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TITLE: NR4A Family as Markers and Mediators of B Cell Tolerance in SLE: From Antigen Discovery to Treatment

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CONTRACTING ORGANIZATION: University of California, San Francisco, CA

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14. ABSTRACT
B cells play a key role in the pathogenesis of multiple autoimmune diseases by differentiating into autoantibody-secreting plasma cells and serving as antigen presenting cells for pathogenic T cells. Indeed, inhibition and/or depletion of B cells represent promising new treatments for systemic lupus erythematosus (SLE). Importantly, SLE exhibits considerable clinical heterogeneity for which clinicians lack adequate biomarkers. Discovery of novel autoantibodies (Abs) and autoantigens (Ags) holds the potential to subset patients according to clinical manifestations, to identify those at greatest risk for progression to end-organ damage, and to provide key insights into pathogenesis of disease. Moreover, selective targeting of pathogenic, self-reactive B cells would allow clinicians to maximize clinical benefit while minimizing off-target immunosuppression and infectious complications. However, although identifying self-reactive B cells and the antigens that they recognize is of critical importance, this task poses a major scientific and technical hurdle. The NR4A family of orphan nuclear hormone receptors (Nur77, Nurr, and Nor1, encoded by NR4A1-3) are upregulated by both acute and chronic antigen stimulation. They represent novel markers of self-reactive lymphocytes and have tolerogenic functions. This proposal harnesses the biology of the NR4A family of orphan nuclear hormone receptors to identify and manipulate self-reactive B cells, and couples this with our existing and well-validated phage-display pipeline for autoAb and autoAg discovery. Focus Area: Understand SLE disease heterogeneity, subtype patients, and understand lupus disease mechanisms Hypothesis: We hypothesize that the expression of NR4A family members may mark pathogenic B cells in SLE patients without a priori identification of self-antigens, and may also function to impose B cell tolerance.

15. SUBJECT TERMS
None listed.

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
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1. INTRODUCTION:

B cells play a key role in the pathogenesis of SLE by differentiating into autoantibody-secreting plasma cells, producing inflammatory cytokines, and serving as antigen presenting cells for pathogenic T cells. SLE exhibits remarkable clinical heterogeneity for which clinicians lack adequate biomarkers. Discovery of novel autoantibodies (Abs) and auto-antigens (Ags) holds the potential to subset patients according to clinical manifestations, to identify those at greatest risk for progression to end-organ damage, and to provide key insights into disease pathogenesis, **particularly for SLE patients with central nervous system (CNS) lupus**. Although identifying self-reactive B cells and the Ags that they recognize is of critical importance, this task poses a major scientific and technical hurdle, especially in SLE because of the remarkable diversity of antigenic targets recognized by pathogenic B cells. This proposal harnesses the dynamic expression pattern of the NR4A family of orphan nuclear hormone receptors to identify self-reactive B cells, and couples this with our existing and well-validated phage-display pipeline for autoAb and autoAg discovery. NR4A genes are rapidly upregulated by antigen receptor signaling and we hypothesize that their expression may mark self-reactive B cells in SLE without a priori identification of self-Ags. We intend to exploit the expression of NR4A genes to identify pathogenic self-reactive B cell clones, discover their transcriptional signature, and take advantage of our existing human peptidome phage display pipeline for autoAb and autoAg identification. With our phage display library we are able to precisely and reproducibly map target epitopes, and use patient CSF or serum to identify novel Abs and their antigenic targets. We aim to perform high-throughput and comprehensive profiling of serum and CSF Abs, as well as monoclonal Abs (mAbs) derived from NR4A-expressing SLE B cells in order to identify their antigenic targets. Our long-term goals are to define new autoAbs and autoAgs in SLE that serve as biomarkers of disease subsets in SLE with prognostic and therapeutic significance, ***with emphasis on CNS SLE***.

2. KEYWORDS:

SLE, NPSLE (neuropsychiatric SLE), autoantibody, autoantigen discovery, phage display, peptidome, CNS, B cells, NR4A nuclear receptors, Nur77

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Specific Aims

Aim 1. Autoantigen, autoantibody and autoreactive B cell discovery in SLE.
In this aim we seek to capture transcriptional signature of chronic Ag stimulation (marked by NR4A family expression) in SLE patient samples in order to identify self-reactive B cells and integrate this with our autoAb and Ag identification phage display pipeline. We will take an unbiased approach to identify novel neural-specific autoAbs and their targets in a subset of SLE patients with neuroinflammatory syndromes by assessing self-reactivity of CSF samples via immunohistochemical (IHC) staining of mouse brain sections, followed by high throughput screening of our human peptidome phage display library. In parallel, we will identify self-reactive naïve and memory B cells on the basis of NR4A gene expression from matched CSF and peripheral blood samples using paired single cell RNA-Seq (scRNA-Seq) and sc immunoglobulin heavy and light chain sequences. We will then generate recombinant monoclonal Abs (rmAbs) that are screened against mouse brain sections, and against our phage display library to identify B cell clones with self-reactive BCRs. We will then apply this approach for discovery of autoreactive B cells and Abs in a broad panel of SLE patients for whom serum and PBMCs are available.

Aim 2. Transcriptional profiling of single self-reactive B cells in SLE.
Typically, assessing gene expression in self-reactive B cell clones from patients with autoimmune disease requires capture of such clones via Ag binding. In this aim we will exploit NR4A expression as a 'mark' of self-reactivity in order to mine existing and newly generated scRNA-Seq data in naïve and memory B cells from SLE and control patients. This will enable us to enrich samples for self-reactivity without a priori information about Ag specificity. This approach will allow us to identify an anergy-associated program in human B cells from healthy donors, and then compare this program with that induced in SLE patients to discover dysregulated gene programs in an unbiased manner.

What was accomplished under these goals?

In the first year of this award, our team has focused on Aim 1. We have to-date enrolled a total of 31 patients with confirmed SLE and a wide range of neuropsychiatric complications. Of these, 21 have been screened for anti-neural antibodies using rodent brain immunostaining. 16 cerebrospinal fluid (CSF) samples exhibited specific CNS staining patterns suggestive of bona fide tissue-specific autoantibodies (Fig 1). We also screened many of the CSF samlpes from these cases for specific autoantibodies using our whole human peptidome phage immunoprecipitation sequencing (PhIP-seq) assay (Fig 2) and will describe the results from one of these cases (NID-0063) in depth below (Fig 3).

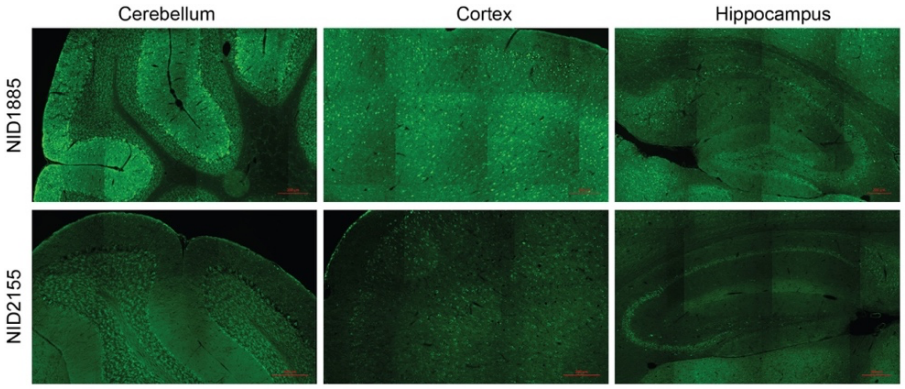

Figure 1: Mouse brain staining of two NSLE cases with CSF staining at 1:25 dilution with distinct patterns. **NID1885** reveals prominent cytoplasmic staining of Purkinje neurons in cerebellum with dendritic stainingin the molecular layer. In the cortex there is a mixture of cytoplasmic and nuclear staining. In the hippocampus there is staining of excitatory neurons of the pyramidal and dentate granule cell layers. **NID2155** reveals cytoplasmic staining in Purkinje neurons and granule cell layer of the cerebellum. In the cortex there is widespread cortical cytoplasmic staining. In the hippocampus there is prominentinterneuron cytoplasmic staining as well as synaptic terminal staining in the pyramidal layers (likely interneuron terminals).

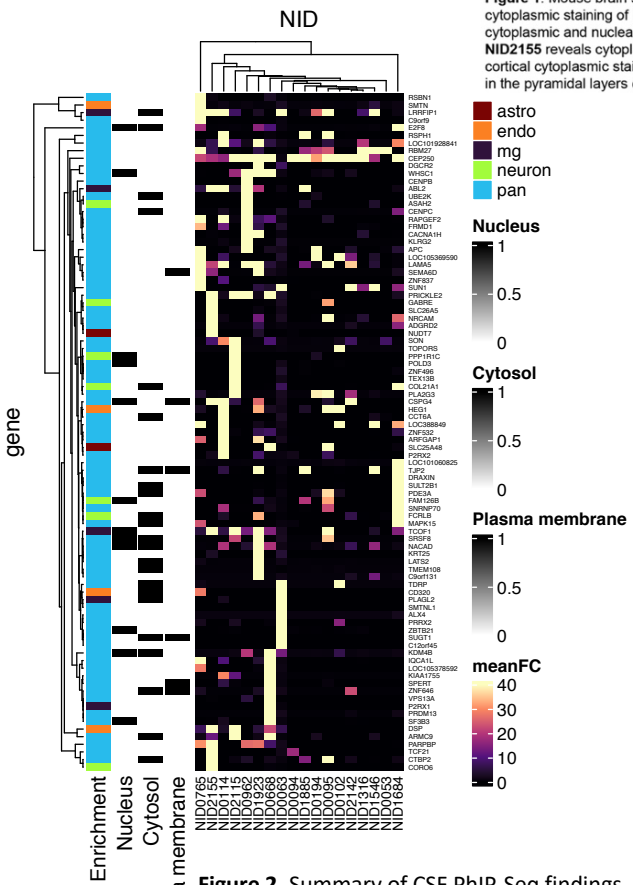

Figure 2. Summary of CSF PhIP-Seq findings for individual SLE patients. Each column represents a patient sample with each row showing the proteins enriched by each sample, color coded by mean fold change (meanFC) above the background.

A 67-year-old woman with SLE presented with subacute ataxia, tremor, weakness, and scanning speech. Magnetic resonance imaging revealed bilateral symmetric T2-weighted hyperintensities with gadolinium enhancement involving the cerebellar peduncles and dorsal brainstem. She achieved partial recovery with empiric steroid treatment and immunosuppressive therapy for her SLE. Clinical autoimmune encephalopathy antibody testing was negative. As described above, her CSF was screened for the presence of neurotropic autoantibodies by immunofluorescent staining of mouse brain tissue (Fig 3). PhIP-seq was used to screen for novel antigens and identified that *overlapping peptides* were enriched from CD320, the transcobalamin receptor (Fig 3). CSF

autoantibodies to CD320 were validated by cell-based overexpression and CRISPR Cas9-mediated knockout (Fig 3). An *in vitro* holo-transcobalamin trafficking assay was developed to evaluate the function of these CSF autoantibodies. Incubation of human cells with NID-0063 CSF impaired the uptake of holo-transcobalamin, and depletion of the IgG from the CSF restored uptake to basal levels suggesting a functional impact of CD320 autoantibodies on transcobalamin trafficking. *Vitamin B12 was measured by a competitive enzyme-linked immunosorbent assay and was found to be nearly undetectable in the CSF of this patient despite normal levels of B12 in the serum.* Transcobalamin receptor autoantibodies may be a novel and unappreciated cause of cerebral vitamin B12 deficiency via inhibition of transport across the blood-brain barrier in NPSLE. Similar to identification of NMO-spectrum disease in patients with NPSLE, it is also possible that autoAbs to CD320 could cause autoimmune neurologic disease in patients without SLE.

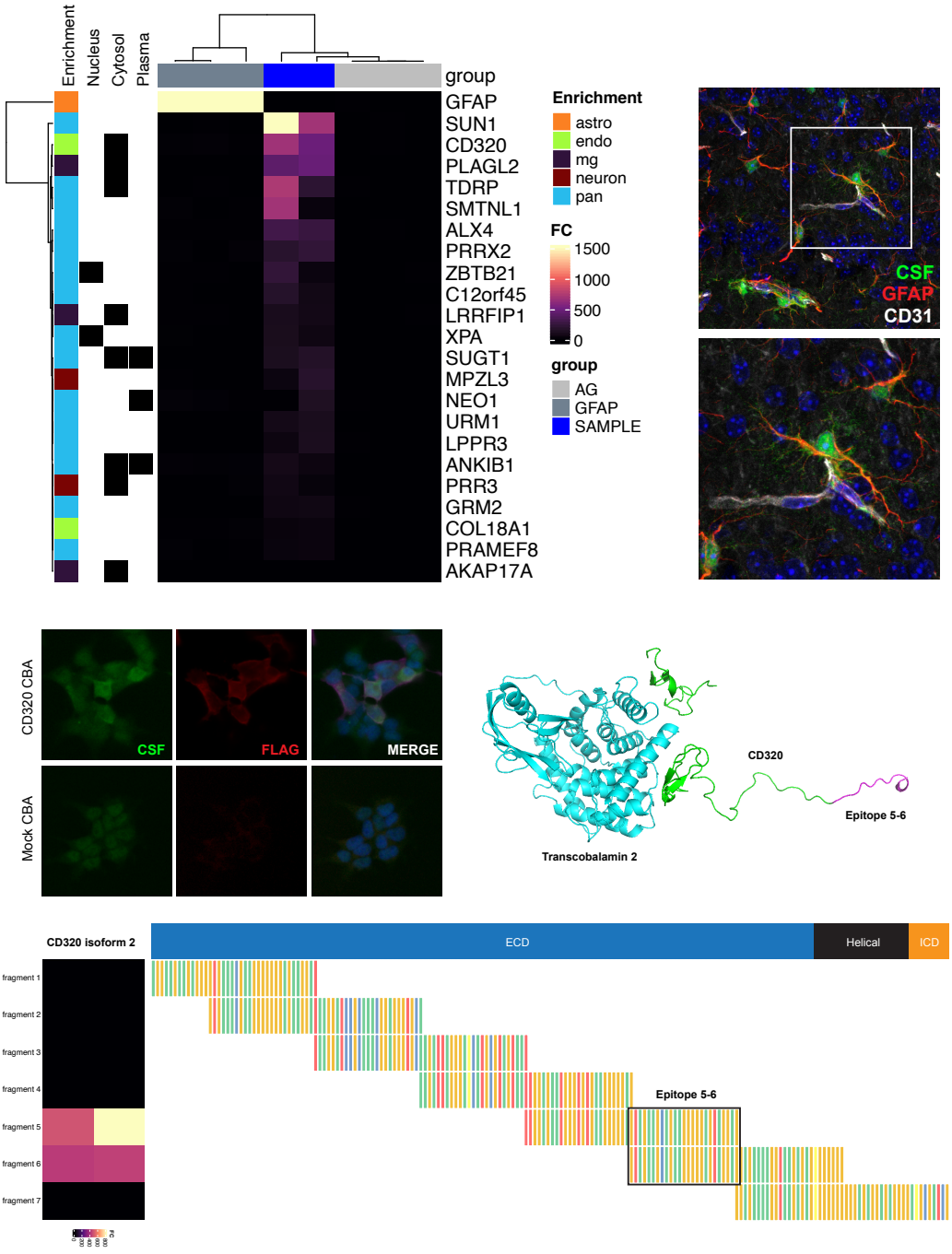

Figure 3. CSF staining of mouse brain tissue revealed immunoreactivity against astrocytes and endothelial cells at the blood-brain barrier. An autoantibody targeting the transcobalamin receptor (CD320) was identified by PhIP-Seq.

What opportunities for training and professional development has the project provided?

Nothing to Report

How were the results disseminated to communities of interest?

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?

To follow-up on the identification of CD320/transcobalamin receptor autoantibodies in one NP-SLE patient, we will screen the remaining N=30 NPSLE CSF samples for this specificity by ELISA and will cross-reference with PhIPseq data generated with larger non-SLE neuroinflammatory diseases (NID) samples as well as SLE patient samples from CLUES cohort without known NP clinical manifestations. Using both Phage-Ag-based capture as well as transcriptional and clonal-expansion-based prioritization, scRNAseq data on NID-0063 CSF B cells, the team will seek to clone the BCR encoding anti-CD320 specificity and generate recombinant mAb from this sequence in order to study its pathogenicity in mouse models in future work.

In addition to N=21 NPSLE CSF samples run on PhIPseq platform as described in Fig 2 above, peripheral blood samples from the complete cohort of CLUES lupus patient samples have been run on PhIP-Seq (>800 samples from almost 300 patients, along with controls). In the coming funding period, our research team will focus on bioinformatic analysis and validation (as in Fig 3) of candidate autoantigens identified in this data set.

Additionally we will pursue additional objectives in Aim 1 by pursuing scRNAseq of CSF B cells to prioritize rmAb generation on basis of Nr4a gene expression signature, and we will pursue Aim 2 by mining pre-existing large single cell data set generated with samples from the CLUES patient cohort and recently published (PMID: 35389781).

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Nothing to report

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?
/

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

5. CHANGES/PROBLEMS:

:

Changes in approach and reasons for change

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them

Early in the project from 9/30/201 until spring 2022 our formal start was delayed by requirement to complete DoD-specific human subjects protocol and receive DoD approval for this.

Changes that had a significant impact on expenditures

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals

Significant changes in use of biohazards and/or select agents

No bioharards or select agents

6. PRODUCTS:

- **Publications, conference papers, and presentations**
- **Journal publications.**

Nothing to report

Books or other non-periodical, one-time publications.

Nothing to report

Other publications, conference papers and presentations.

Nothing to report

- **Website(s) or other Internet site(s)**

Nothing to report

- **Technologies or techniques**.

Nothing to report

- **Inventions, patent applications, and/or licenses**

Nothing to report

- **Other Products**

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name	Calmos	Role	Contribution
DANDEKAR,RAVI	1	Bioinformatician	Ravi Dandekar is the staff bioinformatician in the Wilson Lab who has developed their in house scRNASeg and scTCR and scBCR analysis pipelines. He works on the scRNAseq data analysis (Aims 2)
MUELLER,JAMES L	2	SRA	James Mueller handles and prepares patient samples for sequencing together with Noah Perlmutter below (SRA I), and performs ELISAs, IFAs for autoantibody detection.
PERLMUTTER,NOAH	2	SRA	Noah Perlmutter works closely under co-PI Dr. Judith Ashouri's supervision to prepare patient samples for 5' 10x single cell sequencing
ZHOU,JING	2	Postdoc	Jing Zhou works closely with Dr. Sam Pleasure to perform rodent brain IHC assays and recombinant antibody cloning and generation (Aim 1).
ZIKHERMAN,JULIE	1	PI	Dr Zikherman serves as the PI to coordinate efforts of collaborators, co-PIs, and staff. She and her lab have expertise in cellular and molecular immunology, and her lab has a focus on the study of B cell tolerance, and the biology of the NR4A family in lymphocytes.

Other co-PIs are not drawing effort from this funding source.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES: