II-02 NEUROPSYCHIATRIC LUPUS IS DEPENDENT ON LIPOCALIN-2

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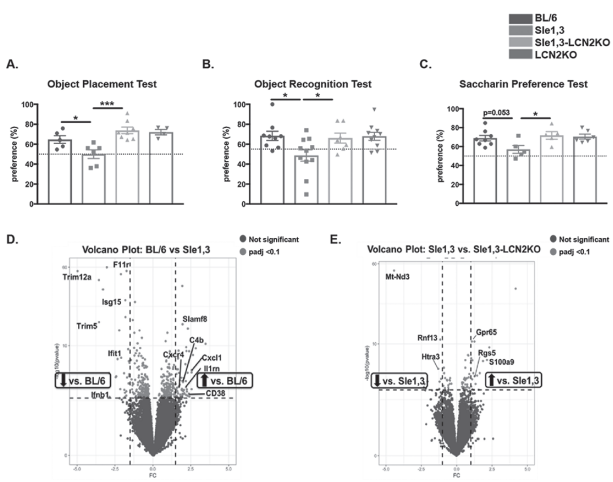
Background Neuropsychiatric manifestations of systemic lupus erythematosus (NPSLE) affect approximately 40% of patients. Lipocalin-2 (LCN2), an acute-phase reactant protein, is an established urinary biomarker in lupus patients. Previous studies using LCN2-deficient mice have demonstrated its role in glial migration and chemokine regulation in the brain. We therefore hypothesized that LCN2 is involved in NPSLE pathogenesis.

Methods We investigated the lupus prone B6.Sle1.Sle3 (Sle1,3) mouse and the effects of LCN2 deficiency on the development of the neuropsychiatric phenotype exhibited by this strain. Sle1,3, B6.LCN2KO, B6, and Sle1,3-LCN2KO mice (6–10 month old; n=5–10/group) underwent comprehensive neurobehavioral assessment, and brains were evaluated by flow cytometry and RNA sequencing.

Results Sle1,3 mice exhibited significant impairment in spatial ($p<0.04$, figure 1A) and recognition ($p<0.02$, figure 1B) memory when compared with B6 mice, and these deficits were attenuated in Sle1,3-LCN2KO mice ($p<0.001$, $p<0.02$, figure 1A-B). Furthermore, Sle1,3 mice demonstrated anhedonia, and this depression-like behavior was significantly reduced with LCN2 deficiency ($p=0.01$, figure 1C). Flow cytometry showed a significant increase in brain infiltrating CD8+ T cells in Sle1,3 mice, with a reduction in infiltration in the Sle1,3-LCN2KO strain ($p=0.06$). Preliminary analysis of RNA sequencing from sorted microglia revealed differential expression of genes between B6 and Sle1,3 mice (figure 1D) and between Sle1,3 and Sle1,3-LCN2KO mice (figure 1E). Moreover, genes involved in cognition and memory that were differentially expressed in Sle1,3 mice were restored to background B6 expression levels in Sle1,3-LCN2KO mice.

Conclusions Our findings establish the Sle1,3 mouse as an NPSLE model and demonstrate that LCN2 deficiency attenuates neurobehavioral deficits and regulates microglial expression of genes essential to NPSLE development.



Abstract II-02 Figure 1 Sle1,3 mice exhibit reduced preference for objects in novel position in the object placement test (A) and for novel objects in the object recognition test (B), and these spatial memory (A) and recognition memory (B) deficits are attenuated by LCN2 deficiency. (C) Sle1,3 mice tend to demonstrate anhedonia through lack of preference for saccharin-treated water, and LCN2 deficiency ameliorates this depression-like behavior. Data are shown as mean ±SEM. * $p<0.05$. Volcano plot demonstrates genes differentially expressed between microglia from BL/6 and Sle1,3 mice (D) and between Sle1,3 mice and Sle1,3-LCN2KO mice (E). Red genes are highly significant genes determined by DEseq2 with a false discovery rate of 10%. Red genes to the right or left of the dashed lines are significant genes with a 3-fold (D) or 2-fold (E) change in expression

II-03 GENERATION OF HUMAN MYELOID DENDRITIC CELLS FROM INDUCED PLURIPOTENT STEM CELLS FOR THE EVALUATION OF GENE POLYMORPHISM FUNCTION IN LUPUS

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Background Functional analysis of gene polymorphisms associated with increased lupus risk cannot be adequately addressed using mouse systems and it is difficult to obtain sufficient relevant immune cells from individuals with these polymorphisms to perform the necessary studies. Furthermore, such studies are complicated by the strong linkage disequilibrium that exists between certain gene polymorphisms as well as by the inherent genetic variability between individual donors unrelated to the gene polymorphisms themselves. To address these issues, we propose that having an abundant supply of isogenic lupus-relevant immune cells in which gene-editing can be done would enable such functional analyses of gene polymorphism function to be performed.

Methods We reprogrammed human peripheral blood mononuclear cells with a lentiviral vector containing the Yamanaka transcription factors Oct4, Klf4, Sox2 and cMyc to obtain induced pluripotent stem (iPS) cells. The iPS cells were treated with a cocktail of growth factors and cytokines to generate definitive mesoderm and subsequently myeloid dendritic cells (mDC), utilizing feeder-free, chemically defined media.

Results Differentiated cells expressed the mDC markers CD11c, CD1c, and Zbtb46. The differentiated cells produced a cytokine profile characteristic of primary human mDCs in response to a panel of Toll-like receptor (TLR) agonists and were also able to effectively activate T cells in a mixed lymphocyte reaction. In contrast, differentiated cells generated from iPS cells with a loss of function mutation in Unc93B, a key component in endosomal TLR signaling, did not respond to endosomal TLR stimulation but responded normally to cell surface TLR activation.

Conclusions The use of mDC generated from iPS cells may provide a way to study polymorphism function in a lupus-relevant immune cell type by direct gene editing in the iPS cells. Gene editing enables the generation of isogenic lines that are genetically identical, except for the polymorphism of interest, and showing differences in the function of mDC derived from these isogenic lines would be the most definitive demonstration that a particular gene polymorphism induces functional effects that could plausibly be relevant to lupus pathogenesis. In addition, as mDC constitute less than 0.5% of peripheral

blood mononuclear cells, this methodology may be valuable not only for the study of human genetic variants but also for providing sufficient numbers of mDC for the study of human myeloid dendritic cell function more generally.

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II-04 BONE MARROW MESENCHYMAL STEM CELLS FROM PATIENTS WITH SLE MAINTAIN AN INTERFERON SIGNATURE DURING IN VITRO CULTURE

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Background We have previously shown that SLE BMSCs have a pro-inflammatory mediated by a MAVS and IFNβ feedback loop. Compared to healthy controls, SLE BMSCs produced increased amounts of IFNβ and had increased mRNA for several genes induced relatively specifically by IFNβ. They also had decreased proliferation, increased ROS, increased DNA damage and repair (DDR), a senescence associated secretory phenotype, and increased senescence-associated β-galactosidase. To better understand the phenotype of SLE BMSCs we conducted RNA sequencing.

Methods Patients fulfilling SLE classification criteria and age and sex matched healthy controls were recruited under an Institutional Review Board approved protocol (6 pairs). Bone marrow aspirates and peripheral blood samples were obtained. BMSCs were isolated with low density Ficoll/Hypaque and grown in tissue culture. Purity of BMSC cultures were verified by flow cytometry. Early passage BMSC were harvested and mRNA samples were sent for RNAseq through the University of Rochester Genomics Core. Serum samples with assayed for IFNβ using an ELISA from PBL.

Results Hierarchical clustering of normalized RNAseq profiles found SLE patients with high levels of serum IFNβ (>13 units per XXX) grouped together while SLE patients with low levels of IFNβ grouped together with healthy controls. Principal component analysis found the majority of the variance could be explained by PC1 (32.3%) and PC2 (25.5%). While PC1 did not separate SLE or SLE IFNβ high from the other samples, PC2 clearly differentiated SLE IFNβ high samples from SLE IFNβlow and control samples. SLE IFNβ low and control sample overlapped in both PC1 and PC2. The top upregulated genes in PC2 were RSAD2, MX1, IFIT1, IFIT2, OAS1, CMPK2, OASL, IFIT3, ISG15, IDO1, IFI6, MX2, HERC5, and IFIH1 … all type I interferon-induced genes.

Conclusions BMSCs from SLE patients are heterogeneous. A subgroup of SLE BMSC are distinguished from other SLE BMSC and from controls by increased levels of mRNAs induced by type I interferons. Moreover, SLE BMSC with increased levels of mRNA for type I interferon-induced genes when grown *in vitro* are derived from patients with increased levels of IFNβ *in vivo*.

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II-05 B CELLS FROM SLE PATIENTS HAVE INCREASED ENDOGENOUS PRODUCTION OF IFNβ WHICH IS STIMULATED BY BCR SIGNALING AND IS REQUIRED FOR SURVIVAL OF AUTOREACTIVE B CELLS

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Background Increased type I interferon (IFN) has been shown to affect survival and activation of B cells in SLE. This study investigated novel mechanisms of endogenous production and autocrine activity of IFNβ in SLE B cells at the single-cell level.

Methods IFNβ in B cells from SLE patients was analyzed using t-SNE platform based high dimensional flow cytometry. Intracellular IFNβ expression was visualized and analyzed by super-resolution confocal imaging and ImageStream analysis. Single cell gene expression analysis was carried out using the Fluidigm/BioMark system for targeted expression of low abundance genes, and the 10x Chromium platform for unbiased transcriptome analysis of up to 4,000 B cells per subject. Functional production of type I IFNs by B cells was analyzed using a human type I IFNs SEAP reporter HEK293 cell line.

Results High dimension flow cytometry analysis identified intracellular IFNβ expression in pDCs, B cells, and CD4 T cells. B-cell endogenous IFNβ was required for optimal *in vitro* BCR and TLR7-induced activation and survival of B cells. Using a Fluidigm targeted-gene approach, B cells could be divided into type I IFN expressing (*IFN*+) or type I IFN stimulated gene (*ISG*+) subpopulations, suggesting B cells not only respond to type I IFNs but also express type I IFNs including *IFNB* and different *IFNA* genes. *TLR7* and *TLR3* were mainly expressed by *IFN* +cells whereas *TLR9* was mainly expressed by *ISG* +B cells. The production of functional IFNβ and IFNα protein by single B cells from SLE subjects and was verified using a novel alkaline phosphatase live staining of HEK-blue reporter cells. There was enhanced IFNAR signaling by reporter cells in direct contact with SLE B cells which was blocked by anti-IFNβ and anti-IFNα. Interesting, anti-Ig crosslinking was required for optimal B-cell endogenous type I IFNs to stimulate responder cells. Unbiased single cells transcriptome analysis of SLE B cells using the 5' 10X Chromium platform and Loupe V(D)J Browser indicated that gene clusters in type I IFN expressing or responding SLE B cells exhibited unique heavy- and light-chain gene expression repertoires.

Conclusion (i) B cells are an important source of type I IFNs in modulating TLR and BCR responses in SLE; (ii) well-orchestrated and distinct programs in type I expression and responses genes in subsets of B cells, and (iii) distinct pathways of B cell survival and activation based on combined signaling through BCR, TLR, and IFNAR with a distinct BCR heavy- and light-chain repertoire.

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