated mice. Immunohistochemistry showed that deposits of C5a and C5b-9 were significantly decreased in the MSCT group. Immunofluorescence examination showed that the expression of IgG, C3, C1q, and properdin was significantly decreased in MSCT treated mice, meanwhile, the expression of MBL was also significantly reduced in these mice.

Conclusions: The activation of the complement system was obviously involved in the glomerulonephritis in lupus mice. Allogeneic UC-MSC transplantation can effectively improve the clinical outcome of lupus mice. Possible mechanism of MSCT might be related to inhibit the activation of complement C5 in the circulation and local kidney via interrupting the classical, alternative, and lectin pathways. The potential involved contributors of UC-MSC are currently under study.

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THU0233 IN VITRO INDUCED REGULATORY T-CELLS CAN AMELIORATE SEVERITY OF PRISTANE INDUCED LUPUS (PIL)

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Background: Pristane induced lupus (PIL) is a well-established murine model for environmentally induced systemic lupus erythematosus (SLE). Mice develop specific autoantibodies and show symptoms of SLE including arthritis, diffuse proliferative immune complex glomerulonephritis and haemorrhagic pulmonary capillaritis.

Objectives: To investigate the therapeutic effects of in vitro -induced regulatory T cells (iTreg) in the murine model of PIL.

Methods: BALB/c mice were injected i.p. with either 0.5ml of pristane (PIL) or PBS (controls). Naive CD4⁺ thymocytes were sorted and cultured and cell suspensions with >80% of CD4⁺FoxP3⁺ cells (iTreg) were injected intravenously (i) once when PIL was induced (5x10⁶ iTreg (iTreg-single)) or (ii) every 4 weeks (1x10⁶ iTreg, iTreg-rep). Animals were monitored for paw swelling and grip strength. After 8 months histological analysis evaluated for cartilage degradation, number of osteoclasts and the extent of inflammation and bone erosion. Glomerulonephritis and pneumonitis were quantified using the kidney biopsy score and a newly adapted histomorphometric image analysis system; inflammatory tissue was further analyzed by tissue cytometry. Serum levels of anti-dsDNA, anti-histone and anti-chromatin antibodies were measured by ELISA.

Results: Monthly injections of 1x10⁶ iTreg reduced the clinical as well as the histological severity of PIL-arthritis, seen by a higher mean grip strength (2.96±0.02 vs. 2.73±0.06, p<0.01), less mean paw swelling (0.04±0.02 vs. 0.36±0.07, p<0.01) and retardation of the symptom onset (Figure A). 62% of PIL-mice and 33% of iTreg-rep mice had erosive arthritis. There was a significant reduction of arthritis severity in all histological parameters (inflammatory area in mm² 0.19±0.06 vs. 0.69±0.11; erosive area in mm² 0.01±0.01 vs. 0.07±0.02; number of osteoclasts 2±1.13 vs. 9.14±2; cartilage degradation in mm² 0.06±0.01 vs. 0.19±0.03, all p<0.01, Figure B). The single boost of 5x10⁶ iTreg could not prevent joint manifestations. However, a slight retardation in "loss of grip strength" and a significantly less erosive area was observed. In regards to the cellular composition of the inflammatory tissue in paws, a significantly increased relative amount of CD4⁺Foxp3⁺ cells was seen in the iTreg-rep group compared to the PIL group (in % 5.2±2.3 vs. 0.6±0.2, p<0.01).

Repeatedly injected mice (iTreg-rep) had significant less pulmonary involvement (perivascular inflammatory area in mm²/mm² lung area 0.003±0.001 vs. 0.007±0.001, p<0.01) and renal disease (glomerular activity score 1.17±0.31 vs. 2.43±0.39, p<0.05) compared to PIL (Figure C). Corresponding, iTreg-rep mice had significantly lower serum levels of disease-associated auto-antibodies (Figure D).

Conclusions: Repeated injections of iTreg ameliorate the clinical and histological severity of PIL- manifestations. A single boost of iTreg at the time of disease induction does not prevent manifestations, but retards the onset of symptoms. Thus iTreg have significant positive effects on PIL, which may have consequences for future approaches in treating SLE.

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THU0234 EPIGENETIC CELL COUNTING: A NOVEL TOOL TO QUANTIFY IMMUNE CELLS IN SALIVARY GLANDS DETECTS ROBUST CORRELATIONS OF TFH CELLS WITH IMMUNOPATHOLOGY

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Background: Histological analysis of salivary glands for decades has been a valuable tool in the characterization of patients with primary Sjögren's syndrome (pSS) and non-Sjögren's sicca (nSS) patients. Importantly, it has helped in understanding the immunopathology of sicca patients. Nonetheless, standardization of histological assessments, e.g. to quantify lymphocytic foci or germinal centers is lacking, contributing to improper classification of disease and assessment of risk of lymphoma for example. Also, detailed and reproducible quantification of the heterogeneity of inflammatory cells and their contribution to immunopathology is lacking. Recent progress in epigenetics has revealed that cell-specific DNA methylation profiles can be applied to reliably quantify numbers of cells in blood and tissues.

Objectives: To investigate whether epigenetic cell counting can serve as a novel reliable tool to quantify immune cells in salivary glands of sicca patients.

Methods: DNA was isolated from frozen tissue sections of 13 nSS, 12 probable SS, 29 pSS and 7 overlap SS patients. Bisulfite conversion of demethylated DNA sites was followed by cell specific qPCR that was used to calculate the percentage of cell subsets related to the total number of cells quantified by housekeeping gene expression. Percentages of epigenetically counted cells were correlated to gene expression generated by RNA-Seq analysis of matched salivary gland tissue and histological and clinical parameters (LFS, %IgA+ plasma cells, serum IgG, SSA positivity).

Results: Strongly increased percentages of epigenetically quantified percentages of CD3, CD4, CD8, B cells, T follicular helper (Tfh) cells and Treg cells in pSS vs nSS patients were observed (all p<0.001, CD8 p<0.01). These inflammatory cell types all strongly correlated with LFS (all at least p<0.001, CD8 p=0.014), local B cell hyperactivity (% IgA+ cells, all p<0.001, except CD8 p=0.06 and B cells, p=0.127) and systemic B cell hyperactivity (all at least p<0.01, except CD8 p=0.051). Th17 cells were not significantly different between nSS and pSS patients. Only CD8 T cells were significantly increased in probable SS patients as compared to nSS patients (p<0.05). Percentages of CD3 and B cells positively correlated with CD3 and CD19 RNA expression (r=0.608, p<0.0001; r=0.597, p<0.0001, resp.). Interestingly, percentages of Tfh cells correlated with CXCL13 (r=0.789, p<0.0001), IL7R, CXCR5 and ICOS RNA expression (all p<0.001) and were strongly associated with autoimmunity (SSA positivity, p<0.001).