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.

References:

[1] Vignesh P, et al. Clin Chim Acta. 2016.

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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).