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

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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

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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.