EFIS session: Emerging concepts how B cells drive systemic autoimmunity: from bench to clinic

OP0320

CHARACTERISTICS OF PERIPHERAL BLOOD B-CELL SUBSETS IN PATIENTS WITH SJOGREN SYNDROME

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Background: B-cells play a pivotal role in primary Sjogren's syndrome (SS) pathogenesis. Recent studies have shown disturbances in the peripheral B cell populations in primary SS.

Objectives: To examine B-cell subsets in peripheral blood of SS patients (pts) and to analyze the association between B-cell subsets and SS activity.

Methods: Twenty active SS pts (19F/1M): median age 42 years (range) (32-54); disease duration 3 (2-10) years; ESSDAI score ≥ 5 in 6 pts, < 5 in 14 pts), were included. SS was diagnosed based on the ACR-EULAR 2016 criteria. Twenty healthy donors composed the control group. CD19+B cells, memory B-cells (CD19+CD27+), non-switched memory B-cells (CD19+IgD+CD27+), switched memory B-cells (CD19+IgD-CD27+), naïve (CD19+IgD+CD27-), double negative (CD19+IgD-CD27-), transitional (CD19+IgD+CD10+CD38+) B-cells, plasmablasts (CD19+CD38++IgD-CD27+), and plasma cells (CD19+CD38+) were analyzed by multicolor flow cytometry using cytometer Navios (Beckman Coulter, USA).

The nonparametric Mann-Whitney test, the unpaired Student's t test for group comparison, and the Pearson's χ^2 criterion were used for statistical analysis. Data were shown as median (Me) with an interquartile range of 25 - 75 percentile. The differences were considered statistically significant when $p < 0.05$. Statistica 10 for Windows (StatSoft Inc., USA) package was used for statistical data processing.

Results: The percentages/absolute numbers of plasmablasts, plasma cells and transitional B-cells in SS were significantly higher than in healthy donors, $p < 0.01$ for all cases (Table 1).

Table 1. B-cell subsets in patients with SS, SS and MALT lymphoma and healthy donors.

B-cell subsets	SS		HD		Pt 1		FN
	%	Abs. cells/ μ l	%	Abs. cells/ μ l	%	Abs. cells/ μ l	
B lymphocyte	12,8 (7,9 - 19,7)	151 (95-112)	8,5 (7,2-11,0)	200 (100-200)	4,5	31	**
plasma cells	6,9 (4,2-10)	12 (6-22)	0,1 (0,05-0,1)	0,1 (0-0,2)	5,7	13	*
plasmablasts	0,88 (0,37-1,8)	2 (1-3)	0,1 (0,1-0,2)	0,2 (0,1-0,4)	26,6	12	*/**
transitional B-cells	5,7 (0,9-17)	8 (4-32)	0,1 (0-0,1)	0,1 (0-0,3)	0	0	*
switched cells	9,1 (5,9-20,7)	18 (11-29)	12,8 (9,3-17,0)	20 (10-40)	52	16	**
non-switched mem-ory B-cells	4,13 (2,7-5,4)	7 (3-15)	7,4 (3,7-11,1)	10 (5-20)	16	5	**

SS Sjogren's patients; HD healthy donors, Pt 1 patient with SS and MALT lymphoma* SS and HD groups compared, $p < 0.01$ ** Pt 1 and SS group compared

One of the patients (Pt 1) was diagnosed with MALT lymphoma of the parotid salivary gland; she had a distinct B-lymphocyte subpopulations distribution, which was different from the rest of the SS group. She had the lowest percentage / absolute B lymphocyte count of all patients with SS, the highest percentage of switched cells and non-switched memory B-cells, and the highest percentages/ absolute numbers of plasmablasts. For this reason, the patient was excluded from the subsequent analysis (Table 1).

Patients with activity of SS by ESSDAI ≥ 5 had a significantly higher percentage of B cells ($p = 0.007$), and significantly higher absolute B lymphocyte count of naïve cells ($p = 0.04$) and plasmablasts ($p = 0.048$).

Conclusion: Immunophenotyping showed disturbed homeostasis of the B-cells subpopulations in our SS cohort. A significant increase in plasmablasts in SS, as well as a positive correlation of the level of plasmablasts with the SS activity and presence of lymphoma could suggest the important role of these cells in the pathogenesis of SS.

Disclosure of Interests: None declared.

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OP0321

DELINEATING THE IMMUNOGENIC DOMAINS OF MDA5 USING PATIENT DERIVED AUTOANTIBODIES

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Background: The presence of myositis specific anti-melanoma differentiation associated protein 5 (MDA5) autoantibodies is associated with mucocutaneous ulcerations, rapidly progressing interstitial lung disease (RPILD), arthritis and mild muscle involvement in patients. RPILD is the major cause of mortality. At present it is unknown which domain of the MDA5 protein is the main elicitor of an immunogenic response.

Objectives: The aim of this study is to delineate the domains in the MDA5 protein that are the target of autoantibodies.

Methods: Anti-MDA5 IgG were isolated from MDA5(+) patient plasma (7 UPMC, 1 KI and 1 KULeuven) by affinity chromatography using an in-house affinity column as described earlier in Ossipova et al, 2014⁽¹⁾. 8 constructs covering different regions of the MDA5 protein were recombinantly produced in *E.coli* (Uniprot ID Q9BYX4, Figure 1). An in-house ELISA was developed to identify the domains with the main epitope(s) by measuring the reactivity of the plasma samples and purified autoantibodies against these MDA5 protein constructs, similar to what was reported by Fernandes-Cerqueira et al, 2018⁽²⁾. The biotinylated MDA5 proteins were immobilized on streptavidin coated plates and subsequently incubated with primary antibodies (purified autoantibodies⁽²⁾ or original plasma) and a HRP-conjugated secondary antibody. The ELISA was developed by the addition of TMB substrate and the optical density (OD) was measured at 450 nm.

Figure 1. Graphical presentation of the constructs representing different (combinations of) domains of the MDA5 protein.

Results: The preliminary data suggest the main reactivity of the plasma samples and the corresponding purified autoantibodies is directed towards the heli-case domains and that there is variability between the patients in the reactivity towards domains located at the end of the protein.

Conclusion: The study aims to resolve the main immunogenic domain of the MDA5 protein, which will lead to more insight in the disease mechanisms. The preliminary results suggest this domain is in the center of the MDA5 protein, but further experiments are necessary. We will use this set up to study differences in reactivity between patients (from different cohorts) and assess if differences in antibody reactivity could be linked to clinical features such as RPILD. Such correlations might be beneficial to predict the disease progression and to apply personal treatment approaches.

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