hypothesized that lymphatic dysfunction could contribute to photosensitivity in SLE.
Methods We examined MRL/lpr lupus prone mice for lymphatic function by injecting Evan's Blue into the ear and measuring retention. Ear thickness and flow cytometric analysis were used to assess photosensitivity. Lymphatic drainage was manipulated using two approaches. First, we used manual lymphatic drainage (MLD) in the MRL/lpr mice. MLD improved lymphatic drainage and reduced photosensitivity. Second, we induced a lupus phenotype in a novel mouse model with enhanced lymphatic function (inducible lymphatic endothelial cell specific PTEN KO) using topical imiquimod.
Results MRL/lpr mice had greater Evan's blue retention compared to controls suggesting lupus prone mice have impaired lymphatic drainage. MLD improved lymphatic drainage and reduced photosensitivity. PTEN KO mice had reduced photosensitivity and reduced systemic immune activation.
Conclusions This data suggests that lymphatic dysfunction contributes to photosensitivity in murine lupus and improving lymphatic flow, even with simple MLD, can ameliorate photosensitivity. Future studies will determine the etiology of lymphatic dysfunction in murine lupus and the mechanism of lessened photosensitivity with improved lymphatic drainage. If similar immune circuitry defects are present in patients with SLE, altering lymphatics could be a novel target for new therapeutics.
Acknowledgments Lupus Research Alliance

300 – Transcriptomics

301 DIFFERENTIALLY EXPRESSED TRANSCRIPTS ASSOCIATED WITH LUPUS RISK LOCI IDENTIFY PATHOGENIC DISEASE PATHWAYS

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Background Genome-wide association studies (GWAS) have identified single nucleotide polymorphisms (SNPs) that tag genomic sites with a high statistical association with a diagnosis of SLE. As most risk SNPs mark regulatory rather than coding regions of the genome, the functional impact of the risk loci on molecular mechanisms and pathways has not been clearly defined. In addition, how disease-associated loci differentially impact pathogenic mechanisms in females and males with SLE has not been explored. We analyzed RNA sequencing data relevant to previously identified risk loci to understand the molecular pathways impacted by genetic variation.
Methods Forty subjects (10 SLE females; 10 SLE males; 10 healthy females; 10 healthy males) were identified for study, with careful matching of SLE and healthy donor subjects for age and ethnicity. Next generation RNA sequencing was performed and unsupervised clustering of transcript expression displayed for genes previously identified in GWAS as near SNPs associated with a diagnosis of SLE. Identified pathways were also investigated using an expanded RNAseq dataset, including 30 patients with lupus nephritis, 29 patients with non-renal SLE and 3 healthy donors.
Results Four major clusters of gene transcripts were identified, along with multiple subclusters. The most striking transcript subcluster preferentially expressed in lupus males identified mechanisms involved in nucleic acid sensing and type I interferon production (IRF7, IFIH1, IKBKE, TLR7, JAK2, CXorf21/TASL, and SLC15A4). Another subcluster that favored males included T and B cell transcripts associated with generation of autoantibody producing B cells (ITGAX, IRF5, SH2B3, TET3, NOTCH4, ICAM4). The cluster with transcripts showing decreased expression in SLE included DEF6 and PHRF1, potentially contributing to impaired transcriptional regulation of lymphocyte function in patients. Genes located in the MHC (ATF6B, NOTCH4, MICB, TNXB, ITPR3) or an X chromosome risk locus (IRAK1, TMEM187, MECP2, NAA10, HCFC1) were distributed among different transcript subclusters, suggesting the broad impact of risk haplotypes at those loci on regulation of multiple disease-associated pathways. Of interest is the observation that PHRF1 and IRF7, adjacent genes identified by a risk SNP (rs4963128), distributed to distinct clusters, with PHRF1 decreased and IRF7 increased in SLE patients. The expanded SLE dataset was also analyzed to further characterize lupus risk-associated functional transcript clusters.
Conclusion Genes located near lupus risk SNPs are differentially expressed in patients with SLE and cluster based on functional relationships. Transcripts differentially expressed in male patients suggest important involvement of nucleic acid sensing pathways in their disease.

302 TRANSCRIPTOMIC ANALYSIS REVEALS A CRITICAL REGULATORY ROLE FOR CD8 T CELLS IN A MOUSE MODEL OF SYSTEMIC LUPUS ERYTHEMATOSUS

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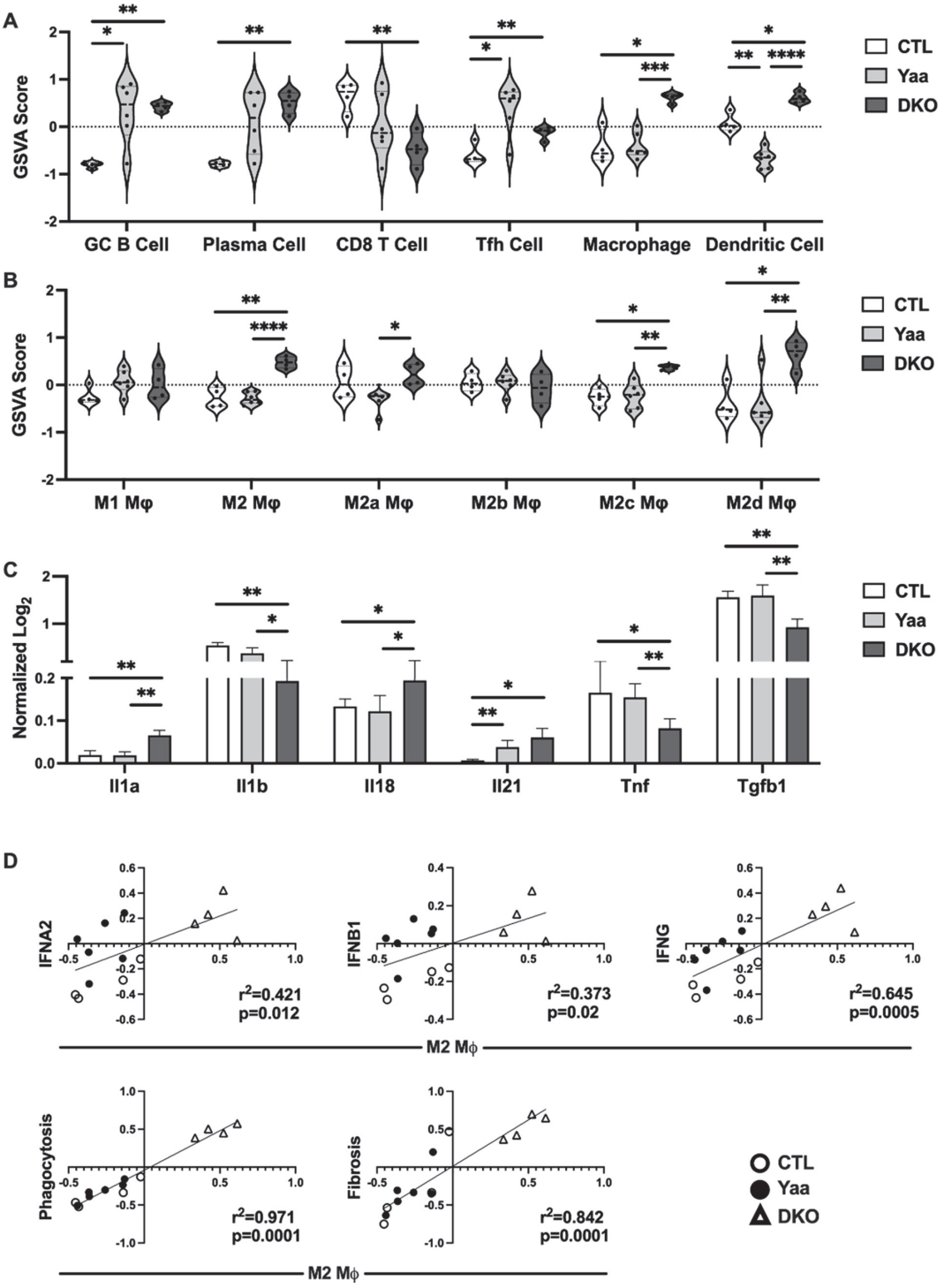
Background Pre-clinical mouse models are invaluable tools to investigate the mechanisms driving systemic lupus erythematosus (SLE) and identify therapeutic targets to treat human disease. The BXSB.Yaa (Yaa) mouse spontaneously develops SLE-like disease, that is accelerated by the loss of CD8 T cells in BXSB.Yaa.CD8a$^{-/-}$IL15$^{-/-}$ (DKO) mice, suggesting that CD8 T cells have a protective role against autoimmunity in this model. The role of CD8 T cells in human SLE remains unclear as studies have found conflicting associations with increased disease severity or with a loss of regulatory activity. Therefore, we carried out transcriptome analysis of Yaa and DKO mice to clarify the role of CD8 T cells in SLE.
Methods Six week old 'pre-disease' mice were examined to determine the impact of CD8 T cells before the development of inflammatory disease. Gene expression profiles from spleens of 6 week old BXSB.B6 (CTL), Yaa, and DKO mice were obtained by RNA-seq. Log_2 gene expression values were normalized between datasets using housekeeping genes. Differential enrichment of immune cell and pathway gene modules curated for analysis of lupus mouse datasets was determined by Gene Set Variation Analysis (GSVA). Immune cell population GSVA scores were then correlated with functional pathways by linear regression.
Results Spleens from 6 week BXSB.Yaa mice showed evidence of extensive immune activation. GSVA revealed shared enrichment of SLE-associated lymphocyte populations including germinal center (GC) B cells and T follicular helper (Tfh) cells in

Yaa compared to healthy CTL mice that was not further accentuated in DKO mice (figure 1A). Notably, plasma cells (PCs) were only enriched in DKO mice. Myeloid cells and specifically macrophages (Mϕs) and dendritic cells (DCs) were not enriched in Yaa, but significantly enriched in DKO over both Yaa and CTL mice. Furthermore, enrichment of Mϕ signatures in DKO mice was restricted to M2 but not M1 polarization markers and specifically to M2a, M2c, and M2d subsets (figure 1B). Comparison of normalized log_2 gene expression of pro- and anti-inflammatory cytokines revealed that *Il1a*, *Il18*, and *Il21* were elevated, whereas *Il1b*, *Tnf*, and *Tgfb1* were decreased in DKO as compared to CTL and Yaa mice (figure 1C). The enriched M2 population significantly correlated with a number of inflammatory pathway signatures related to Mϕ function including IFNA1, IFNB2, IFNG, phagocytosis, and fibrosis (figure 1D).



Abstract 302 Figure 1 Enrichment of Mϕ populations in the absence of CD8 T cells in BXSB.Yaa mice. (A and B) GSVA of BXSB CTL, Yaa, and DKO mice for enrichment of immune cell gene signatures. Enrichment scores are shown as violin plots. (C) Housekeeping gene normalized log_2 gene expression values for a panel of inflammatory cytokines. (D) Linear regression analysis between M2 Mϕ GSVA scores and functional pathway GSVA scores from BXSB CTL, Yaa, and DKO mice. Correlations with p<0.05 were considered significant. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Conclusions Transcriptomic analysis of Yaa mice before onset of SLE like clinical disease and DKO mice that develop accelerated disease uncovered differences in immune profiles, which point to a role for CD8 T cells in protection from autoimmunity in this model. In the absence of CD8 T cells, DKO mice exhibited an increase in gene signatures of M2-like Mϕs representing a unique functional subset correlated with pathologic pathway signatures. This analysis provides evidence for a protective, regulatory function of CD8 T cells against autoimmune pathology with implications for understanding their role in human SLE.

303 A STEPWISE TRANSCRIPTOMIC ANALYSIS USING GENE MODULES AND IMMUNE CELL SIGNATURES TO STRATIFY SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS AND IDENTIFY POTENTIAL TREATMENT TARGETS

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Background A major challenge in drug development for systemic lupus erythematosus (SLE) is the heterogeneous clinical presentation of SLE patients, which necessitates personalized treatment strategies. We aimed to identify clusters of SLE patients based on molecular transcriptomic signatures associated with clinical phenotypes to help address this challenge.
Methods To address this question, we developed an integrated pipeline that defines subsets of patients based on cell type-specific gene expression in blood. Gene expression profiles from two large independent SLE trials, ILLUMINATE-1 and ILLUMINATE-2, were analyzed to identify SLE patient clusters. We first performed a gene expression correlation network analysis to identify co-expressed gene modules. Then, unsupervised consensus clustering was performed on the modules to identify molecular clusters. We correlated cluster membership with clinical phenotypes and immune cell signatures from high resolution scRNA-seq data. We also determined whether immune cell signatures were stable over time.
Results We identified four molecular clusters of SLE patients. Cluster 1 exhibited high signature scores for T cells, B cells, plasma cells, macrophages, and monocytes. Conversely, Cluster 2 exhibited low signature scores for the aforementioned cells. Cluster 3 had high T and B cell signature scores. Cluster 4 had a high signature score for neutrophils. Clinically, Cluster 3 subjects exhibited the lowest disease severity compared to other clusters. We validated these four molecular clusters in three additional independent SLE cohorts. We identified four molecular clusters of SLE patients that were consistent across five independent genomics datasets totaling 2,100 patients. For individual patients, cluster membership was not necessarily stable over time.
Conclusions We have established methods to address SLE heterogeneity in a data-driven, unbiased manner using transcriptomic data. We have uncovered reproducible patterns in stratifying SLE patients using this method and connected SLE patient subsets to cellular alterations in the blood. Our findings have important implications for personalized treatment of SLE and provide guidance for clinical trials in this highly heterogeneous disease.
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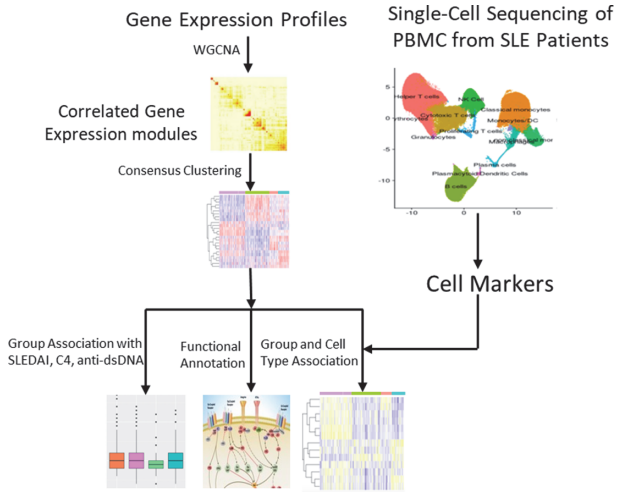

Abstract 303 Figure 1 Overview of analysis

304 METABOLIC DYSREGULATION CHARACTERIZES THE TISSUE RESPONSE TO IMMUNE INJURY IN SYSTEMIC LUPUS ERYTHEMATOSUS AND INFLAMMATORY SKIN DISEASES

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Background Autoimmune and inflammatory diseases such as systemic lupus erythematous (SLE) affect tissues throughout the body. Alterations to inflammatory cell metabolism are often cited as a contributing factor in diseases such as lupus[1], however, changes to metabolism in diseased tissues are poorly understood. Therefore, we investigated changes to cellular metabolic processes in the tissues affected by SLE as well as samples from other inflammatory skin diseases.
Methods Gene expression data collected from patients with lupus nephritis (LN) glomerulus (GL), LN tubulointerstitium (TI), discoid lupus erythematosus (DLE), psoriasis (PSO), atopic dermatitis (AD), and systemic sclerosis (SSc), or murine LN was obtained from Gene Expression Omnibus. Enrichment of metabolic and cellular signature in individual samples was analyzed using Gene Set Variation Analysis (GSVA). Stepwise regression and classification and regression tree (CART) analyses were performed to determine correlations between each metabolic signature and all cellular signatures in each diseased tissue.
Results Comprehensive gene expression analysis of samples derived from glomerular and tubulointerstitial LN kidneys, and DLE, PSO, AD, and SSc skin revealed concurrent changes to genes reflective of cellular metabolic processes and cellular transcripts. In lupus-affected tissues there were shared decreases to metabolic gene signatures[2], whereas in other inflammatory skin diseases some metabolic transcripts were increased. In glomerular LN, decreased glycolysis gene expression was correlated with increased endothelial cell transcripts[2].