indicated that patients with autoimmune conditions such as systemic lupus erythematosus (SLE) and the antiphospholipid syndrome (APS) have antibodies against mitochondria in their blood stream. Presence of these antibodies was associated with increased disease activity and clinical manifestations of these diseases (e.g. kidney disease, arterial vessel disease). In this study, we studied blood samples harvested by an international group dedicated to the study of SLE [i.e., the SLE International Collaborating Clinics (SLICC) cohort] and observed that patients may be clustered into groups, upon their levels of antibodies and/or sex, allowing to have a better appreciation of their risks of death, vascular events, and kidney disease. These results might lead to improved diagnosis and/or prognosis in SLE and thus, in improved care and quality of life for the people living with lupus.

104 SLE AUTOANTIBODIES TO CASEIN KINASE II: POTENTIAL MEDIATORS OF IMMUNOPATHOLOGY

[1]Daniel H Shu, [2]Joan T Merrill, [1]Philip L Cohen. *[1]Lewis Katz School of Medicine at Temple University; [2]Oklahoma Medical Research Foundation*

10.1136/lupus-2022-lupus21century.4

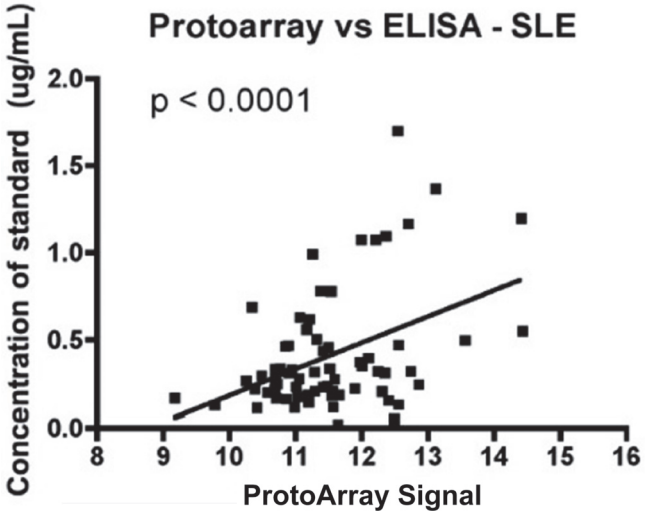
Background Casein kinase II (CSNK2) is a key regulator of cell activation, proliferation, and apoptosis. While primarily an intracellular molecule, it has recently been appreciated that CSNK2 is expressed on cell surfaces, where it functions as an ectoenzyme with broad regulatory activity. Its presence of cell surfaces raises the possibility that autoantibodies to CSNK2 might interfere with its function and mediate immunopathology. A role for CSNK2 autoantibodies was suggested 30 years ago in animal models. More recently, we have used protein array technology (Protoarray, Invitrogen Technologies) to investigate the full spectrum of SLE autoantibodies. A study of patient sera from three different cohorts (Philadelphia, Romania, and People's Republic of China) showed that autoantibodies to the alpha (catalytic) subunit of CSNK2 were among the top seventeen self specificities found in common among these diverse lupus sera. In the present study, we developed a formal ELISA to investigate autoantibodies to CSNK2 in two well studied collections of SLE sera.

Methods We developed an ELISA binding assay for CSNK2 autoantibodies. We inhibited the ELISA to show antigen specificity, and we performed Western blots to show that the lupus sera recognized recombinant antigen. We used the clinical information about both cohorts to look for clinical correlations with the presence of this autoantibody.

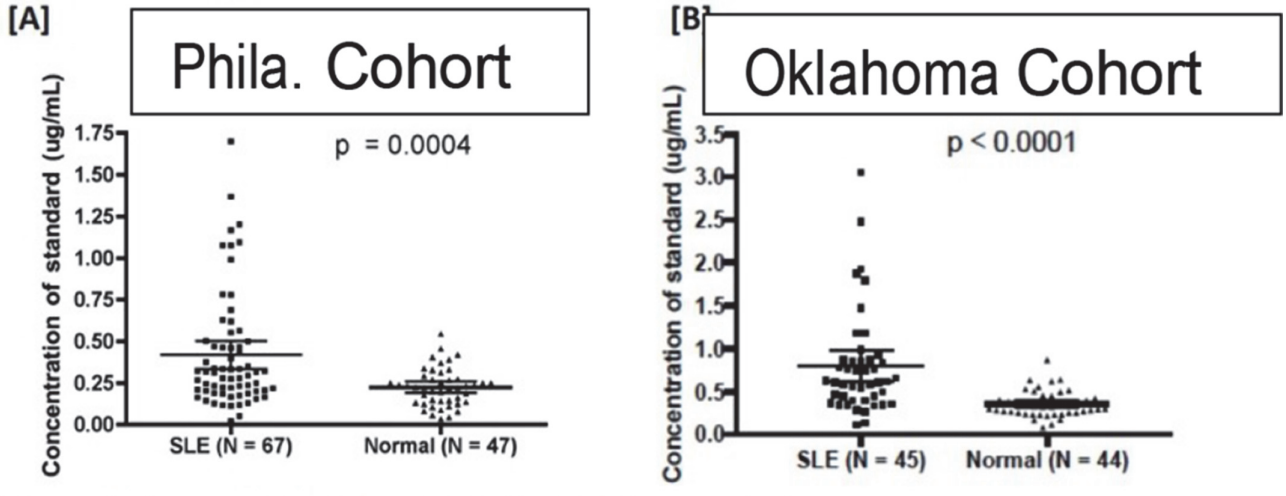
Results Using recombinant human alpha subunit of human CSNK2 (Lifespan Technologies), we developed an ELISA to test binding of lupus and control sera to CSNK2. We examined 114 SLE sera and age and sex matched controls from Philadelphia, and 99 paired lupus sera from Oklahoma City.

We showed by Western blot that lupus sera bound to the same molecular weight recombinant CSNK2 as rabbit antisera to this protein.

The presence of autoantibodies to CSNK2 correlated with overall SLEDAI and with new onset arthritis in the two groups. Other correlations were inconsistent.



Abstract 104 Figure 2 Protoarray correlates with ELISA



Abstract 104 Figure 1 Indirect ELISA for anti-CSNK2α2 in two cohorts. Panel A shows ELISA signal strength for α-CSNK2α2 in temple Lupus cohort and matched controls (N=114). Panel B shows ELISA signal strength for α-CSNK2α2 in Oklahoma Lupus cohort and matched controls (N=99).

Conclusions Most SLE autoantibodies are directed against intracellular (often intranuclear) antigens. The prevalence in SLE of autoantibodies to a key regulatory ectoenzyme is of interest and raises the possibility that such antibodies might play a role in pathogenesis.

Antibodies to the CSNK2 catalytic subunit may interfere with its function in regulating cell growth, apoptosis, and activation. As the ectoenzyme is expressed on endothelial cells, lymphocytes, neutrophils, and monocyte-macrophages, there is the potential for broad effects on inflammation and immunity. The antibodies we have described deserve further investigation.

201 *EZH2* KNOCKOUT IN B CELLS IMPAIRS PLASMABLAST DIFFERENTIATION AND AMELIORATES LUPUS-LIKE DISEASE IN MRL/*LPR* MICE

[1]Xiaoqing Zheng, [2]Mikhail G Dozmorov, [1]Colleen E Strohlein, [3]Sheldon Bastacky, [1,4,5,6]Amr H Sawalha. *[1]Division of Rheumatology, Department of Pediatrics, Children's Hospital of Pittsburgh, University of Pittsburgh, Pittsburgh, Pennsylvania, USA; [2]Department of Biostatistics, Virginia Commonwealth University, Richmond, VA, USA; [3]Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA; [4]Division of Rheumatology and Clinical Immunology, Department of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania, USA; [5]Lupus Center of Excellence, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA; [6]Department of Immunology, University of Pittsburgh, Pittsburgh, Pennsylvania, USA*

10.1136/lupus-2022-lupus21century.5

Enhancer of zeste homolog 2 (EZH2) has been shown to regulate early B cell development and the differentiation of antibody secreting cells (ASCs). We have previously demonstrated increased EZH2 expression in peripheral blood mononuclear cells isolated from lupus patients, and that pharmacological inhibition of EZH2 alleviates lupus-like disease in mouse models. In this study, we generated a conditional knockout mouse to examine the effect of EZH2 deficiency in B cells in the MRL/*lpr* lupus-prone mouse. We show that *Ezh2* deletion in B cells significantly decreased autoantibody production and improved glomerulonephritis. B cell development was altered in the bone marrow and spleen in EZH2-deficient mice. Differentiation of ASCs was impaired. Single cell RNA sequencing showed that XBP1, a key transcription factor in B cell development, is downregulated in the absence of EZH2. Inhibiting XBP1 *in vitro* impairs ASC development similar to EZH2-deficient mice. Single cell B cell receptor RNA sequencing revealed defective immunoglobulin class switch recombination in EZH2- deficient mice. In human lupus B cells, we observed a strong correlation between EZH2 and XBP1 mRNA expression levels. Taken together, our results suggest that EZH2 overexpression in B cells contributes to disease pathogenesis in lupus.

Lay Summary Epigenetics refers to the mechanisms that regulate gene expression. DNA methylation is a key epigenetic mechanism that is dysregulated in lupus cells. We have previously revealed a central role for EZH2, a key epigenetic modifier, in modulating epigenetic changes in lupus. We have shown that lupus immune cells, including T and B lymphocytes, overexpress EZH2. In this study we generated a mouse model to delete the gene encoding EZH2 in B cells. We show that EZH2-deficient lupus-prone mice are significantly protected from lupus-like disease, with a reduction in autoantibody production and renal involvement. EZH2 deficiency impair B cell development by downregulating XBP1 which plays an important role in B cell differentiation.

202 TRANSCRIPTOMIC DIVERSITY AND OVERLAPPING CLONALITY ACROSS SUBSETS OF ANTIBODY-SECRETING AND MEMORY B CELLS FROM SPONTANEOUS GERMINAL CENTERS

[1]Carlos Castrillon, [1]Lea Simoni, [1]Theo van den Broek, [1]Cees van der Poel, [1,3,4]Elliot H Akama-Garren, [1]Minghe Ma, [1,2,3]Michael C Carroll. *[1]PCMM, Boston Children's Hospital, Harvard Medical School, Boston; [2]Harvard-MIT MD/PhD Program, Cambridge, MA, USA*

10.1136/lupus-2022-lupus21century.6

Background Affinity matured self-reactive antibodies are a hallmark of autoimmune diseases like systemic lupus erythematous. Earlier studies using a mixed-bone marrow transplant (BM) model system identified spontaneous germinal centers (GC) as sites for epitope spreading.

Moreover, this autoimmune model system revealed that autoreactive GC B cells compete for self-antigen and undergo clonal selection much like that identified for foreign antigen specific B cells. However, the results raised questions about other subsets of autoreactive B cells such as memory B cells (MemB)?

Methods BM from reporter mice (S1PR2 cre_TOM) was mixed with BM from 564 Igi autoimmune mice to generate a model in which spontaneous GC B cells were marked with TOM and were derived primarily from WT background. In parallel, mixed BM chimeras were prepared with WT BM and immunized with a T-dependent antigen. Single cell transcriptomics coupled to antibody repertoire analysis was used to characterize the post germinal center (GC) B cell compartment in the two groups of mice.

Results Antibody secreting cells (ASCs) and memory B cells (MemBs) from spontaneous GCs grouped into multiple subclusters. ASCs matured into two terminal clusters, with distinct secretion, antibody repertoire and metabolic profiles. MemBs contained FCRL5+ and CD23+ subsets, with different in vivo localization in the spleen. Interestingly IgM pos GC derived FCRL5+ MemBs share transcriptomic and repertoire properties with atypical B cells found in aging and infection and localize to the marginal zone.

Differential gene expression and repertoire analysis showed that autoreactive MemBs were similar to foreign antigen immune mice.

Conclusions Autoreactive ASC and MemB cells differentiate into subsets similar to that identified in foreign antigen immune mice. Of the two major subsets of MemB, the FCRL5+ subset is primarily IgM pos and localize to the marginal zone. Moreover, clonal redundancy between all MemB and ASC cell clusters was observed.

Lay Summary A mouse model of lupus was used to show that autoreactive B cells form memory similar to those following vaccination. Thus, autoreactive memory B cells are an important therapeutic target.