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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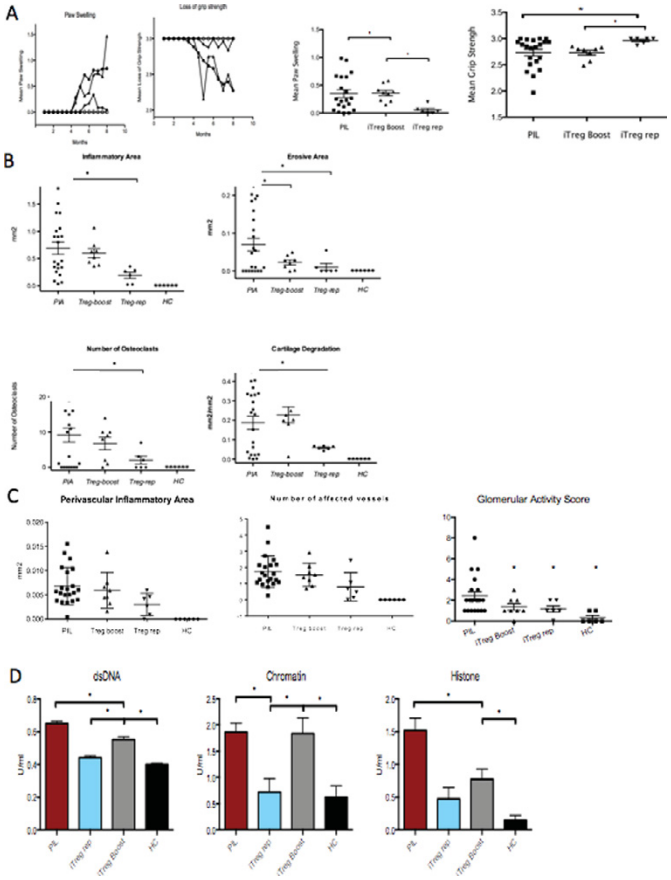
Background: Pristane induced lupus (PIL) is a well-established murine model for environmentally induced systemic lupus erythematosus (SLE). Mice develop specific autoantibodies and show symptoms of SLE including arthritis, diffuse proliferative immune complex glomerulonephritis and haemorrhagic pulmonary 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 ($5x10^6$ iTreg (iTreg-single)) or (ii) every 4 weeks ($1x10^6$ 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 $1x10^6$ 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 mm^2 0.19±0.06 vs. 0.69±0.11; erosive area in mm^2 0.01±0.01 vs. 0.07±0.02; number of osteoclasts 2±1.13 vs. 9.14±2; cartilage degradation in mm^2 0.06±0.01 vs. 0.19±0.03, all $p<0.01$, Figure B). The single boost of $5x10^6$ 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 mm^2/mm^2 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.

Disclosure of Interest: None declared

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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 $p\leq0.001$) and were strongly associated with autoimmunity (SSA positivity, $p<0.001$).

Conclusions: Epigenetic cell counting is a promising novel tool to reproducibly and easily quantify immune cells in the (inflamed) labial salivary gland of sicca patients with relatively low amount of tissue needed (<1 mm^3). Considering the potential of this technique to include a huge number of (cell-specific) biomarkers we believe this opens up new standardized ways for salivary gland analysis with high relevance for patient classification, understanding of immunopathology and clinical trials.
Disclosure of Interest: None declared
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THU0235 EXOSOME-DELIVERED MIR-146A REGULATES SENESCENCE OF BONE MARROW- MESENCHYMAL STEM CELLS FROM SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS THROUGH TARGETING IRAK1 AND TRAF6

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Background: Exosomes are membrane nano-vesicles secreted by a multitude of cells that harbor biological constituents such as proteins, lipids, mRNA and microRNA. Recent study suggests that microRNAs can be transferred between cells and mediate target gene repression. Our research group revealed the senescence of bone marrow-mesenchymal stem cells from systemic lupus erythematosus patients,which participated in the development of SLE. However, the relationship between senescence of MSCs and miRNAs remains unclear.
Objectives: In this study, we investigated whether exosomes act as intercellular messengers delivering microRNA that modulate the senescence of BM-MSCs from SLE patients and its possible mechanism.
Methods: Tewelve female SLE patients and healthy subjects were enrolled in the study. All patients were females, and their age distribution was similar to that of the cases. Serum were collected from these persons. All BM-MSCs were Isolated by density gradient centrifugation. Serum-derived exosomes were extracted by Total Exosome Isolation reagent and confirmed by transmission electron microscope and western blot. The internalization of exosomes was detected by immunofluorescence. QRT-PCR was used to distinguish the difference of expression of miR-146a in exosomes between normal group and SLE group. Different exosomes stimulated normal BM-MSCs, then detecting expression of miR146a by qRT-PCR, detecting expression of IRAK1 and TRAF6 by WB, observing the activity of β-gal of cells, the changes of cytoskeletal structure by F-actin staining and the distribution of cell cycle by flow cytometry. We used miRNA mimics and miRNA inhibitor to interfere the expression of miR-146a.
Results: Serum-derived exosomes could be taken up by BM-MSCs through the plasma membrane due to treatment of BM-MSCs with exosomes. After stimulation of exosomes in normal MSCs, miR146a was decreased, but, IRAK1 and TRAF6 was activated. And, the cell volume and the number of SA-β-gal positive in SLE BM-MSCs was increased. The organization of cytoskeleton was neatly disordered. The rate of cell proliferation was decreased. The miR-146a mimics in SLE BM-MSCs can significantly reverse the senescence.
Conclusions: Exosomes-delivered miR-146a in the serum of SLE patients can promote the senescence of BM-MSCs through targeting IRAK1 and TRAF6. Exosomes play an important role in the pathogenesis of SLE.
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THU0236 ANTIBODIES TOWARDS ATP-BINDING CASSETTE TRANSPORTER ABCA1: A NEW MECHANISM FOR ATHEROSCLEROSIS IN SLE?

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Background: Systemic Lupus Erythematosus (SLE) is considered an independent risk factor for cardiovascular disease and patients with SLE have an increased burden of atherosclerotic vascular disease[1]. High-density lipoproteins (HDL) are the plasma lipoproteins responsible for reverse cholesterol transport[2]. HDL protective effect on cardiovascular disease is attributed to the cholesterol efflux capacity as well as to its anti-oxidant and anti-inflammatory properties[3]. Dyslipidemia is frequent amongst patients with SLE, characteristically with low HDL levels.
ATP-binding cassette transporter ABCA1, also known as the cholesterol efflux regulator protein, is a ubiquitous cholesterol transporter that is highly expressed in macrophages. Its main function is to donate cholesterol to apolipoprotein A-I (ApoA-I) in lipid-poor HDL particles. As such, ABCA1 closely influences HDL levels and its role in atherosclerosis has been increasingly studied[4].
Objectives: This study was undertaken to determine if antibodies against ABCA1 can be detected in patients with SLE through enzyme-linked immunosorbent assay (ELISA).
Methods: Patients with SLE were divided in two groups: group A, with low damage (based on less than 4 SLICC criteria), and group B, with high damage (based on the presence of at least 4 SLICC criteria). Groups A and B were compared with a control group. 48 patients were enrolled (13 in group A and 35 in group B), and 18 age and gender-matched healthy controls were included in the control group. IgG anti-ABCA1 and anti-HDL antibodies were assessed by home-made ELISAs, using commercially available ABCA1 synthetic peptide and HDL from healthy donors.
Results: There were no differences between group A and the control group. Group B had higher titers of anti-ABCA1 antibodies when compared with group A (p=0.016) and the control group (p=0.022). For positivity we considered values superior to 3 standard deviations above the mean of healthy controls. Four patients showed positive anti-ABCA1 titers (11.4%).
Conclusions: This is the first time that naturally occurring antibodies against ABCA1 are detected by ELISA. These antibodies are increased in patients with SLE that have higher damage, measured by SLICC classification criteria. Future studies will determine their pathogenic role and the potential use of a standardized ELISA to detect anti-ABCA1 antibodies in clinical practice.
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THU0237 WHOLE TRANSCRIPTOME ANALYSIS OF APL TREATED HUVECS MAPS PROINFLAMMATORY AND PROCOAGULANT PATHWAYS

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Background: Antiphospholipid syndrome is an autoimmune thrombophillia characterized by recurrent thromboembolism and or pregrancy morbidity in the presence of antiphospholipid antibodies (aPL). β2GPI which is the major autoantigen in the syndrome forms complexes with anti-B2GPI autoantibodies that activate platelets, monocytes and endothelial cells. Previous studies have shown that anti-β2GPI-β2GPI complexes activate TLR4 and TLR6 on endothelial cells leading to NFκB, MAPK activation and Tissue Factor and proinflammatory cytokine expression.[1,2]
Objectives: To evaluate the whole transcriptome of endothelial cells that have been stimulated with aPL-B2GPI complexes.
Methods: Human umbilical Vein Endothelial cells (HUVECs) were isolated from 2 APS patients and 4 Healthy control women upon delivery. Healthy donor HUVEC were stimulated with IgG isolated from APS patients with high aPL titers and healthy individuals in the presence of B2GPI. Consequently total mRNA was isolated, cDNA libraries were created and whole transcriptome sequencing (RNASeq) was performed. Gene expression data were validated in protein levels with immunohistochemistry in placenta tissues from APS patients and healthy individuals.
Results: Whole transcriptome analysis of HUVECs stimulated with aPL-β2GPI complexes and IgG from healthy individuals revealed 680 differentially expressed genes, among which 377 were upregulated and 303 downregulated in the aPL stimulated endothelial cells. Characteristic examples of the upregulated genes are IL-6, IL-8, VCAM1, SELE and TGFB2 and TGFBR1. Bioinformatics analysis revealed that the upregulated genes belong mainly to the cytokine-cytokine receptor interaction (hsa053323), MAPK signaling pathway (hsa04010), TNF signaling pathway (hsa04668) and NOD-like receptor pathway (hsa04621). Characteristic examples of the downregulated genes include the CBX4, CBX8, BCOR and HDAC7 genes. Interestingly some of the proteins encoded by these genes play role in the epigenetic modification of DNA. Immunohistochemical staining on placenta biopsies from APS patients and healthy individuals for IL-6, IL-8, IL-18, NFkB, TF, TNF-a, E-SELECTIN, MAPK8, TGFB2 and TGFBR1 showed increased intensity in the signal of endothelial cells on APS specimens validating thus the RNASeq results in the tissues.
Conclusions: RNASeq of endothelial cells treated with aPL and B2GPI reveals a thoroughly analysed proinflamatory and procoagulant phenotype. Moreover differential expression of DNA modifying proteins suggests the possible epigenetic regulation of gene expression on endothelial cells in APS syndrome. Ongoing experiments aim to analyze histone acetylation and methylation status of the promoters of the selected genes that were shown to be differentially expressed.
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