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

[1]Melissa E Munroe, [1,2]Rufei Lu, [1]Samantha R Slight-Webb, [1]Joel M Guthridge, [3]Timothy B Niewold, [4]George C Tsokos, [5]Michael P Keith, [6]John B Harley, [1,2]**Judith A James***. *[1]Arthritis and Clinical Immunology, Oklahoma Medical Research Foundation, Oklahoma City, OK, USA; [2]Medicine and Pathology, University of Oklahoma Health Sciences Centre, Oklahoma City, OK, USA; [3]Department of Immunology and Division of Rheumatology, Mayo Clinic, Rochester, MN, USA; [4]Rheumatology, Beth Israel Deaconess Medical Centre, Harvard Medical School, Boston, MA, USA; [5]Rheumatology, Walter Reed National Military Medical Centre, Bethesda, MD, USA; [6]Cincinnati Children's Hospital Medical Centre and US Department of Veterans Affairs Medical Centre, Cincinnati, OH, USA*

10.1136/lupus-2016-000179.68

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.

Acknowledgements This study was supported by funding from the National Institutes of Health, including the National Institute of Allergy, Immunology, and Infectious Diseases, Office of Research on Women's Health, National Institute of General Medical Sciences, and National Institute of Arthritis, Musculoskeletal and Skin Diseases, and the US Department of Veterans Affairs. The views and conclusions contained herein are the views of the authors and do not necessarily represent the official views of the Departments of the Army, Navy, or Defense, the Department of Veterans Affairs, or the NIH.

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

[1]Jennie A Hamilton, [1]Qi Wu, [1]Ping Ar Yang, [1]Bao Luo, [1]Shanrun Liu, [1]Jun Li, [2]Ignacio Sanz, [1]W Winn Chatham, [1]Hui-Chen Hsu, [1,3]**John D Mountz***. *[1]Department of Medicine, University of Alabama at Birmingham, Birmingham, AL 35294; [2]Department of Medicine, Emory University; [3]Department of Medicine, Birmingham VA Medical Centre, Birmingham, AL 35233*

10.1136/lupus-2016-000179.69

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.

In combination with BCR signalling, this facilitates survival and maturation of autoreactive B cells that otherwise will be deleted. Identification of the molecular mechanism leading to INFb autocrine stimulation in transitional B cells should unveil new pathways for development of autoreactive B cells.

Acknowledgements This worked was supported by grants from the NIH (grants R01-AI-071110 to Dr. Mountz, R01-AI-083705 to Dr. Hsu, P30-AR-048311 and P30-AI-027767 to the UAB Comprehensive Flow Cytometry Core), the Department of Veterans Affairs Merit Review grant 1I01BX000600-01 to Dr. Mountz, P30-AR-048311 P&F Project support to Dr. Jun Li, the Lupus Foundation of America Finzi Summer Fellowship and the UAB Immunology T32 training grant support to Jennie Hamilton, and the Lupus Research Institute Novel Research Award to Dr. Hsu. Dr. Sanz is supported by NIH R37AI049660 and NIH U19 AI110483 Autoimmunity Centre of Excellence.

I-05 DUAL FUNCTIONS OF TREX1 IN AUTOIMMUNE DISEASES

Nan Yan*. *Department of Immunology and Microbiology, University of Texas Southwestern Medical Centre, Dallas, TX, USA*

10.1136/lupus-2016-000179.70

Background TREX1 is an endoplasmic reticulum (ER)-associated exonuclease and a critical negative regulator of innate immunity. TREX1 mutations are associated with several autoimmune and autoinflammatory diseases, including Aicardi–Goutières syndrome (AGS), familial chilblain lupus, systemic lupus erythematosus (SLE), and retinal vasculopathy with cerebral leukodystrophy (RVCL). Both DNase-dependent and –independent functions have been described for TREX1 N-terminal DNase domain and C-terminal ER localization domain, respectively. Biallelic mutations abrogating DNase activity cause autoimmunity by allowing immunogenic self-DNA to activate the cGAS-STING-TBK1 signalling pathway leading to type I interferon (IFN) response and autoimmunity.

Methods and results We recently showed that inhibiting TBK1 by a potent small molecule inhibitor, Compound II, was able to ameliorate autoimmune disease phenotypes of *Trex1*$^{-/-}$ mice, increase mouse survival, and dampen the IFN gene signature in *TREX1* mutant patient lymphoblasts. We are also interested in a group of dominant frame-shift (fs) mutations that encode DNase-active but mislocalized proteins. We found that TREX1 C-terminus suppressed immune activation by interacting with the ER oligosaccharyltransferase (OST) complex and stabilising its catalytic integrity. C-terminal truncation of TREX1 by fs mutations dysregulated the OST complex, leading to free glycan release, immune activation and autoantibody production. Proper glycosylation of proteins in immunity is critical for their function, and protein glycosylation is also important for preventing self-immune recognition and production of autoantibodies. We recently established the TREX1-V235fs knock-in mouse to better understand the disease associated with *TREX1-fs* mutations.

Conclusion Together, our past and ongoing studies reveal dual functions of TREX1 in regulating self-DNA and self-glycan metabolism, and suggest potential therapeutic targets and options for *TREX1* mutant-associated autoimmune diseases.

I-06 LONG INTERSPERSED NUCLEAR ELEMENT-1 RETROELEMENTS ARE EXPRESSED IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS AND PRIMARY SJOGREN'S SYNDROME AND INDUCE TYPE I INTERFERON

[1,2,3]Clio P Mavragani, [1]Irina Sagalovskiy, [1]Qiu Guo, [2]Adrianos Nezos, [3]Efstathia K Kapsogeorgou, [1]Pin Lu, [1]Jun Liang Zhou, [1]Kyriakos A Kirou, [4]Surya V Seshan, [3]Haralampos M Moutsopoulos, [1]**Mary K Crow***. *[1]Mary Kirkland Centre for Lupus Research, Hospital for Special Surgery, USA; [2]Department of Physiology, School of Medicine, National and Kapodistrian University of Athens, Greece; [3]Department of Pathophysiology, School of Medicine, National and Kapodistrian University of Athens, Greece; [4]Department of Pathology, Weill Cornell Medical College, USA*

10.1136/lupus-2016-000179.71

Background Recent studies have documented numerous common and several rare genetic variants that are associated with SLE, but the endogenous and exogenous triggers that initiate and perpetuate disease have not yet been defined. The presence of elevated serum type I interferon (IFN-I) activity and a broad signature of IFN-I-induced gene transcripts and proteins in blood and tissue of patients with lupus and other systemic autoimmune diseases, including primary Sjogren's syndrome (SS), are consistent with a viral trigger, but available data have not identified an exogenous virus as an etiologic agent in these diseases. To identify disease-relevant triggers of the IFN-I pathway we investigated whether endogenous virus-like genomic repeat elements, normally silent, might be expressed in patients with systemic autoimmune disease, activate an innate immune response and induce IFN-I.

Materials and methods Expression of IFN-I and long interspersed nuclear element-1 (LINE-1; L1) was studied in kidney tissue from lupus patients and minor salivary gland (MSG) tissue from patients with primary SS by PCR, western blot and immunohistochemistry. Induction of IFN-I by L1 was investigated by transfection of plasmacytoid dendritic cells (pDCs) or monocytes with an L1-encoding plasmid or L1 RNA. Involvement of innate immune pathways and altered L1 methylation were assessed.

Results L1 mRNA transcripts were increased in lupus nephritis kidneys and in MSG from SS patients and correlated with IFN-I expression. Using bisulfite-PCR pyrosequencing, a negative correlation of L1 expression with% L1 methylation was documented for the majority of L1 promoter CpG sites tested, suggesting that augmented demethylation processes, or alternatively impaired remethylation, might account for the observed L1 overexpression in SS MSG tissues. L1 open reading frame 1/p40 protein and IFN-beta were expressed in MSG ductal epithelial cells and in lupus kidneys, and IFN-alpha was detected in infiltrating pDCs. Transfection of pDCs or monocytes with L1-encoding DNA or RNA or U1 RNA, but not hY3 RNA, induced IFN-I. Inhibition of TLR7/8 reduced L1 induction of IFN-alpha in pDCs, and an inhibitor of IKK-epsilon/TBK1 abrogated induction of IFN-I by L1 RNA in monocytes.

Conclusions L1 genomic repeat elements represent endogenous nucleic acid triggers of the IFN-I pathway in SLE and SS and may contribute to initiation or amplification of autoimmune disease. Investigation of the genetic and environmental factors that alter regulation of L1 elements and increase availability of these endogenous immunostimulatory factors should suggest novel therapeutic interventions in SLE and related diseases.

Acknowledgements We acknowledge Evi Poulou and Effie Papageorgiou for assistance in immunohistochemical and Western Blot stainings.