8. SLE, Sjögren's and APS - etiology, pathogenesis and animal models

AB0122

DETECTION OF CIRCULATING M3 MUSCARINIC ACETYLCHOLINE RECEPTOR REACTIVE TH17 CELLS IN PATIENTS WITH PRIMARY SJÖGREN'S SYNDROME

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Background: Sjögren's syndrome (SS) is an autoimmune disease which is characterized by lymphocytic infiltration including CD4⁺IL-17 producing helper T (Th17) cells to the lacrimal and salivary glands. We previously detected anti-M3 muscarinic acetylcholine receptor (M3R) antibodies (1) and M3R reactive CD4⁺IFN γ producing helper T (Th1) cells (2) in SS patients. Moreover, we clarified that M3R reactive Th1 and Th17 cells had pathogenic roles in the development of auto-immune sialadenitis in SS mouse model (3).

Objectives: The purpose of this study was to identify circulating M3R reactive Th17 cells among primary SS (pSS) patients, and to determine functional properties of those cells.

Methods:

- 1) Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood of 10 pSS patients, age gender matched 10 healthy controls (HC), and 5 IgG4-related disease (IgG4-RD) patients. According to their HLA-DRB1 typing, top 10 ranked 20 mer peptides from the full length of M3R, which were highly predicted to bind to each HLA molecules according to the immune epitope database website, were selected for each subjects. PBMCs were stimulated with these selected M3R peptides mixed for 40 hours, and M3R peptide reactive IL-17 secreting cells were detected by IL-17 enzyme-linked immunospot assay (ELISpot).
- 2) PBMCs from 5 pSS patients who were positive for M3R specific IL-17 secreting cells, were stimulated with selected 12-20 mer M3R peptides separately, to identify the dominant M3R peptides responsible for IL-17 secretion by ELISpot.
- 3) To identify whether detected IL-17 secreting cells were Th17 cells or not, isolated CD4⁺ T cells from 3 pSS patients who were positive for M3R specific IL-17 secreting cells, were co-cultured with auto-monocyte derived dendritic cells (DCs), and stimulated with the dominant IL-17 secreting M3R peptides detected in method 2.
- 4) Anti-M3R antibodies were examined using ELISA method.
- 5) Clinical features were compared between M3R specific Th17 cells positive and negative pSS patients.

Results:

- 1) 5 of 10 (50%) pSS patients, while none of 10 (0%) HC, and 5 (0%) IgG4-RD patients, showed significantly increased IL-17 positive spots against selected M3R peptides mixed stimulation compared with non-stimulation in ELISpot (Figure 1). M3R specific IL-17 secreting cells were detected significantly more frequently in pSS (5/10, 50%) than in HC (0/10, 0%) ($p=0.03$).
- 2) All 5 pSS patients, who were positive for M3R specific IL-17 secreting cells, showed significantly increased IL-17 positive spots against M3R AA76-95 peptides.
- 3) Co-culturing CD4⁺ T cells with DCs, stimulated with identified dominant M3R peptides in method 2, showed significantly increased spots, clarifying that IL-17 secreting cells were peripheral M3R reactive Th17 cells.
- 4) Titers of anti-M3R antibodies were significantly higher among M3R reactive Th17 cells positive pSS patients than negative pSS patients.
- 5) 5 pSS patients positive for M3R reactive Th17 cells had significantly higher disease activity score (ESSDAI: 8.0 ± 4.3) than 5 negative pSS patients (2.8 ± 1.7) ($P=0.01$).

Conclusion: We detected circulating M3R reactive Th17 cells in pSS patients using ELISpot, whose T cell epitopes were shown to be included in M3R AA76-95. Moreover, M3R reactive Th17 cells might correlate with higher disease activity and production of anti-M3R antibodies in pSS patients.

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Figure 1

Figure1: Representative photomicrographs of IL-17 ELISpot assay. Significantly increased spots compared with non-stimulation were detected after incubating with selected M3R peptides mixture in M3R reactive Th17 cells positive patient (pSS8; lower row), whereas no spots were detected in those negative pSS patient (pSS2; upper row). M5R; control peptide, PHA; positive control

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AB0123

CHANGES IN CELLULAR GLYCOSYLATION AS A KEY FACTOR IN THE IMMUNOPATHOGENESIS OF SYSTEMIC LUPUS ERYTHEMATOSUS

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Background: Systemic Lupus Erythematosus (SLE) is one of the most challenging autoimmune diseases as it may be presented as a severe, relapsing and disabling immune-mediated disorder still remaining incurable (1,2).

Protein glycosylation is an essential post translational modification that participate in the correct recognition of cells by the immune system (3–5). Moreover, glycosylation changes in T cells (specifically loss of branched N-glycans mediated by GnTV, encoded by *MGAT5* gene) have been shown to impact its intrinsic function and activity in a diverse panel of autoimmune diseases (3,4,6)

Objectives: To evaluate the impact of glycans in the cellular and molecular mechanisms underlying the loss of immune-tolerance, envisioning the identification of a new targeted-specific mechanism.

Methods: We have analysed the profile of cellular glycosylation of a subset of biopsy-proven lupus nephritis from SLE patients and normal kidney tissue (from two Porto Centre Hospitals), through immunohistochemistry (IHC) as well as by real time PCR using RNA extracted from paraffin tissues. Blood samples were collected and were analysed by flow cytometry. *Mgat5* null mice with 15-months old were monitored for autoimmune signs by evaluating proteinuria, weight loss and colon and kidney tissues were analysed by IHC and FACS.

Results: SLE patients revealed a significant decreased expression of complex N-glycans in the renal parenchyma, when compared to healthy kidney. In addition, we have identified in lupus nephritis patients a unique subset of circulatory CD3⁺T cells with an abnormal glycosignature and displaying an increased expression of specific glycan-binding receptors. Interestingly, *Mgat5* null mice develop clinical signs compatible within autoimmune-like syndrome together with an increased infiltration of specific CD3⁺T cells subset identified in SLE patients.

Conclusion: These findings point towards the identification of a novel immune player with increased ability to sense abnormal N-glycans, modulating the surrounding immune response. We propose glycosylation as a regulatory mechanism that tips the balance between homeostasis/self-tolerance and autoimmunity opening a potential novel targeted-specific mechanism in SLE pathogenesis.

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AB0124

NO EFFECTS OF HIGH DOSE 25OH-VITAMIN D SUPPLEMENTATION ON LUPUS NEPHRITIS IN AN ANIMAL MODEL OF SYSTEMIC LUPUS ERYTHEMATOSUS

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Background: 25OH Vitamin D (25-OH-D3) is a fat-soluble steroid-derived molecule involved in the calcium homeostasis. Low levels of 25-OH-D3 are commonly found in patients with systemic lupus erythematosus (SLE) and have been correlated to higher disease activity and severity. Several recent studies have demonstrated that high dose Vitamin D may influence several aspects of the innate and adaptive immune response and some authors hypothesized that high dose 25-OH-D3 may have a role in the treatment of SLE. Despite these observations, the immunomodulatory effect of high dose 25-OH-D3 *in vivo* still needs to be demonstrated.

Objectives: The aim of our study was to identify the effect of 25-OH-D3 on proteinuria, survival and renal biopsy in New Zealand Black/White F1 mice (NZ), that develop a disease very similar to human SLE nephritis.

Methods: We administered to 20 NZ mice a diet enriched with high dose 25-OH-D3 10,000 UI/Kg starting from 8 weeks of age. Mice were divided in 7 experimental groups (5 mice each). The first group was sacrificed before the start of the treatment (8 week of age), three groups were treated (treated mice – TM) with 25-OH-D3 and sacrificed at 16, 26 and 36 weeks of age. The other three groups were enrolled as controls and sacrificed at 16, 26 and 36 weeks of age respectively (untreated mice – UM). The parameters collected included: total urinary protein and kidney histology for the evaluation of lupus nephritis (LN): glomerulonephritis, interstitial nephritis and vascular lesions according to a 5 points scale to obtain a total score (ranging from 0 to 12).

Results: In UM, proteinuria tended to increase over 1 mg/day at 12 weeks of age (1.7 ± 0.43 mg/day) and further increased until to reach a plateau after 28 weeks of age (10 ± 2.0 mg/day).

In TM, a significant increase in proteinuria over 1 mg/day was observed at 24 weeks, when the mean proteinuria was 1.7 ± 1.33 which was lower than controls at the same age although without statistical significance (2.9 ± 2.6); thereafter proteinuria started to increase also in treated mice and at week 30 was higher in TM compared with UM (10.3 ± 8.8 vs 4.3 ± 3.5 $p=0.05$). Figure 1. Kidney histology showed, in mice sacrificed before the start of the treatment no signs of LN. In mice sacrificed at 16 weeks minimal interstitial nephritis (score 1) was identified in 2 mice only in UM. At 26 weeks of age, a higher total LN score was identified in TM compared with UM (3.4 ± 3.8 vs 0.4 ± 0.9) with higher score for all three parameters analyzed. At 36 weeks of age, the TM group maintained a higher total LN score compared to UM (6.5 ± 1.7 vs 6.0 ± 2.6) with higher score for glomerulonephritis and interstitial nephritis.

In the TM group, three mice spontaneously died at 26, 30 and 32 weeks of age, while in the UM only one mouse died at 36 weeks of age.

Conclusion: Our data suggest that, in this animal model of SLE, 25-OH-D3 administration seems to delay the onset of proteinuria, although has no effect on the overall disease control. In addition, it may have a negative effect on renal histology and survival with earlier development of LN.

Figure:

Disclosure of Interests: None declared

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AB0125

EXPRESSION OF INTERFERON TYPE I- AND TYPE II-INDUCED GENES IN PATIENTS WITH SJOÖREN'S SYNDROME WITH AND WITHOUT EXTRAGLANDULAR INVOLVEMENT

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Background: It is well known that Sjögren's syndrome (SjS) is characterized by an upregulation of interferon (IFN)-induced genes. Namely, IFN type I signature has been reported in peripheral blood mononuclear cells (PBMCs) and in salivary glands of patients with this disease. However, few data are available on possible variability of IFN-induced gene upregulation in different clinical phenotypes of SjS.

Objectives: To verify whether upregulation of IFN-induced genes is comparable in patients with SjS characterized by different clinical phenotypes, i.e., patients with systemic extraglandular manifestations (EGMs) versus patients with a disease limited to glandular features (GFs) and with widespread pain (WP).

Methods: The study population was composed by 11 patients with SjS and EGMs (1 male, age range 18-78 years), and 10 patients with only GFs and WP (all females, age range 46-81 years), all classified according to ACR-EULAR criteria. The prevalence of anti-SSA(Ro) antibodies was 11/11 and 8/10, respectively. Lip biopsy was positive in all cases. Six healthy normal subjects were also included in the study as control population.

Four IFN type I- and 5 IFN type II-induced genes were chosen for the study on the basis of previous literature data. Total RNA from each patient and control was isolated from purified PBMCs, followed by cDNA preparation and real time quantitative-PCR (RQ-PCR) analysis, using specific primer/probe sets. For calculation of relative expression, all samples were normalised against expression of a household gene (beta actin). A further normalization was performed against the mean value of relative expression obtained in the normal controls. Final fold change values were determined from the double-normalised values using the $2^{-\Delta\Delta CT}$ method (Applied Biosystems).

Results: Fold change values of gene expression of both IFN type I- and type II-induced genes in PBMCs were different in the two clinical phenotypes of SjS. Fold change values of IFN type I-induced genes appeared strongly higher in patients with EGM, and some of them only moderately increased in those with only GF and WP. The expression of some of IFN type II-induced genes were slightly increased in patients belonging to both clinical phenotypes. Results are detailed in the table.