

non-responders. After adjustment for rapid changes, early increases or no return after a rapid expansion in CD20+CD27+ memory B cells portended subsequent severe flares (HR: 1.58; 95% CI: 1.18–2.11). In stratified analysis, patients on ST developing renal flares exhibited a rapid decrease in the memory B cells (-34.8% vs 0.0%; P=0.006), while belimumab induced increases in memory B cells irrespective of renal flaring.

SRI-4 responders on belimumab displayed rapid reductions in anti-dsDNA (-14.8% vs -8.7%; P=0.043) and increases in C3 (+4.9% vs +2.1%; P=0.014) and C4 levels (+11.5% vs +8.3%; P=0.017). Patients who developed flares of any severity showed no or less prominent rapid or early (P<0.001 for both) decreases in anti-dsDNA levels. Conversely, early changes in serological biomarkers did not distinguish patients developing renal flares.

Conclusions Specific changes in composition and pattern of circulating B cell subsets upon treatment for active SLE can portend disease flares or treatment response. Importantly, most B cell changes are related to treatment effectiveness rather than to treatment targets, although anti-B cell therapies can incite certain peculiar alterations which require awareness i.e. increase in memory B cells. Modifications in circulating B cell subsets occur soon after treatment initiation and may therefore prove a useful complement in SLE patient surveillance and early treatment evaluation.

PO.2.28 INTRACELLULAR OXIDATIVE STRESS IN T-CELLS IS ASSOCIATED WITH THE DISEASE ACTIVITY OF LUPUS

¹A Tandon*, ¹A Sharma, ²A Bhatnagar. ¹PGIMER ~ Chandigarh ~ India; ²Department of Biochemistry, Panjab University ~ Chandigarh ~ India there is significant implication of oxidative stress in pathology of SLE. The abnormality in cell mediated immunity largely contributes to many autoimmune diseases like SLE. To understand the association between intracellular oxidative stress and antioxidant status, alterations in proportions (TH, Tc and Treg) and severity of Lupus, the cell-specific study was carried out in T-cell subtypes of SLE patients

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Methods Lupus Patient and healthy subjects: Lupus patients were enrolled from outpatient department (OPD) of rheumatology clinic, PGIMER, Chandigarh.

Flowcytometric analysis PBMC isolated were incubated with antibodies conjugated to APC, PE, PerCP/cy5.5 for surface staining of antigens CD3,CD4,CD8 and CD25(Biolegend,USA). Primary antibodies for Keap1/Nrf2 (from Santacruz biotech, USA) were utilized for intracellular staining followed by FITC labelled Goat anti-mouse IgG(santacruz biotech,USA). DCFDA dye method used for analysis of oxidative stress.

Magnet-associated cell sorting for isolation of CD4+ and CD8+ T-cells: CD4+ and CD8+ T-cells were sorted using isolation Kits manufactured by StemcellTM, USA,

Quantitative real-time polymerase chain reaction cDNA was synthesized from RNA separated from these sorted subtypes and were utilized for evaluating expression of genes Keap1/Nrf2/HMOX-1. Quantitative PCR was done on the StepOne-Plus RealTime PCR Systems and was analyzed with StepOne Software V2.1 (Applied Biosystems New York, USA).

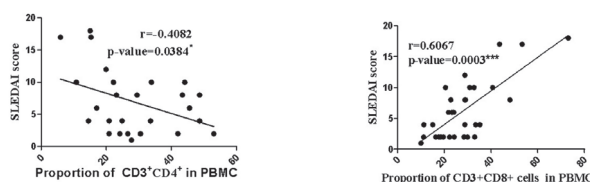
Statistical analysis Quantitative data were presented as mean ± standard error mean (SEM), were analyzed using unpaired Student's T-tests for parametric quantitative data and Mann-Whitney U test for non-parametric data. Likewise, the correlational study of parametric data was done with Pearson's

correlation and for non-parametric data Spearman's rank correlation. The software Graphpad prism version5.1 was used for analysis

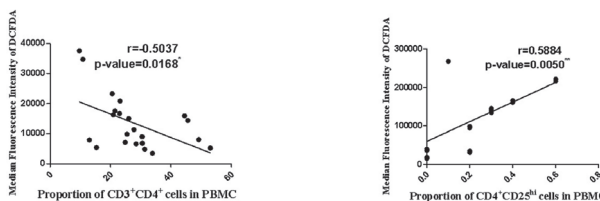
Results

- Intracellular oxidative stress was higher in T-cell subtypes: CD3+CD4+, CD3+CD8+ and CD4+ CD25hi cells of lupus patients.
- Keap1levels were significantly higher in CD3+CD8+ and CD4+ CD25Hi in SLE patients.
- Intracellular concentration of Nrf2 were significantly higher in CD3+CD8+ of SLE patients.
- Relative mRNA expression of Nrf2 in CD8+ cells were higher in SLE patients as compared to healthy controls
- Relative mRNA expression of HMOX-1 was higher in CD4+ and CD8+ of SLE patients
- Proportion of CD3+CD4+ , CD3+CD8+ and CD4+ CD25hi were significantly reduced in SLE patients.
- Median Fluorescence Intensity (MFI)of Dcfda(or ROS levels) were directly correlated with disease activity score in CD3 +CD4+ & CD3+CD8+ of SLE patients.
- Intracellular levels of Nrf2 directly correlates with proportion of CD3+CD4+ and CD3+CD8+ cells.
- Concentration of Keap1 in CD3+CD4+ and CD3+CD8+ and relative mRNA of Keap1 expression in CD4+ & CD8+

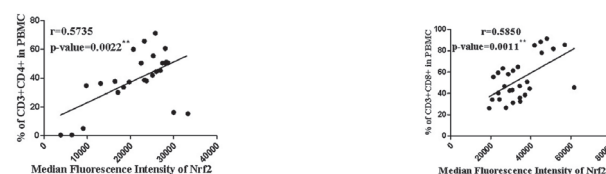
➤ Proportion of helper cells and CD3⁺CD56^{dim} cells had negative correlation with SLEDAI score whereas cytotoxic T-cells had positive correlation with SLEDAI score



➤ The proportion of CD3⁺CD4⁺ cells in SLE patients associates negatively with intracellular oxidative stress while proportions of CD3⁺CD4⁺ and CD4⁺CD25^{hi} correlates positively with the oxidative stress



➤ Nrf2 had cytoprotective role in lupus patients



Abstract PO.2.28 Figure 1

cells and relative mRNA expression of Nrf2 positively correlated with SLEDAI score.

Conclusion Our study clearly elucidates that intracellular oxidative stress was elevated in the subtypes of T-cells and their was alteration in Keap1/Nrf2/HMOX-1 and proportions, found to be associated with disease activity score(SLEDAI).

PO.2.29 SERUM LEVELS OF B-CELL RELATED FACTORS BELONGING TO THE TNF/TNFR SUPERFAMILY ARE LOWER IN ANTIPHOSPHOLIPID-RELATED SYNDROMES THAN IN SYSTEMIC LUPUS ERYTHEMATOSUS

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Purpose B-cell tolerance checkpoint defects are part of the pathomechanism in humoral autoimmune disorders, such as systemic lupus erythematosus (SLE) and antiphospholipid syndrome (APS).¹ In SLE, less stringent selection of autoreactive B cells at the transitional stage are potentially propagated by dysregulated BAFF (B-lymphocyte stimulator) homeostasis.² In APS, slight changes in circulating B-cell subsets were described, although with restricted autoantibody repertoire.³ A recent study demonstrated that differences in the naïve B-cell repertoire could explain the higher number and variety of autoantibodies in SLE in comparison to APS, especially in those with obstetric complications.⁴

Increased levels of circulating BAFF were described in SLE and the efficacy of B-cell targeted therapies, such as belimumab and rituximab, was well demonstrated in clinical trials and/or observational studies.⁵ In APS, despite the strong evidence of antibody mediated pathogenesis, specific B cell phenotype abnormalities, and by the development of the disease in patients with inborn errors of immunity involving B cell ontogeny, data on the use of therapies directed toward B cells are still lacking and anticoagulation remains the corner stone of management.⁶

The aim of this study was to measure serum levels of TNF/TNFR superfamily factors which are involved in B-cell homeostasis, looking for differences among diseases.

Methods Seventy-one patients [20 SLE, 10 SLE+aPL, 18 SLE+APS and 23 primary APS (PAPS)] were enrolled. The dosage was performed by high-sensitivity ELISA. Data are expressed by mean (in pg/ml, ng/ml for sBCMA). P values≤0.05 were considered statistically significant (*).

Results SLE patients had BAFF serum levels=1925 vs 1415 (in SLE+aPL), 965* (in SLE+APS), 1291 (PAPS);APRIL=8017 vs 3362*, 2502*, 2416*;sBCMA=14126 vs 10113, 8713*, 9145*;sTACI=8187 vs 2987, 1545*, 1570*;CD40L=3965 vs 2943, 1663*, 2098*;TWEAK=11331 vs 4971, 1890, 1573.

The statistical differences in factor levels among the clinical conditions were maintained after analysis by mixed linear model with multiple imputation, controlling for several clinical parameters (sex, race, BMI, blood pressure, hormonal substitution, age, disease duration).

Conclusions Lower serum levels of B-cell related TNF superfamily factors in the aPL-related conditions compared with SLE suggest that selection of autoreactive B cells in the

transitional stage, which is central in SLE, might not be the prominent mechanism for inducing aPL antibodies.

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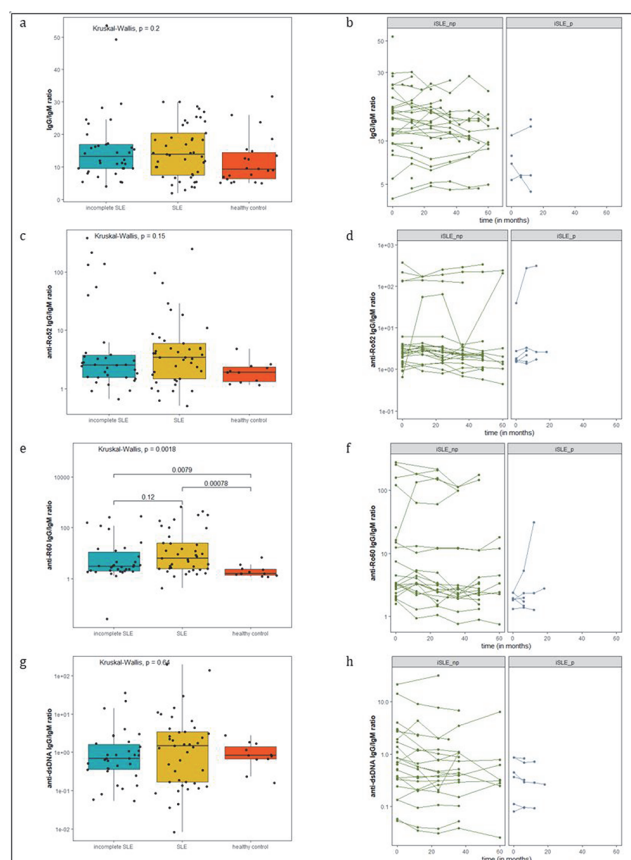
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PO.2.30 IGG/IGM AUTOANTIBODY RATIOS DO NOT RELATE TO PROGRESSION FROM INCOMPLETE SYSTEMIC LUPUS ERYTHEMATOSUS TO SYSTEMIC LUPUS ERYTHEMATOSUS

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Purpose To identify whether IgG/IgM autoantibody ratios differ between patients with incomplete systemic lupus erythematosus (iSLE), patients with SLE and healthy controls (HC) and



Abstract PO.2.30 Figure 1 Median and interquartile ranges are depicted for a, c, e and g. Total IgG/IgM ratios (a), anti-Ro52 IgG/IgM ratios (c) and anti-dsDNA IgG/IgM ratios (g) were not significantly different between groups. Anti-Ro60 IgG/IgM ratios were significantly elevated in iSLE and SLE patients compared to healthy controls. Longitudinal line graphs of total IgG/IgM ratio (b), anti-Ro52 IgG/IgM ratio (d), anti-Ro60 IgG/IgM ratio (f) and anti-dsDNA IgG/IgM ratio (h) are shown for iSLE patients that did not progress to SLE (iSLE_np) and for iSLE patients that did progress to SLE (iSLE_p)