DEFECTIVE IKAPPABNS LEADS TO LOSS OF PERITONEAL B CELLS AND A REDUCTION IN INDUCED BUT NOT SPONTANEOUS AUTOIMMUNE HEMOLYTIC ANAEMIA

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Background The role of the B1a subset in cells in autoimmunity remains controversial. Here we identify a spontaneous mutation in IkappaBNS associated with severe reduction in the peritoneal B1a, but not other B cell subsets and use this mutation to study its contribution to autoimmunity.

Materials and methods NZB, NZB.NZW-Lbu2, B6-Nfkbid<sup>paBNS</sup> (IkappaBNS-deficient mice), and crosses were bred and maintained at TSRI and experiments approved by Scripps IACUC. Flow cytometry, mapping, sequencing, Ig and autoantibody ELISA, and direct Coomb’s test were by standard procedures. For in vivo poly(I:C) stimulation, 200 ug were given i.p. to 6–10 wk-old mice 2x/wk for 8 wks. B cells were stimulated in vitro with 30 ug/ml goat F(ab')<sub>2</sub> anti-mouse IgM in RPMI 10% FCS.

Results A chromosome 4 NZB subcongenic line, NZB.NZW-Lbu2SE, was discovered to exhibit very low B-1a cells in the peritoneal cavity and reduced serum IgM, but with no detectable effect on B cells in other lymphoid compartments including the MZ subset in the spleen. Mapping and a complementation study with Nfkbid (IkappaBNS gene)-deficient mice, identified a spontaneous hypomorphic K100N mutation of IkappaBNS, a member of the nuclear IkappaB family that serves as modulators of NF-κB function. Notably, in contrast to complete deletion of IkappaBNS, which affects multiple immune cell types, the phenotype of the NZB-SE mutation, named <i>lowb1</i>, was limited to peritoneal B-1a cells. The absence of low B-1a cells did not reduce susceptibility to spontaneous autoimmune hemolytic anaemia. However, <i>lowb1</i> mice were resistant to poly(I:C)-induced autoimmune hemolytic anaemia indicating that B-1a cells could play a role in modulating environmental factors.

Conclusions These studies suggest a limited role for B1a cells in autoimmune hemolytic anaemia and identify the nuclear Ikappa family as a modulator of autoimmunity.
by gene ontology (GO) and pathway enrichment analysis that were highly enriched in SLE T cells included mediators of adaptive responses and inflammation, and those regulating co-stimulation. By contrast, negative regulators of cell proliferation and function were found in the healthy control cluster, and diminished in SLE.

Conclusions Our data demonstrate altered transcriptional programs of lupus Tfh and Tcm cells, and therapeutic targets in disease. They also represent the first detailed transcriptional profiling, and single cell transcriptional profiling, of Tfh cells, the necessary and critical driver of humoral immunity in SLE.

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AI-23 MULTIPLEXED MECHANISTIC ASSAYS FOR CHARACTERISING SLE

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Background SLE is a complex disease with very few approved therapeutic options. Unique opportunities exist to characterise blood cells, tissues such as kidney and skin, urine, serum and plasma as part of ongoing longitudinal cohort studies such as Accelerating Medicines Partnership (AMP) and Autoimmunity Centres of Excellence (ACE), and investigator initiated or company-sponsored clinical trials.

Materials and methods SLE blood and tissue samples are being studied using a variety of single cell measurements as well as studies of biofluids. Several of these technologies are becoming firmly established in the SLE field, including single cell RNA Seq of blood and dissociated kidney using recently-developed methods in several consortia related to SLE and cancer; low input RNA-Seq of bulk purified cells; Assay of Transposase Accessible Chromatin (ATAC Seq); CyTOF and EpiCyTOF developed through the ACE consortium; transcript profiling using many methodologies; meta analysis of existing transcript profiling datasets; autoantibody profiling using autoantigen microarrays, and arrays composed of secreted factors such as cytokines and chemokines; multiplexed ion beam imaging (MIIB); and unpublished imaging methods such as CODEX.

Results An overview of multiplexed methods will be presented and will focus on efforts by the Stanford ACE and collaborating investigators to develop methods specifically for the study of SLE. Historical methods will be compared, and ACE datasets on human SLE, and mouse models of SLE characterised as part of ALR studies, will be described that demonstrate unique roles for interferons and STAT signalling in lupus.

Conclusions Big data analyses and multiplexed assays of samples derived from SLE patients, as well as patients with related autoimmune diseases, have tremendous potential and should be included in all clinical trials, with a goal to better understand pathogenesis and to identify novel therapeutic targets.

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