of family members and improved therapy for patients and families.

Acknowledgements Childhood Arthritis and Rheumatology Research Alliance (CARRA) Small Grant, McLaughlin Centre, University of Toronto.

GG-12 ABSTRACT WITHDRAWN

GG-13 'EPITOF' – A NEW METHOD FOR CHARACTERIZING THE EPIGENETIC LANDSCAPE IN SLE

Paul J Utz*. *Department of Medicine, Division of Immunology and Rheumatology, Institute for Immunity, Transplantation and Infection, Stanford University School of Medicine, Stanford, CA*

10.1136/lupus-2018-lsm.99

Background SLE is a complex disease with very few approved therapeutic options. Unique opportunities exist to characterize 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. The epigenome, in particular, is an area of great interest.

Materials and methods We have recently published a new method called Epigenetic Time of Flight (EpiTOF), a mass cytometry based method that enables broad characterization of posttranslational modifications (PTMs) of histones in health and disease. Our initial studies demonstrated marked heterogeneity in younger vs older healthy adults. Twin studies showed that ~70% of variation is related to environment. Twenty-two different populations of blood cells were profiled, and the PTMs alone were sufficient to identify cell populations, even in the absence of cell surface markers.

Results Blood derived from multiple diseases was provided through the ACE Collaborative Network and local Stanford investigators and subjected to EpiTOF analysis followed by complex computational analysis. I will present ongoing studies in SLE, SSc, RA, IBD, JIA, vaccines, and infectious diseases using EpiTOF.

Conclusions EpiTOF and other multiplexed assays (such as autoantibody profiling) 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.

Acknowledgements NIAID/DAIT, Henry Gustav Floren Trust, Baxter Foundation, and many patients with SLE who have provided samples.

Innate Immunity

II-01 TLR9-DEFICIENCY EXACERBATES AUTOIMMUNE DISEASE IN MODELS OF SLE AND CUTANEOUS LUPUS THROUGH B CELL INDEPENDENT MECHANISMS

[1]Kerstin Nundel, [2]Anette Christ, [1]Purvi Mande, [3]Wei-Che Ko, [3]John E Harris, [2]Eicke Latz, [1]Ann Marshak-Rothstein*. *[1]Department of Medicine, University of Massachusetts School of Medicine, Worcester, Massachusetts, USA; [2]Institute of Innate Immunity, University Hospital Bonn, University of Bonn, Bonn, Germany; [3]Department of Dermatology, University of Massachusetts School of Medicine, Worcester, Massachusetts, USA*

10.1136/lupus-2018-lsm.100

Background TLR9 appears to play both a protective and a disease-promoting role in animal models of SLE. Even though TLR9 is required for the production of anti-dsDNA and anti-nucleosome autoantibodies, TLR9-deficient autoimmune-prone mice invariably develop more severe disease than their TLR9-sufficient counterparts. Molecular mechanisms that account for this paradoxical function of TLR9 have mainly been explored in cell lines to a large extent have focused on competition between TLR7 and TLR9 for binding to Unc93B1 and the ability to access to the appropriate signaling compartment. Our own *in vitro* comparison of bone marrow derived macrophages and bone marrow derived dendritic cells, obtained from TLR9-sufficient vs TLR9-deficient mice and stimulated with TLR7 ligands, suggested that the impact of TLR9-deficiency might be highly cell type specific, and led us to focus on primary cells obtained fro animal models of systemic autoimmunity.

Methods We initially used pristane-injected BALB/c mice as a model of SLE, and found that TLR9-deficiency let to exacerbated renal disease and the accumulation of an unusual myeloid subset in the kidneys of these mice. We have directly examined the contribution of TLR9-deficient and TLR-sufficient cells in these mice using a mixed bone marrow chimera strategy. We have also developed an inducible rapid onset model of cutaneous lupus that depends on the injection of OVA-specific T cells into mice that express an OVA fusion protein on class II+cells; here, TLR9-deficient and TLR7-sufficient recipients develop cutaneous lesions with many of the features of discoid lupus within 4 weeks of T cell injection. Cells isolated from the kidneys of the BALB/c pristane mice and the skin of the cutaneous lupus mice have been further characterized by flow cytometry and gene expression.

Results These studies have identified a myeloid subset present at sites of inflammation and in normal peripheral blood that appears to be uniquely impacted by the loss of TLR9. Functional properties of these cells will be discussed.

Conclusions TLR9 deficiency impacts very specific myeloid subsets apart from its effects on B cell development and differentiation.

Acknowledgements This project has been supported by the Lupus Research Alliance and NIAMS.

II-02 NEUROPSYCHIATRIC LUPUS IS DEPENDENT ON LIPOCALIN-2

[1]Elise Mike, [2]Carla Cuda, [2]Hadijat Makinde, [2]Harris Perlman, [1,3]Chaim Putterman*. *[1]Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY, USA; [2]Division of Rheumatology, Northwestern University Feinberg School of Medicine, Chicago, IL, USA; [3]Division of Rheumatology, Albert Einstein College of Medicine, Bronx, NY, USA*

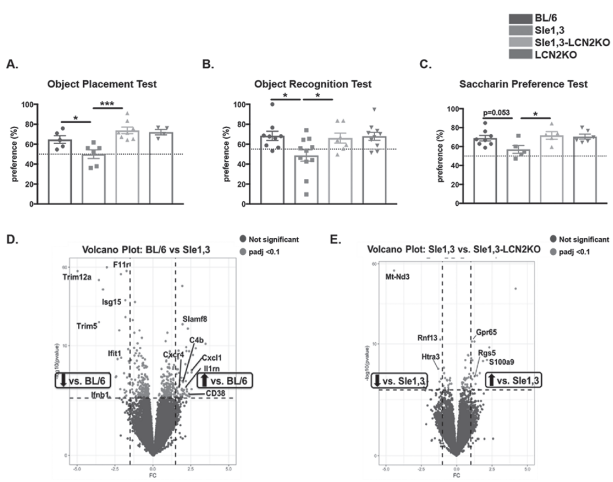
10.1136/lupus-2018-lsm.101

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

[1,2]Margaret A Baker, [2,3]Seonmi M Park, [1]Kei Yasuda, [2,3]Gustavo Mostoslavsky, [1,4]Ian R Rifkin*. *[1]Renal Section; [2]Center for Regenerative Medicine; [3]Section of Gastroenterology, Department of Medicine, Boston University School of Medicine; [4]Renal Section, Department of Medicine, VA Boston Healthcare System, Boston, Massachusetts, U.S.A*

10.1136/lupus-2018-lsm.102

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