I-03 DYSREGULATION OF T HELPER-TYPE CYTOKINES AND INTERFERONS APPEAR DURING EARLY SYSTEMIC LUPUS ERYTHEMATOSUS PATHOGENESIS AND CONTRIBUTE TO CLINICAL DISEASE DEVELOPMENT

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Background Systemic lupus erythematosus (SLE) is a complex autoimmune disease stemming from a poorly understood preclinical stage of autoantibody and symptom accrual. Antinuclear autoantibodies (ANAs) accumulate during this preclinical period. As many healthy individuals are also ANA-positive, this study aimed to identify further immune dysregulation that may contribute to disease pathogenesis.

Materials and methods SLE-associated autoantibodies, serum IFN-alpha activity and soluble mediators from multiple immune pathways were measured in serial serum samples from the Department of Defense Serum Repository by bead-based assays and cell-based reporter assays. Eighty-four patients with samples available pre- and post-SLE classification (average timespan = 5.98 years) were compared to 86 matched healthy controls. Temporal and predictive connexions between autoantibodies, soluble mediators, and SLE classification were determined by mixed linear regression, growth curve modelling, path analysis, analysis of covariance and random forest analyses.

Results In cases, but not matched controls, autoantibody specificities and IFN-associated mediators accumulated over a period of years, plateauing near the time of disease classification ($p < 0.001$). Nine soluble mediators, including IL-5 ($q = 4.35 \times 10^{-6}$) and IL-6 ($q = 8.26 \times 10^{-6}$), were significantly elevated in cases vs. controls >3.5 years pre-classification. Th_1-type, Th_{17}-type, and TNF superfamily soluble mediators increased longitudinally in cases approaching SLE classification, but not in controls ($q < 0.05$). In particular, levels of BLyS and APRIL were comparable in cases and controls until <10 months pre-classification ($q = 0.003$ and $q = 0.019$, respectively). During the early preclinical stage, random forest models incorporating IL-5 and IL-6 levels (79–82% accuracy) distinguished future SLE patients better than models with ANA alone (58% accuracy). Autoantibody positivity coincided with or followed type II IFN dysregulation, preceding IFN-α activity in growth curve models, with elevated IFN-α activity and BLyS levels occurring shortly before SLE classification ($p \leq 0.005$). Cases were distinguished by multivariate random forest models incorporating IFN-γ, MCP-3, anti-chromatin and anti-spliceosome antibodies (accuracy 93% >4 years pre-classification; 97% within 2 years of SLE classification).

Conclusions Years before SLE classification, enhancement of the type II IFN pathway allows for accumulation of autoantibodies and subsequent elevations in IFN-α activity immediately precede SLE classification. These and other serologic mediators demonstrate a long progression of immune dysregulation leading to SLE classification. Immunological profiles that distinguish individuals who develop clinical SLE may be useful for delineating early pathogenesis, discovering therapeutic targets, and designing prevention trials.

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I-04 AUTOCRINE STIMULATION BY INTRACELLULAR TYPE I IFN PRODUCED BY TRANSITIONAL T1 B CELLS IS A NOVEL BIOMARKER FOR SURVIVAL OF AUTOREACTIVE B CELLS

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Background Abnormal selection of self-reactive B cells has been shown to occur at the transitional B cell stage, the tolerance checkpoint II, in systemic lupus erythematosus (SLE). This study investigated novel mechanisms of IFN-beta (IFNb)-dependent tolerance loss of transitional B cells.

Materials and methods Using a La-peptide specific tetramer, La13-27 autoreactive B cells from the spleens of B6 and autoimmune BXD2 mice were analysed for the development of CD93+ transitional B cell subsets. Mice were treated with IFNa, IFNb, or anti-IFNAR to induce or block type I IFNs. qRT-PCR was used to determine expression of IFN and genes involved in type I IFN induction and responses. IFNB1 expression in human SLE patients and mouse B cells was determined by intracellular flow cytometry analysis.

Results Enhanced IFNAR provided a needed signal to promote transitional (CD93+) autoreactive (La13-27+) B cell maturation and survival in BXD2 mice. IFNb, compared to IFNa, exhibited a more potent effect to stimulate BXD2 transitional B cells. Surprisingly, there was abnormal elevation of IFNb in transitional T1 B cells of BXD2 mice. Autocrine production and stimulation by type I IFN was necessary for optimal anti-IgM-induced transitional B cell activation in purified B cells from BXD2, and the effect was abrogated by IFNAR blockade. Despite the higher expression of IFNb, there was lower expression of genes involved in nucleic acid sensing and TLR pathway (Rig1, Mda5, Pkr, Zbp1, Irf3, and Irf7) in BXD2 T1 B cells, compared to B6 T1 B cells, suggesting non-conventional induction of Ifnb in BXD2 T1 B cells. Interestingly, in vivo immune complex stimulation enhanced Ifnb levels in BXD2 T1 cells. Further, BXD2 but not B6 T1 B cells were susceptible to anti-IgM induction of IFNb. Higher expression of Ifnb1 was also found in La(+) B cells compared to La(-) B cells, suggesting that BCR stimulation may provide a signal to enhance type I IFN expression in BXD2 B cells. Similar to the mouse finding, elevation of IFNb was identified in 9G4+ transitional B cells from SLE patient, compared to B cells from healthy controls.

Conclusions These results suggest that transitional B cells from BXD2 mice exhibit autocrine stimulation by intracellular IFNb.