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

1John C Scatizzi, 1Hua Huang, 2Bruce Beutler, 1Argyrios N Theophilopoulos, 1Dwight H Kono*. 1Department of Immunology and Microbial Science, The Scripps Research Institute, La Jolla, CA 92037; 2Center for Genetics of Host Disease, Department of Immunology, UT Southwestern, Dallas, TX 75390

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**Background** Genetic and epigenetic mechanisms that may contribute to lupus susceptibility in humans and mouse models are of interest especially if they provide enzymes that could function as potential therapeutic targets. We have discovered an enzymatically mediated O-acetylation event found prominently in B cells in humans with lupus and in MRL/+ mice. Detailed studies in MRL/+ mice indicate that this modification may allow for a break in B cell tolerance and may be a major component of lupus susceptibility in these mice as well as possibly in humans as well.

**Materials and methods** We have used a catalytically dead Influenza C hemagglutinin esterase Ig fusion protein and a bovine coronavirus hemagglutinin esterase – Ig fusion protein as tools to respectively identify and remove 9-O-acetylated sialic acid on B cells in humans and in MRL/+ mice. Enzymatic approaches were used to identify the type of glycoconjugate that exhibits enhanced 9-O-acetylation of sialic acid moieties. We created a CasD1 knockout mouse to examine the role of this enzyme in O-acetylated sialic acid moieties in vivo. Genetics, whole genome sequencing and RNA-seq approaches are being used to identify the mechanism underlying this alteration and its link to lupus susceptibility.

**Results** Increased 9-O-acetylation of sialic acid on naive B cells is observed on approximately two-thirds of subjects with active SLE. Markedly increased levels of 9-O-acetyl sialic acid are also observed in the earliest B lineage cells in lupus prone MRL/+ mice and this high level is maintained throughout B cell development and well before these mice exhibit any features of disease. This increased 9-O-acetylation of sialic acid was not observed on glycoproteins or mucins on MRL/+ B cells but was restricted to gangliosides. Acetylated gangliosides protected these B cells from BCR-dependent apoptosis. Deacetylation of sialic acid on MRL/+ B cells restored anti-IgM mediated apoptosis to wild type levels. We used a CasD1 knockout mouse to establish that this enzyme is required for the 9-O-acetylation of sialic acid in vivo. Increased 9-O-acetylation of sialic acid in MRL/+ mice is dominantly inherited and the molecular basis of this striking change is being investigated using genetics and whole genome sequencing.

**Conclusions** Enhanced 9-O-acetylation of sialic acid on B cells in lupus prone mice and in humans may represent a potential mechanism by which B cell tolerance is abrogated in lupus prone mice and in humans may represent a potential therapeutic target of relevance in lupus.

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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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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 Centers 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 (MiBi); 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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