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.

References:

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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

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2020-eular.1642

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.