analysis. Our data provide clues to the molecular pathways contributing to the glanular and systemic manifestations of pSS and to potential therapeutic targets for different pSS subgroups.

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OP0213
CHARACTERISTIC ENRICHMENT OF ANTIPHOSPHOLIPID-REACTIVE B CELLS AMONG ATYPICAL MEMORY SUBSETS OF PRIMARY APS SUGGEST ONGOING INDUCTION IN EXTRAFOLLICULAR SITES

Keywords: Adaptive immunity, Autoantibodies, Anti-phospholipid syndrome

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Background: Antiphospholipid syndrome (APS) is an autoimmune disease defined by arterial or venous events or pregnancy complications in association with autoantibodies against phospholipids. Despite these autoreactive antibodies, B lineage cells remain poorly studied in APS.[1] Besides triple-potency of autoantibodies and the occurrence of lupus anticoagulant, there are no biomarkers for risk assessment in APS.[2] Characterization of autoantigen-specific B cells hold promise for better understanding APS pathogenesis which may guide targeted immune therapies including identification of biomarkers.

Objectives: The study characterized B cell subsets among patients with primary (pAPS) and secondary APS (sAPS) compared to healthy controls. In particular, we analyzed phosphatidylcholine (PcC)-specific B cells for their frequency of occurrence and immunophenotype related to disease characteristics.

Methods: We analyzed PBMCs from 20 HCs, 25 pAPS and 16 sAPS patients. Using multi-dimensional flow cytometry, we analyzed the expression of 15 surface markers on B cells including the frequency of PcC-specific B lineage cells. We established a novel assay to detect antigen-specific B cells against PIC as a potential correlate of antiphospholipid antibodies as previously reported for autoimmune B cells in mice. [3] The obtained cellular subsets and autoimmune cells were subjected to the FlowSOM algorithm in R to identify B cell clusters through an unbiased strategy. Results: pAPS and sAPS patients showed increased frequencies of atypical CD21low with as well as CD21low/CD11c+ B cells, most prominent within the IgD+/CD27+ memory compartment (CD21low: p<0.01, CD21low/CD11c+: p<0.001). We found higher frequencies of total PIC-specific B cells compared to HCs among pAPS in contrast to sAPS patients. In HCs, PIC-specific B cells were found mainly among naïve B cells, while they were significantly enriched within IgD+/CD27+ pre-switch memory B cells in pAPS patients (p<0.01). Most notably, high frequencies of PIC-specific IgD+/CD27+ pre-switch memory B cells were associated with a high-risk APS profile according to the EULAR classification criteria (p<0.05). Unsupervised FlowSOM clustering identified eight distinct B cell clusters. Remarkably, PIC-reactive memory B cells mainly resided in a unique cluster of IgD+/CD27+/CD11c+ memory B cells in pAPS. The data indicate that there is ongoing induction of autoantigen-specific atypical memory B cells apparently induced outside the germinal centers escaping negative selection.

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OP0214
A MULTI-DIMENSIONAL APPROACH REVEALS A DYSREGULATED SYSTEMIC LUPUS ERYTHEMATOSUS IMMUNE RHEOSTAT WITH AN ABNORMAL IMMUNOREGULATORY RESPONSE AND REDUCED CTLA4 EXPRESSION IN EFFECTOR T CELLS

Keywords: Adaptive immunity, Systemic lupus erythematosus, Biomarkers

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Background: Systemic Lupus Erythematosus (SLE) immunopathogenesis involves a complex network of regulatory and effector cells with its balance constituting an immune rheostat that maintains immune homeostasis. Thus, the disease must be interrogated holistically to identify mechanistically and clinically relevant immune cell subsets for its pathogenesis. There is a crucial unmet need to examine the mechanism of tolerance loss in lupus and identify novel targets for potential therapeutic intervention to improve outcomes.

Objectives: To characterise the SLE immune rheostat and address our hypothesis that SLE is driven dually by an impaired immunoregulatory axis and perturbed immune effector system.

Methods: Forty-one peripheral blood mononuclear cell (PBMC) samples from 26 adult SLE patients and 27 age-matched healthy controls were studied with a 43 markers mass cytometry panel. The SLE patients (23 females) had a median age of 40 (interquartile range [IQR]: 28 to 54) years with a median SLEDAI 2K score of 4 (IQR: 0 to 6). Quality check, batch-effect correction, cell clustering, annotation and visualisation after t-distributed stochastic neighbour embedding (tSNE) dimensional reduction was done using our extended polydimensional immunome characterisation (EPIC) pipeline [1]. Features are expressed as a percentage of total CD45+ PBMC or ratio and described with median and IQR. Statistical significance is defined as p<0.05 (Mann-Whitney U test with Bonferroni correction).

Results: Our multi-parametric approach reveals multiple derangements in all the major immune lineages but predominantly in the CD4+ T cell population (Figure 1). Interestingly, there were no significant differences in the memory (CD45RO+) and naïve (CD45RA+) Treg (CD3+CD4+CD25+Foxp3+CTLA4+) subsets between SLE and controls. Instead, an enrichment of an activated memory Treg-like T subset (CD3+CD4+CD45RO+CD25lowFoxp3+CTLA4+ICOS+) was found in SLE (SLE...
Primary Sjögren’s syndrome (pSS) is a chronic autoimmune disorder characterized by lymphocytic infiltration and functional impairment of exocrine glands, resulting in dryness of the eyes, mouth and extra-glandular manifestations in numerous organs. Immuno-pathology in pSS is associated with prominent B cell hyperactivity reflected by elevated serum IgG levels, autoantibody production, immune complex formation, and complement activation[1]. In a recent double blinded placebo controlled randomized trial, we demonstrated unprece-dented robust inhibition of B cell hyperactivity and different disease activity parameters (including primary endpoint ESSDAI) by leflunomide-hydroxychloroquine (LEF-HCQ) combination therapy in the treatment of patients with pSS[2].

Objectives: To assess the potential of LEF-HCQ combination therapy to normalize dysregulated inflammatory cytokine levels in pSS patients, and to identify biomarkers that reflect inflammation, monitor changes in disease activity and predict the treatment response.

Methods: We employed a high-throughput protein Olink proteomic assay (Immuno-Oncology panel) in order to determine the blood serum concentrations of 92 immune biomarkers, assessed at baseline and at the clinical endpoint in placebo (n=8) and treated patients (n=21). Welch 2-sample t-test and paired t-test were used to calculate differences in unpaired and paired data, respectively. For corrected p values were assessed using the Olink Analyze package available on CRAN.

Results: Of the 92 immune biomarkers, 34 were significantly upregulated in pSS patients compared to healthy controls at the onset of the trial (all at least p<0.05). Moreover, the majority of these inflammatory mediators (24/34) were downregulated after 24 weeks of LEF-HCQ treatment (LGLAS9, CCL19, IL10, CXCL10, CXCL11, CXCL13, GZMA, CXCL9, and soluble CD28, CD83, CD27, PDCD1, ADGR1, PD-1, CRTAM) with 9 returning to healthy control levels (TNF, IL12, GZMA, and soluble LAG3, IL12RBI, TNFSF9/4-1BB, CD8, KLRD1, CD70). Conversely, no significant alterations were observed in the placebo group. Interestingly, changes in ESSDAI scores were significantly (all at least p<0.05) associated with changes in IL13, TNF, PTN, CXCL9, CXCL11 and soluble LAG3 concentrations. In addition, changes in serum IgG and Rheumatoid Factor levels were both significantly associated with changes in soluble CXCL13, GZMA, CXCL9, soluble CD4 and CD28 concentrations. Based on a machine learning approach (sPLS-DA) we identified baseline levels of CXCL9/10/11 as key markers of the treatment response.

Conclusion: LEF-HCQ treatment robustly inhibits inflammatory activity in pSS patients. The correlation of several mediators with clinical activity parameters indicates that these may have value in monitoring the disease activity. Furthermore, the clinical outcome upon LEF-HCQ treatment seems predetermined by an inflammatory endotype, with circulating biomarkers such as CXCR3 ligands as key determinants. These findings represent an important advance in our understanding of the underlying immunopathology of pSS and could help in the development of more personalized and effective treatment approaches for pSS and potentially other rheumatic diseases.

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