HELPERS AND CYTOTOXIC FOLLICULAR T-CELLS IN SJÖGREN'S SYNDROME

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Background: Follicular T-cells, characterized by the expression of CXCR5, secrete interleukin 21 (IL-21) and help B-cell differentiation in lymphoid follicles. They are also found in circulation and may play a key role in Sjögren’s syndrome (pSS) chronic autoimmune epithelium.

Objective: To characterize circulating follicular helper (Tfh) and cytotoxic (Tc) T-cells in peripheral blood (PB) from pSS patients, Rheumatoid Arthritis (RA) patients and healthy controls (HC), and to investigate how they correlate with B-cell subsets. We also aimed to explore associations between the Tfh and Tc cells and clinical and laboratory features of pSS.

Methods: PB from 57 pSS patients, 20 RA patients and 24 HC was analysed by flow cytometry to characterize T- and B-cell subsets, with CXCR5 defining Tfh and Tc within CD4 and CD8 T-cells, respectively. A stimulation assay was used to assess the production of IL-21 by CD4+ and CD8+ T-cells.

Results: Compared to HC, pSS and RA patients presented significantly lower lymphoplasma blast and IL-21+ cells (1615 and 1935 cells/μL respectively compared to 2228 cells/μL in HC, p<0.001 for HC vs pSS, p=0.018 for HC vs RA), and percentages (29.9% in pSS, 24.0% in RA, and 35.4% in HC, p=0.022 for HC vs RA, p=0.001 for HC vs RA). B- and T-cell absolute counts were also lower in both groups of patients compared to HC in (HC in vs pSS, p=0.011 for B-cells and p=0.001 for T-cells; in RA vs HC, p=0.018 for B-cells and p=0.018 for T-cells). B-cells absolute counts and percentages in pSS were higher than in RA (177 vs 132 cells/μL, p=0.013; 9.7 vs 6.4%, p=0.001). There were no differences in CD8 T-cells percentages between groups, but pSS had lower CD4+ T-cell percentages compared to HC (p=0.004).

pSS patients presented lower absolute counts of CXCR5+ Tfh compared to RA (134 vs 181 cells/μL, p=0.039) and HC (134 vs 241 cells/μL, p=0.001). No differences were found in Tfh percentages. RA patients had lower percentages of CXCR5+ Tc compared to pSS (2.1 vs 2.6%, p=0.113) and HC (2.1 vs 3.0%, p=0.046).

pSS patients exhibited higher percentages of IL21+CD4 and IL21+CD8 T-cells (IL21+CD4 T cells: 12.4% in pSS vs 9.0% in RA, p=0.046; vs 9.7% in HC, p=0.028; IL21+CD8 T cells: 4.1% in pSS vs 2.3% in RA, p=0.001; vs 2.8% in HC, p=0.030).

In pSS patients, CXCR5+ Tfh correlated positively with the percentages of plasmablasts (r=0.262 for CD24-CD38++ cells, and r=0.282 for IgM-/CD38+ cells). IL21+CD8 T cells correlated positively with the Naïve/CD45RA+Th1 cells (Th1: G1vsG3, p=0.003 and G2vsG3, p=0.067).

Conclusion: pSS patients present a profile of circulating Tfh and Tc different from RA and HC, both phenotypically and functionally. Moreover, our study reveals for the first time the association of EBV with circulating B and T cells. Particularly, pSS patients seem to affect circulating B and T cells. In contrast, RA patients seem to affect circulating T cells, and in fact we found that these cells were also augmented in G2 patients compared to G1 (p=0.024). Moreover, plasmablasts (Bm3+Bm4) were increased in G1 and G2 patients compared to G3 (%: G1vsG3, p=0.088 and G2vsG3, p=0.003; absolute counts, G1vsG3, p=0.020 and G2vsG3, p=0.003). Plasmablasts were also slightly augmented in G2 patients compared to G1 (p=0.099). Our study shows a higher prevalence of EA-IgG in pSS patients. Moreover, the distinct EBV serological profiles observed in pSS patients seem to affect circulating B and T cells. Particularly, pSS sera with serological evidence for late acute/chronic active infection show a more proinflammatory pattern, with increased Th1 cells, and elevated transitional B cells with increased plasmablast differentiation.

Disclosure of Interests: Filipe Barcelos Consultant for: Pfizer; Ely-Lilly, Speakers bureau: Novartis, Catarina Martins: None declared, Ricardo Monteiro: None declared, Tiziano Prusiani: None declared, Miguel Silma: None declared, José Vaz-Pat1: None declared, Luis Miguel Borrego: None declared, Jaime Branco: None declared, Luis Miguel Borrego Grant/research support from: MSD, Consultant for: MSD, Tecnifar; Paid instructor for: MSD; AstraZeneca, Speakers bureau: MSD; Tecnifar; AstraZeneca

EBV SEROLOGICAL PROFILE AFFECTS CIRCULATING LYMPHOCYTES IN PSS

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Background: Genetic, hormonal, and environmental triggers have been related to the dysregulated autoimmune response observed in primary Sjögren Syndrome (pSS), but its aetiology is still unestablished. Epstein-Barr virus (EBV) is a strong candidate as a trigger for the autoimmune epithelitis in pSS.

Objective: To evaluate the EBV serology in pSS patients, and to compare it with other autoimmune patients and healthy controls (HC). EBV-seroepidemiology, in parallel, we have characterized the immune profile of pSS patients, in order to assess if the viral background affects the circulating lymphocytes.

Methods: We have recruited 34 pSS patients (2002 AECG criteria), 20 Rheumatoid arthritis (RA) patients (ACR/EULAR classification criteria) and 20 HC. Immunoenzymatic assays were used to determine the serum levels of IgG, IgA and IgM antibodies against EBV Nuclear Antigen (EBNA), Early Antigen (EA) and Capsid antigen (VCA). Flow cytometry was used for the characterization of T and B cells, including regulatory (Tregs) and follicular (Tfh) T cells, T helper and Bm1-Bm5 subsets. Significance was considered for p<0.05.

Results: ELISA sera were positive for VCA-IgM. Only 2 HC and 3 RA were positive for EA-IgA. For VCA-IgG and EBNA-IgG we found no differences in positive cases between groups (all participants, except 2 pSS were positive for VCA-IgG; 26/34 pSS, 16/20 RA and 13/20 HC were positive for EBNA-IgG). However, pSS patients evidenced more positive sera for EA-IgG than HC (32% vs 5%; p=0.0215) and RA (32% vs 20%; p=0.2312), suggesting a more prevalent state of late acute/chronic active infection in pSS.

Thus, according to the serologic pattern observed, we divided pSS patients in 3 groups: G1 (n=18), past infection (EA-IgG+, EBNA IgG+); G2 (n=11), late acute/chronic active infection (EA IgG+, EBNA IgG-), and G3 (n=5), without serological evidence of active infection (EA IgG-, EBNA IgG+). Within the Tfh compartment, G2 patients presented increased percentages Tregs compared to G1 pSS patients (p=0.031). G1 and G2 patients presented increased CXCR3+ Th1 cells and CXCR3+ Tfh1 compared to G3 (Th1: G1vsG3, p=0.120 and G2vsG3, p=0.008; Th1v: G1vsG3, p=0.003 and G2vsG3, p=0.067).

As for B cells, pSS patients are known to have increased percentages of transitional Bm2 cells in circulation, and in fact we found that these cells were also augmented in G2 patients compared to G1 (p=0.024). Moreover, plasmablasts (Bm3+Bm4) were increased in G1 and G2 patients compared to G3 (%: G1vsG3, p=0.088 and G2vsG3, p=0.003; absolute counts, G1vsG3, p=0.020 and G2vsG3, p=0.003). Plasmablasts were also slightly augmented in G2 patients compared to G1 (p=0.099).

Conclusion: Our study shows a higher prevalence of EA-IgG in pSS patients. Moreover, the distinct EBV serological profiles observed in pSS patients seem to affect circulating B and T cells. Particularly, pSS sera with serological evidence for late acute/chronic active infection show a more proinflammatory pattern, with increased Th1 cells, and elevated transitional B cells with increased plasmablast differentiation.

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