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TITLE: Understanding Plasma Cell Differentiation in SLE

PRINCIPAL INVESTIGATOR: Betty Diamond

CONTRACTING ORGANIZATION: The Feinstein Institutes for Medical Research
Manhasset, NY

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14. ABSTRACT Our immune system functions to eradicate pathogens from our body. One arm of this defense is through antibodies which facilitate pathogen clearance. However, if antibodies target our own body, autoimmune diseases, such as Systemic Lupus Erythematosus (SLE), can arise. Most SLE patients have anti-nuclear antibodies (ANA). These antibodies recognize an array of molecules that normally reside in the nucleus of cells. However, when an injury or infection occurs, these molecules can be released from the nucleus of dying cells, thereby becoming accessible to binding by ANA, which usually results in an inflammatory response. Plasma cells are the immune cells that produce antibodies, and they can arise from B cells through different pathways. Our previous results have shown that the differentiation of most ANA+ B cells to plasma cells is prevented in healthy individuals, a process we call tolerance. In SLE, this tolerance process is unsuccessful, thereby leading to secretion of ANA and to subsequent inflammatory reactions throughout the body. Our previous research has shown that SLE patients can be separated in two groups based on their pattern of ANA+ plasma cells. In the proposed project, we aim to study the pathways that lead to the emergence of ANA+ plasma cells in SLE. Included among ANA+ PCs are cells making anti-DNA and anti-Sm antibodies, both of which are known to contribute to disease in SLE. We will compare the two groups of patients; we hypothesize that each group has a distinct pathway for the development of ANA+ plasma cells. Moreover, we will determine how to cause the differentiation of a naïve or memory ANA+ cells to PCs to provide insights into prevention.					
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1. Introduction

We have previously shown that a major breach to B cell tolerance in patients with systemic lupus erythematosus (SLE) is the enhanced differentiation of IgG plasma cells. Based on this observation, the goal of this study is to understand the induction of plasma cells in SLE. We propose to determine if there is a difference in plasma cell differentiation when they arise through an extrafollicular vs a germinal center pathway, and whether there is a difference in the differentiation of plasma cells directed to nuclear antigens (ANA+) and those without this auto-specificity. We also propose to develop approaches to blocking IgG plasma cell differentiation.

2. Keywords

Systemic lupus erythematosus; B cells: plasmablasts/plasma cells; IgG; extrafollicular; germinal center; B cell repertoire

3. Accomplishments

- What were the major goals of the project?

Specific Aim 1 Cellular origin of ANA+ PCs in SLE patients	Percentage of completion
Major Task 1: Deep sequencing of the ANA+ plasmablast/ B cell repertoire from SLE patients	70%
Specific Aim 1 Cellular origin of ANA+ PCs in SLE patients	Percentage of completion
Major Task 2: Data analysis: repertoire	50%
Specific Aim 2 Phenotype of ANA+ PCs related to PC differentiation pathway	Percentage of completion
Major Task 3: Phenotype of ANA+ plasmablasts in SLE	50%

Specific Aim 2 Phenotype of ANA+ PCs related to PC differentiation pathway	Percentage of completion
Major Task 4: RNA sequencing of ANA+ plasmablasts	10%
Specific Aim 3 BCR AND TLR7/9 stimulation pathways for PC differentiation	Percentage of completion
Major Task 5: Induction of ANA+ plasmablasts	25%

- **What was accomplished under these goals?**

Specific Aim 1:

We have been studying whether we can determine through molecular analyses of the B cell receptor (BCR) the pathway for the generation of plasma cells in SLE patients. We have isolated ANA+ and ANA- naïve, memory and age/autoimmunity associated B cells and plasmablasts from SLE patients and subjected these to DNA sequencing of the B cell receptor.

We identify two groups of patients. In one group, plasma cells are most clonally related to naïve B cells; we believe these are patients in whom plasma cells are generated through an extrafollicular pathway. In the second group, plasma cells are clonally most related to memory B cells; we believe plasma cells in these individuals arise through a germinal center pathway. We further observe an increased frequency of somatic mutation in BCRs of those plasmablasts we believe arise from the germinal center response.

Specific Aim 2:

We have begun the transcriptional analysis of ANA+ and ANA- plasmablasts. We obtained these cell populations from 5 healthy and 5 SLE individuals. We are currently applying quality control metrics to the data and analyzing the results.

Specific Aim 3:

We have optimized protocols for the generation of plasma cells from naïve B cells to mimic the extrafollicular pathway and from memory B cells to mimic the germinal center pathways. We have begun to explore potential mechanisms to block plasma cell differentiation. We have found that metformin can prevent plasma cell differentiation. This does not appear to be through metabolic alteration, based on our preliminary data, but rather through cross-linking RAGE and LAIR-1 and preventing the entry of CpG, a factor used in our plasma cell differentiation cocktail, into the cell to engage endosomal toll-like receptors.

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

Yemil Atisha-Fregoso obtained a PhD from the Elmezzi Graduate School based on the studies that led to the application of this award and has continued to work on the project as an Instructor in the laboratory.

- **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?**

We will continue our studies of all three specific aims.

In Aim 1, we will increase our sample size to confirm that there are indeed two patterns of clonal relatedness of plasmablasts to other B cell subsets and will assess parameters of serum autoantibody profile in each group to determine if a broader specificity and a lower affinity for nuclear antigens correlates with an extrafollicular pathway and a more restricted specificity and higher affinity for nuclear antigens correlate with a germinal center pathway.

In Aim 2, we will continue the transcriptional analysis of ANA+ plasmablasts. We want to address 2 questions:

- 1) How do ANA+ compare to ANA- plasmablasts? and 2) How do plasmablasts differ depending on their differentiation pathway.

In Aim 3, we will continue to explore approaches to block IgG plasma cell differentiation, in particular, the mechanism of action of metformin, which is a cheap, safe and approved drug that could be repurposed for use in SLE. We will additionally explore whether we can preferentially block differentiation of IgG rather than IgM plasma cells as we know that IgM autoantibodies are protective in SLE, and suppress the inflammatory milieu.

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**

In Aim 1, we have pursued DNA sequencing of BCR as we were advised that it was more accurate and that there was, in principle, an equal representation of each B cell in the final analysis. We are going to increase the number of individuals analyzed in this fashion but will also obtain BCR sequence data from RNA sequencing.

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

We have obviously been delayed by the Covid-19 pandemic which has limited the access of investigators to laboratories. We have adhered to staggered schedules in order to maintain social distancing. We also had a moratorium on clinical research when we felt it was unsafe to bring patients in to obtain blood. This continued until vaccines were available to all. We have tried very hard to progress rapidly in recent months, especially as it is not known whether the current surge will require new restrictions.

- **Changes that had a significant impact on expenditures**

See above

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

IRB Approval date 05/20/2021

- **Significant changes in use or care of human subjects**

We have instituted precautions to protect patients and staff from Covid-19. This has no impact on the data obtained from the study but has slowed progress.

- **Significant changes in use or care of vertebrate animals.**

Not applicable

- **Significant changes in use of biohazards and/or select agents**

Not applicable

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?

Example:

Name:	Yemil Atisha-Fregoso
Project Role:	Graduate student, currently Instructor
Researcher Identifier (e.g. ORCID ID):	0000-0001-7702-8640
Nearest person month worked:	12 months
Contribution to Project:	Dr. Atisha-Fregoso has been involved in isolating B cell subsets and analyzing repertoire data. In addition he has performed in vitro studies of plasma cell differentiation
Funding Support:	Elmezzi Graduate Program and DOD
Name:	Tamara Moreno-Sosa
Project Role:	Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID):	0000 0002 6637 4852
Nearest person month worked:	5 months
Contribution to Project:	Dr. Moreno-Sosa has been performing the transcriptions analysis of ANA+ B cells and plasma cells
Funding Support:	NIH and DOD

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

What other organizations were involved as partners?

Nothing to Report

- i. **Organization Name:** Broad Institute and University of Texas at Austin
- ii. **Location of Organization:** Domestic
- iii. **Partner's contribution to the project** (*identify one or more*)
 - 1. **Financial support;**
 - 2. **In-kind support** (*e.g., partner makes software, computers, equipment, etc., available to project staff*);
 - 3. **Facilities** (*e.g., project staff use the partner's facilities for project activities*);
 - 4. **Collaboration:** The Broad Institute and the University of Texas supervise the RNA sequencing and B cell receptor repertoires studies.
 - 5. **Personnel exchanges** (*e.g., project staff and/or partner's staff use each other's facilities, work at each other's site*); and
 - 6. **Other.**

8. Special Reporting Requirements

COLLABORATIVE AWARDS:

QUAD CHARTS:



Appendices

DOD -- Diamond, Betty, M.D.

Completed

GMS Kim L Cooper Tel

1P01AI073693

(Diamond)

08/01/08 – 07/31/14

NIH/NIAID – **Anti-NMDA receptor antibodies in adult brain dysfunction and fetal brain development: lessons from lupus**

Project 4

(Diamond)

Preventing anti-NMDAR Ab neurotoxicity: Rational Therapies for NPSLE

This is a study of maintaining the integrity of the blood-brain barrier which does not overlap with the current proposal and a study of peptide therapy which includes partial overlap with the current proposal.

Specific Aims:

We hope to develop the DWEYS peptide or a modification of the peptide as a therapeutic to protect the brain from anti-NMDAR antibodies. We also intend to understand the degree of breach of the blood-brain barrier mediated by LPS and epinephrine.

These studies are critical to the development of an effective therapeutic to protect the brain from anti-NMDAR antibodies.

GMS Yen Thach Tel:

NIH R01AR057084

(Diamond)

07/01/10 – 06/30/15

Toward a new approach to SLE therapy

This is a proposal to develop a small molecule that functions like a peptide mimotope of DNA but can be orally administered and will reduce the inflammation of lupus.

Specific Aims

Systemic Lupus Erythematosus is an autoimmune disease which preferentially affects young and minority women, with a higher incidence and a greater severity among African-Americans and Latinos (1, 2). It is characterized by the production of autoantibodies, many of which have specificity for nuclear antigens (3, 4) and contribute to irreversible damage in multiple target organs (5). Over the past few decades, the survival of lupus patients has increased, reflecting the availability of renal dialysis and better antibiotics to treat the frequent and severe infections that assault the immunosuppressed host (6). Approximately half the deaths in lupus patients are the result of opportunistic infections. Thus, despite the improved prognosis for lupus patients, it is clear that a better approach to therapy is needed: one that eliminates autoreactivity without resulting in generalized immunosuppression.

GMS Yen Thach Tel:

NIH R21 AR065199

(Gregersen)

07/01/13 – 06/30/15

(Diamond co-investigator)

TNIP1 risk haplotypes and immune endophenotypes

Genetic and functional investigations of human TNIP1 haplotypes associated with Systemic Lupus Erythematosus, Systemic Sclerosis and Early Onset Myasthenia Gravis.

Specific Aims:

It is increasingly clear that the vast majority of risk genetic variants for autoimmune diseases are more directly associated with particular immune quantitative traits. These genotype-phenotype relationships need to be clarified before a full understanding of autoimmunity can be achieved. This proposal is directed at carrying out the initial exploratory studies to define the immune quantitative traits, or endophenotypes, that are regulated by *TNIP1*, a gene which has been associated with a variety of autoimmune diseases, including systemic lupus erythematosus (SLE), systemic sclerosis, and psoriasis (1-3). Our recent data using the ImmunoChip shows a modest association ($OR=1.14$, $p=4.04 \times 10^{-5}$) with rheumatoid arthritis (4). Our specific interest in *TNIP1* was catalyzed by our recent finding of a remarkably strong association with Myasthenia Gravis (OR , 1.91; p

= 3.2×10^{-10}) (5) that implicates an amino acid change of Pro to Ala at codon 151 as a possible causative variant.

GMS Shelia Simmons Tel

NIH U34 AR063407

(Mackay)

09/25/13-08/31/15

Treatment of SLE with ajulemic acid, a non-psychoactive cannabinoid derivative

(Diamond co-investigator)

This Phase IIa clinical trial will test the safety and efficacy of a non-psychoactive cannabinoid derivative, ajulemic acid, on patients with lupus that have mild to moderate disease activity.

SPECIFIC AIMS

The goal of the proposed clinical trial is to investigate the anti-inflammatory and analgesic effects and safety of ajulemic acid (AjA) for musculoskeletal pain associated with Systemic Lupus Erythematosus (SLE).

Musculoskeletal pain is one of the most common symptoms reported by SLE patients. Arthritis is the most commonly reported of the 11 criteria suggested by the American College of Rheumatology for the diagnosis of SLE; it is the first manifestation of disease in more than 50% of patients and is the major symptom that responded to belimumab, a very expensive new therapeutic for treatment of non-renal, non-CNS manifestations of SLE. The morbidity associated with chronic pain in SLE is substantial and contributes significantly to depression and reduced quality of life. The inflammatory processes underlying SLE-associated arthritis are likely related to classic mediators of tissue damage in SLE; autoantibodies, cytokines and activated immune cells, as the musculoskeletal system is readily accessible to circulating pro-inflammatory molecules. Additionally, the role of pro-inflammatory mediators such as TNF α , IL-1 β , MCP-1 and metalloproteinases in the pathogenesis and perpetuation of pain and the accelerated atherosclerosis characteristic of SLE has been well-documented. Current treatment strategies for inflammatory pain associated with SLE involve the use of non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids and narcotic analgesia. These therapeutic strategies are limited in their efficacy and are associated with substantial toxicities. AjA is a synthetic non-psychoactive cannabinoid with potent anti-inflammatory and analgesic effects. AjA reduces production of the pro-inflammatory cytokines IL-6, IL-1 β , IFN α and IFN γ and increases production of prostaglandin J2 and lipoxin A4, eicosanoids that facilitate resolution of inflammation. We propose a Phase IIa clinical trial to test the safety and explore the clinical efficacy of two doses of AjA compared to placebo for the treatment of inflammatory arthritis associated with SLE. We anticipate that results from the proposed clinical trial will be used to power a larger Phase III clinical trial for clinical efficacy of AjA. The U34 Planning Grant will allow us to complete protocol development and set up all of the administrative and regulatory structures necessary for the implementation of a clinical trial of this magnitude.

GMS Alessandro Bellucci Tel

Empire Clinical Research Investigator Program (ECRIP) 01/15/14-02/15/15

An evolutionary approach to SLE therapy

Project Director (Diamond)

This is a study of anti-DNA antibodies in patients with malaria.

The theme of this research project entitled "An evolutionary approach to SLE therapy" is to understand how malaria infection protects against development of systemic lupus erythematosus (SLE). SLE is an autoimmune disease characterized by the production of anti-nuclear antibodies. In Western Europe and North America, individuals of West African descent have a 3 fold greater incidence of lupus than Caucasians. Paradoxically, West Africans in sub-Saharan Africa appear to have a lower incidence of lupus, and all available studies suggest a milder disease with less nephritis.

We hypothesize that West Africans are genetically predisposed to lupus because lupus susceptibility genes are protective against malaria, the most potent selective pressure on the genome in endemic regions. We further hypothesize that malaria infection may protect against the development of lupus and mitigate the severity of disease. We propose that the anti-DNA antibodies induced by parasitic infection in the predisposed host decrease activation of pathogenic DNA-reactive B cells, which protects kidneys from being targeted by high affinity, potentially pathogenic autoantibodies.

GMS Margaret G Dowd Tel

Autoantibodies and prolactin participate in a positive feedback loop

This is a study of the role of anti-NMDAR antibodies in triggering autoantibody production.

A subset of antibodies to double stranded (ds) DNA binds also to the NR2A and NR2B subunits of the N-methyl-D-aspartate receptor (NMDAR). These antibodies, termed DNRAbs, are present in approximately 30 to 40% of patients with systemic lupus erythematosus (SLE); their presence in cerebrospinal fluid (CSF) associates with symptoms of neuropsychiatric lupus (NPSLE). NMDARs, the target antigen, are receptors for the neurotransmitter glutamate and are best characterized for their critical role in learning and memory. DNRAbs bind the NR2A and NR2B subunits of the NMDAR by recognizing a consensus pentapeptide D/E W D/E Y S/G (DWEYS) present in a solute-exposed region of the extracellular domain of NR2A and NR2B, but not in NR2C or NR2D. We have shown that the antibodies are not true receptor agonists; they preferentially bind already activated receptors, augmenting the effects of glutamate. Thus, they function as receptor modulators to enhance, but not initiate, NMDAR signaling. Thus DNRAbs augment NMDAR signaling, enhancing excitatory post-synaptic potentials. When they engage receptors containing the NR2A subunit, they induce neuronal apoptosis. These antibodies can lead to manifestations of neuropsychiatric lupus in a mouse model. They cause cognitive impairment when they access hippocampal neurons and behavioral impairment when they access neurons in the amygdala, but only if they are able to penetrate the blood-brain barrier (BBB). DNRAbs are present in 30-40% of lupus patients.

In this project we will:

- 1) Determine if DNRAbs regulate prolactin synthesis by pituitary neurons.
- 2) Determine whether decoy antigens that block antibody from binding NMDAR can decrease prolactin levels *in vivo*.
- 3) Determine if decreasing prolactin leads to a decrease in B cell autoreactivity.

These studies will extend our understanding of neural endocrine and immune interactions. Importantly, if we determine that DNRAbs upregulate prolactin levels, we will have a mechanistic biomarker for a rapid, focused, proof-of-principle, clinical trial of a DNRAb blocker that we have recently developed which protects kidney and brain and can be tested for its ability to reduce serum prolactin and restore a compromised B cell tolerance checkpoint. If we can show DNRAbs consistently augment serum prolactin, we will have a marker for effective antibody neutralization in a clinical trial.

GMS Shoenfeld Yehuda Tel

Binational Science Foundation (BSF)(subcontract Dr. Ilan Ben Zv) 10/01/14 – 09/30/17

The role of HMGB1 and TLRs 2 and 4 in the pathogenesis of inflammation in familial Mediterranean fever

(Diamond co-investigator)

This is a study of HMGB1 in familial Mediterranean fever.

Autoinflammatory diseases are a group of genetic disorders characterized by spontaneous activation of the innate immune system, resulting in periodic bursts of short-lasting inflammation involving the serous membranes, skin, joints and other systems, and accompanied by high fever. Familial Mediterranean fever (FMF) is the most common and prototypical of the autoinflammatory diseases, resulting from mutation in the MEFV gene, leading to mutated pyrin protein and activation of an inflammasome, culminating in the release of IL1 β and an inflammatory attack. To date, the mechanism by which mutated pyrin induces inflammation in FMF is still not clear and the triggers for the spontaneous acute inflammatory attacks in FMF have yet to be elucidated. In this project we will study the role of toll-like receptors 2+4 and HMGB1 in triggering attacks of inflammation in FMF. We will study their activation-status in FMF-PBMCs and in THP1 cells that are deficient in the MEFV gene. We will also develop novel HMGB1 blockers, and assess whether HMGB1 blockade can inhibit the inflammatory reaction in cells from FMF patients, substantiating a rationale for a clinical trial with HMGB1 blockers in FMF patients.

This project will contribute to elucidating the molecular mechanism of FMF, and other diseases involving the innate immune system, mainly the autoinflammatory syndromes.

GMS Mark E Langer Tel

R21 AR063929

(Diamond)

07/01/13-06/30/17

Nelfinavir in SLE: A pilot phase IIa clinical trial

This is a pilot clinical trial of nelfinavir in SLE.

Systemic lupus erythematosus (SLE) is an autoimmune disease that preferentially afflicts young women. SLE harms a variety of organ systems, including the skin, joints, kidney and brain. Management of active SLE frequently requires the use of multiple non-specific immunosuppressive and cytotoxic drugs that have significant associated toxicities and often have limited efficacy. Recently, we have made the surprising discovery that certain FDA-approved Human Immunodeficiency Virus (HIV) protease inhibitors show promise in a variety of preclinical lupus models. Among these, nelfinavir (brand name Viracept), possesses a relatively benign safety profile in HIV patients and is not likely to be immunosuppressive or cause adverse events in lupus patients. On the strength of our preclinical data demonstrating that nelfinavir acts, in part, as a decoy antigen to quench pathogenic lupus anti-dsDNA autoantibodies in critical tissues (e.g., kidney and brain), we propose to assess the safety and preliminary efficacy of nelfinavir as a lupus therapeutic in a pilot feasibility phase IIa clinical trial. We will pre-screen subjects for elevated anti-dsDNA autoantibody titers and incorporate early surrogate efficacy measures into this trial design. These and other innovations will bring a personalized medicine approach to lupus and contribute to the likelihood of the trial's success. Moreover, it has not escaped our notice that the repurposing of an FDA-approved drug into a new indication will facilitate an extremely rapid path into an urgent unmet medical need.

AR130137 Department of Defense

(Diamond)

9/30/14 – 09/29/17

DOD

Maternal brain-reactive antibodies and autism spectrum disorder

To understand the impact of brain-reactive antibodies cloned from mothers of a child with ASD on fetal brain development.

Specific Aims: We have generated approximately 50 monoclonal brain-reactive antibodies from blood B cells of mothers of a child with ASD. These antibodies bind to mouse brain and to human fetal brain lysates; we have identified antigens bound by several of these monoclonal antibodies. We propose to:

- 1) Identify those antibodies that alter fetal brain development and lead to behavioral deficits.
- 2) Screen sera of mothers of an ASD child for specific antigenic reactivity as defined by our monoclonal antibodies to try to correlate antibody specificity with phenotypic characteristics in the child.

GMS Yen Thach Tel

NIH R21 AR067012-01A1

(Diamond)

06/01/15 – 05/31/17

Function of the IRF5 risk allele for SLE in B cells

This is a study of the functionality of the IRF5 risk allele in B cells of healthy individuals.

Specific Aims:

To address these goals, we will:

1. Investigate the expression and function of IRF5 in different B cell subsets as a function of IRF5 genotype.
2. Investigate the effects of estradiol alone and in conjunction with type 1 interferon on B cell function as a function of IRF5 genotype. We will determine if B cells with the IRF5 risk allele exhibit an enhanced response to TLR-7 and 9 ligands and if this is further enhanced by exposure to estrogen and type 1 interferon.

The innovation in this proposal is to examine IRF5 in B cells, as opposed to myeloid cells, as the function of this gene in B cells in SLE may be equally important. Studies such as this are highly significant in that they may permit us to identify patient subsets that have unique therapeutic targets for maintenance of remission.

GMS Yen Thach Tel
NIH R01 AR065209
(Diamond co-investigator)

(Kim)

04/01/14-03/31/18

The Blimp-1 SLE risk variant regulates inflammatory function in dendritic cells

This grant is to study the function of the PRDM1 SNP rs548234 in dendritic cells for predisposition to SLE.

Specific Aims:

Systemic Lupus Erythematosus (SLE) is an autoimmune disease which primarily affects young women and is characterized by the generation of self-reactive antibodies. Many of the autoantibodies have specificity for nuclear antigens and contribute to irreversible damage in multiple organs. As is characteristic of most complex diseases, genetic and environmental factors contribute to the development of SLE. Many genome-wide association studies (GWAS) have now helped identify genetic variants associated with the development of SLE. A major challenge remains in translating how these variants alter cell physiology to predispose to risk and progression of autoimmune disease.

Specifically, in healthy subjects, I will address the following aims:

- (1) Identification of the molecular mechanism for decreased expression of Blimp-1 in DCs of risk allele carriers. We hypothesize that the risk allele alters the regulation of Blimp-1 expression in DCs through decreased transcription.
- (2) Identification of new molecular targets of Blimp-1 in DCs which regulate DC differentiation and activation. We hypothesize that the alterations induced by low Blimp-1 enhance antigen presentation ability and skew T cell subsets.
- (3) Identification of the role of estrogen in the regulation of Blimp-1 expression and DC phenotype. Our hypothesis is that estrogen influences Blimp-1 expression and the inflammatory phenotype in DCs, in a PRDM1 risk allele specific fashion.

GMS Gregory P Smith Tel

NIH 1UG3OD023391-0 (Gregersen & Diamond)

09/21/16 -08/31/19

Prenatal autoimmune and inflammatory risk factors for Autism Spectrum Disorders

This is a study of neurodevelopmental abnormalities in offspring of women with autoimmune disease.

SPECIFIC AIMS

In specific aim 1, we will test the hypothesis that clinical or subclinical autoimmunity in the mother at the time of pregnancy predisposes to a child with ASD. We will expand our existing cohort to enroll and serially sample a cohort of 4,500 pregnant mothers, including mothers with evidence of clinical or subclinical autoimmunity, along with control mothers. We will monitor their children for the presence of ASD-related phenotypes and other neurodevelopmental problems at two time points – age 2-2.5 years and 3.5–4 years.

In specific aim 2, we will test the hypothesis that the presence of maternal anti-brain antibodies during pregnancy predisposes to a greater risk of a child with ASD or neurodevelopmental problems.

In specific aim 3, we will explore the hypothesis that maternal immune activation or increased cytokine levels at during pregnancy predisposes to ASD in the child. We will assess maternal serum cytokine levels and modular patterns of whole blood gene expression in the second and third trimesters of women with an ASD child or a typically developing child in order to assess the correlation of maternal immune and inflammatory factors with ASD-related phenotypes in offspring.

GMS Kelvin D Lyons Tel

NIH P01 AI102852

(Diamond & Tracey)

04/01/14 – 03/31/20

1.8 cal mons

NIH/NIAID - **Cognitive and immune impairments in sepsis survivors**

Project 2

(Diamond)

NCE

Immune function in sepsis survivors

Study of immune impairment in monocytes following sepsis

Specific Aims:

We will explore the molecular basis of altered immune function in sepsis surviving mice through the following three aims:

Aim 1: To determine the contribution of splenic monocytes/macrophages to immune impairment in sepsis surviving mice. *Hypothesis:* Monocytes/macrophages in sepsis surviving mice exhibit a sustained alteration in phenotype and function that contributes to immune impairment.

We will identify the monocyte/macrophage populations residing in the spleens of sepsis survivors and analyze gene and protein expression and intracellular signaling cascades in those monocyte/macrophage subsets at baseline and in response to LPS challenge, and whether monocyte/macrophage subsets from sepsis-surviving mice can alter immune function in naïve mice. We will ask which alterations observed in sepsis survivors are present in mice given systemic HMGB1, IL-1, TNF or other cytokines that are present at high level during the acute phase of sepsis or during the recovery phase. Finally, we will determine if monocytes/macrophages from sepsis surviving mice can provide the systemic signals to cause memory impairment.

Aim 2: To evaluate the functional competence of acetylcholine producing T cells in sepsis surviving mice. *Hypothesis:* Acetylcholine-producing T cell (ChAT⁺ T cell) number and function are altered in sepsis survivors and no longer suppress splenic monocytes/macrophages.

We will determine the number and phenotype of ChAT⁺ T cells in spleens of sepsis surviving mice and evaluate whether ChAT⁺ T cells in sepsis surviving mice secrete acetylcholine in response to appropriate in vivo or in vitro stimulation. Finally, we will ask whether ChAT-GFP⁺ T cells from sepsis surviving mice function appropriately in nude mice.

Aim 3: To establish where the immunological memory of sepsis resides. *Hypothesis:* Alterations in a long-lived cell lineage serves to maintain abnormal immune function in sepsis surviving mice.

GMS Jenny L Greer

NIH/NIAID 1UM1AI1095650

(Nepom. G)

05/01/14 – 06/30/20

0.6 cal mons

Co-Chair Diamond

CALIBRATE – Rituximab Plus Cyclophosphamide followed by Belimumab

This is a clinical trial to determine if rituximab followed by belimumab reduces autoreactive B cell repertoire.

Study treatment will be rituximab, cyclophosphamide (CTX), and Solumedrol. This treatment will be followed by prednisone and belimumab in one group, and by prednisone without belimumab in the other group.

This trial will be conducted as a prospective randomized phase 2 open label multicenter study in individuals with active lupus nephritis age 18 and older. There will be two treatment arms with 1:1 randomization of a total of 40 participants.

During the treatment phase, all participants will receive infusions of Solumedrol 100mg, rituximab 1000mg, and CTX 750mg intravenously (IV) at week 0 and week 2. Prednisone 40 mg per day will be administered with a guided steroid taper to 10mg per day by week 12.

Participants will be randomized at week 4 to either the Rituximab/Cyclophosphamide (RC) Group or the Rituximab/Cyclophosphamide/Belimumab (RCB) Group.

The RC Group will be maintained on prednisone. The RCB Group will receive IV belimumab 10mg/kg at weeks 4, 6, 8, and then every 4 weeks through week 48 in addition to prednisone.

During the tolerance assessment phase, intravenous study medication will be discontinued after week 48, and all participants will be maintained on prednisone through week 96 of the study.

The primary objective of the study is to assess the safety of belimumab administration following treatment with rituximab and CTX, in terms of infectious adverse events.

GMS Ella Silayeva Tel

The NLM Family Foundation

(Diamond)

04/01/18 – 03/31/20

0.6 cal mons

Maternal antibody as a contributor to Autism Spectrum Disorder

This is a proposal to develop a decoy antigen to protect the fetal brain from autism related antibodies

Specific Aims

We propose the following aims:

Aim 1: We will explore the activation state of microglia in mice exposed in utero to anti-Caspr2 antibody. Our preliminary data suggest on-going microglial activation in these brains. Microglia are a druggable target in other neurologic diseases; therefore, understanding the activation state of microglia in a model of ASD might lead to therapeutic strategies. We will explore microglia using immuno-histochemistry, transcriptional profiling and microPET studies with a ligand that binds activated microglia.

Aim 2: We will explore the gender bias in the anti-Caspr2-induced model of ASD. We are intrigued that we observe that only male offspring exhibit a vulnerability to in utero exposure to anti-Caspr2 antibody as the incidence of ASD in children is strongly biased to males. We have, however, also determined that women with anti-Caspr2 reactivity sometimes have an affected female child, and that the ratio of male to female affected offspring of women with anti-Caspr2 reactivity is reflective of the overall ratio of males to females affected with ASD. We hypothesize that the female fetus will develop anti-Caspr2-mediated neurodevelopmental impairments only if there is a “second hit” and that maternal microbiome or maternal response to infection will render female offspring susceptible to maternal anti-Caspr2-mediated ASD.

GMS Ronald M Burch Tel

NIH 1R3MH106195-SBIR

(Burch, R)

04/01/18 – 03/31/20

1.2 cal mons

Developing a prenatal biologic therapy to mitigate ASD risk from maternal autoantibodies to Casp

Co-Investigator

This proposal is to create a therapeutic biologic designed to neutralize maternal anti-Caspr2 autoantibodies and prevent or reduce the severity of ASD in an anti-Caspr2 autoantibody-relevant mouse model of ASD.

Specific Aims

Aim 1: Evaluate candidate biologics for ability to neutralize Ab that disrupt brain development.

Objectives: Screen a panel of eight candidate biologics for their ability to neutralize Caspr2-reactive antibodies (polyclonal human sera and monoclonal antibodies) *in vitro* and *in vivo*; determine the circulating half-life of candidates. Milestones: The top two candidates will block anti-Caspr2 antibodies in a mouse model of pathologic *in utero* exposure and exhibit circulating $t_{1/2} > 3$ days.

Aim 2: Examine toxicology of lead candidates in adult female mice.

Objectives: Administer repeat doses of candidate to virgin and pregnant female mice; conduct preliminary toxicology and immune complex deposition assessments in blood and tissue. Milestones: Candidates will not show toxicity in adult mice, or trigger spontaneous abortion.

Aim 3: Assess placental transport of lead candidates and toxicology in fetal mice.

Objectives: Measure amount of candidates in fetal circulation and tissues; conduct preliminary toxicology studies.

Milestones: Candidate in fetal:maternal circulation $< 1:10$, no toxicity or fetal abnormalities.

Aim 4: Assess immunotoxicity of lead constructs in mice with a humanized immune system.

Objectives: Conduct immunotoxicity study in mice with humanized immune system.

Milestones: Repeat dosing of candidates does not cause immunotoxicity in humanized mice.

Aim 5: Evaluate efficacy of candidate therapeutics for mitigating ASD phenotypes in a mouse model of ASD.

Objectives: Test efficacy of candidates to block pre-existing, polyclonal Caspr2 immunity during gestation.

Milestones: Treating with candidates rescues brain morphology and behavioral phenotypes in male progeny.

Current

GMS Adam Graham Tel

NIH 1P01 AI073693-06

(Diamond)

09/01/2008 – 06/30/2025

NIH/NIAID –**Anti-NMDA receptor antibodies in adult brain dysfunction, & fetal brain development**

Project 3

(Diamond)

1.2 cal mons **Mechanisms of**

DNRAb-mediated damage to the fetal brain

This is a study of the effect of lupus antibodies on the developing fetal brain.

No overlap

Specific Aims:

Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterized by the production of anti-nuclear antibodies, with anti-DNA antibodies being the most common and both diagnostic and pathogenic. The disease affects multiple organ systems with inflammatory damage initiated by autoantibodies in most, if not all, involved organs. While there has been intensive investigation of kidney disease in SLE, and numerous studies of skin manifestations and abnormalities in the coagulation pathway, there have been fewer studies of neuropsychiatric SLE (NPSLE) and no approved therapies for NPSLE exist. Yet NPSLE is among the classification criteria for the diagnosis of SLE, and a recent FDA-sponsored Patient-Focused Drug Development meeting identified many of the clinical manifestations of NPSLE, especially cognitive impairment, as among the most concerning to patients [1].

We have identified an antibody, DNRAb, which cross-reacts with DNA and the N-methyl D-aspartate receptor (NMDAR). The effect of DNRAb on the adult and fetal brain is the focus of the current proposal in 1) a mouse model of neuropsychiatric lupus (NPSLE); 2) in SLE patients; and 3) in mice exposed in utero to maternal DNRAb.

Project 1: To understand the mechanism of microglial activation following DNRAb-mediated neuronal death, and the mechanism by which ACE inhibitors reverse microglial activation.

Project 2: To understand the correlation of DNRAb with microglial activation and blood-brain barrier breach in the hippocampus and to study longitudinally the progression of metabolic and structural abnormalities in DNRAb+ and DNRAb- patients.

Project 3: To understand how DNRAbs affect fetal health and develop a strategy to protect the fetus from maternal DNRAbs.

These projects are supported by 3 cores. The Administrative Core will facilitate the work of all projects and the scientific cores. The Monoclonal Antibody Core will generate reagents and perform ELISAs to ensure consistencies within and among projects. The Behavioral and Electrophysiology Core will perform behavioral and cognitive assessments and the electrophysiology to ensure comparability among all studies.

These studies will position us to perform among the first clinical trials in NPSLE and the first prevention trials to protect the offspring of DNRAb+ mothers.

NIH 1P01 AI073693 (Adm Supplement) (Diamond)

07/01/2020 – 06/30/2022

0.6 cal mons

Effect of Covid-19 engagement of ACE2 on brain health and pathology

no cost extension

The goal of this study is to understand how the spike protein and its receptor binding domain affect the function of neurons, microglia and brain microvascular epithelial cells, all of which express ACE2, the receptor for Covid-19.

No overlap

We have been interested in the insidious consequences to the central nervous system of peripheral immune activation. These studies have led us to recognize the role of chronic neuroinflammation to altered cognition and behavior. In the course of these studies we have increasingly come to appreciate the role of the renin-angiotensin system (RAS) in modulating neuro-quiescence and neuroinflammation. There is a proinflammatory arm consisting of angiotensin converting enzyme (ACE) which cleaves angiotensin-I to become angiotensin-II.

Angiotensin-II binds to the type-1 receptor (AT1) to activate the release of proinflammatory cytokines, and type-1 interferon. An anti-inflammatory arm consists of angiotensin-II binding to the type-2 receptor (AT2) to suppress the proinflammatory program, and a membrane-bound angiotensin converting enzyme (ACE2), that cleaves both angiotensin-I and angiotensin-II to an inactive form. When angiotensin-II is cleaved, a small peptide ang 1-7, is released that binds Mas, a G-protein coupled receptor that also suppresses an

inflammatory response. That this pathway can be modulated for therapeutic effect has been shown in studies of ACE inhibitors which retard progression of human Alzheimer's disease, and prevent or reverse pathology in a mouse model of neuropsychiatric lupus (NPSLE) which we have extensively studied. Angiotensin receptor blockers (ARBs) also modulate this pathway by preferentially binding to AT1. The system is best studied in microglial cells, but neurons and brain microvascular endothelial cells also express AT1, AT2 and ACE2.

This pathway is now of particular interest as ACE2 is the receptor for several corona viruses, including Covid-19. It is not known, however, whether activated or quiescent microglia are more likely to internalize virus based on the level of expression of ACE2. There is also a key unanswered question for the numerous individuals on ACE inhibitors or ARBs: does the use of these drugs increase or decrease the viral penetration of cells?

To address these questions, we propose to explore healthy mice and two mouse models that mimic human disease: a model of antibody-mediated NPSLE, and sepsis-surviving mice. In each case we have observed microglial activation and pathological pruning of neuronal dendrites.

Specifically, we propose to address the following hypotheses:

Aim 1. There will be diminished ACE2 expression and therefore diminished binding of the Covid-19 trimeric spike protein (S) or the receptor binding domain (RBD) of spike protein to microglia, neurons and brain endothelial cells in mice with chronic neuroinflammation. The rationale for this hypothesis is that elsewhere in the body chronic inflammation leads to decreased ACE2. We will quantitate the binding and uptake of S or RBD by microglia, neurons, and brain microvascular endothelial cells in healthy mice, mice with antibody-induced NPSLE, and sepsis-surviving mice.

Aim 2. There will be no effect of ACE inhibitors on levels of ACE2 in the brain, but there will be an increase of ACE2 induced by ARBs. The presence of S or RBD, however, will diminish the effectiveness of ACE inhibitors and ARBs. These hypotheses are predicated on data showing that these drugs do not alter ACE2 expression in renal tissue, although ARBs increase ACE2 in cardiac tissue, and the data showing that S can activate TACE to diminish levels of membrane-bound ACE2. We will quantitate the impact of ACE inhibitors and ARBs on binding of S or RBD to brain cells. Because we anticipate decreased ACE2 expression in the presence of S and RBD, we hypothesize that ACE inhibitors and ARBs will be less effective at modulating the effects of neuroinflammation in the presence of S or RBD. We will, therefore, assess the alterations in brain pathology induced by ACE inhibitors or ARBs in the presence of S or RBD.

Aim 3. The binding of S or RBD to brain cells will increase the pathology seen in NPSLE and in sepsis surviving mice. This hypothesis is based on the data that S binding to ACE2 increases shedding of the ACE2 ectodomain by activating TNF α converting enzyme (TACE). We will assess whether the binding of S or RBD to brain cells alters their activation state, or alters *in vivo* pathology using immunohistology, transcriptional analysis, and PET scanning.

These studies should advance our understanding of the RAS system in neuropathological conditions, but more importantly they will inform us whether Covid-19 is more likely to infect the healthy brain or a brain exhibiting neuroinflammation and the consequences of frequently used anti-hypertensive medications on Covid-19-induced brain pathology. These studies cannot be performed in human subjects.

GMS Stephanie Kreider Tel NIH NAMS

UH2 AR067688

(Diamond)

09/24/14-05/31/2022

0.6 cal mons

PEARL: Pathway exploration and analysis in renal lupus

no cost extension

This a proposal to apply state of the art genomic and immunophenotyping technologies to lupus nephritis.

No overlap

Specific Aims:

Lupus nephritis (LN) remains an unsolved medical problem. Current therapies are often ineffective, with a significant percent of patients progressing to end stage renal disease. In addition, current therapies invariably have adverse effects, including broad immunosuppression and, in some cases, death due to opportunistic infection, cancer, or other complications of treatment. Regrettably, recent trials of novel biologic approaches to

treatment have failed. Thus, there is a need to refocus our efforts to understand the mechanisms of disease to guide us toward more effective, and safer, treatments.

We will pursue these goals through the following specific aims.

Aim 1: We will establish the infrastructure and SOPs for multi-site studies of LN.

Aim 2: Phase 0: We will determine the high resolution gene expression profile of resident kidney cells in order to understand changes that occur in LN. We will use RNA-seq of glomerular and interstitial tissue to localize particular immune subsets within the kidney. We will optimize techniques for obtaining RNA from cells in the urine, exploring single cell RNA-seq versus RNA-seq of hematopoietic and non-hematopoietic cells. We will additionally study LN biopsies to determine disease-specific changes in cell populations and gene expression profiles.

Aim 3 (Phase 1): We have two Phase 1 projects. We will study kidney biopsy, blood and urine of 20 patients with active nephritis in order to help focus the analyses of the Phase 2 study. We will use blood samples collected in the ITN-funded trial of abatacept in LN to determine those cellular subsets in blood that may predict response to therapy or best reveal therapeutic response in lupus nephritis.

Aim 4 (Phase 2): We will enroll 200 patients in a longitudinal study to obtain kidney tissue, blood and urine at the time of initiation of therapy for LN and blood and urine at frequent intervals thereafter for 24 months. We will execute a focused analysis of cellular and molecular networks that define renal pathology and predict therapeutic response, and of blood and urine surrogates for renal tissue. We will identify early changes in blood and urine that correlate with subsequent therapeutic response.

Pilot Project: We will extend a novel histologic approach (cell distance mapping) to analyze cellular interactions within biopsy tissue using cell markers that are identified in the Phase 0/Phase 1 studies.

Supplement funded to PEARL

09/18/2020 – 05/31/2022

Pearl Pilot Program Supplement

The AMP has been focused on analyzing the transcriptome of both tissue resident and infiltrating cells in kidneys of patients with lupus nephritis (LN). The patient population is from North America, and includes a high percentage of African-American and Hispanic patients. That raises a question concerning how generalizable the results are.

In collaboration with Sang-Cheol Bae of Korea, we have collected 26 LN biopsies of Korean patients obtained using the AMP protocol. Currently, in the AMP phase 2 cohort we have 39 Asian patients, though it is probable that this includes a high percent of Chinese.

We believe it will be instructive to analyze the transcriptome of cells in the biopsies of Korean LN patients. If the data are the same as we see for Asian patients in the US, it suggests that the pathways of disease pathogenesis are independent of environmental factors. If the analysis identifies differences between the AMP and the Korean cohorts, it would propel us to a study of environmental modulators of disease perhaps beginning with the microbiome.

GMS Cynthia M Rojas Tel

NIH U19AI144306

PIs Diamond/Davidson

04/1/2019 – 03/31/2024

1.2 cal mons

Heterogeneous pathways to autoantibody production: Implications for prognosis and therapeutic targeting

This is a study of autoreactive plasma cells in SLE.

No overlap

OVERVIEW Specific Aims:

Our ACE proposal entitled “Heterogeneous pathways to autoantibody production: implications for prognosis and therapeutic targeting” examines basic mechanisms for loss of tolerance to lupus related autoantigens using novel reagents and technologies.

The Principal Project (Dr. Betty Diamond) will use a novel fluorescence-labeled autoantigen reagent to isolate and characterize autoreactive B cells from patients with lupus and to ask about their origins and transcriptional profile, specific to autoreactive B cells, compared with cells that protect against microbial antigens.

The Collaborative Project (Dr. Anne Davidson) will ask how TNF inhibitors that are widely used to treat inflammation in autoimmune diseases break B cell tolerance to induce non-specific and specific lupus related autoantibodies.

The Pilot Project (Dr. Sun Jung Kim) will address the transcriptional, metabolic and functional diversity of circulating T follicular helper cells in patients with lupus and ask whether it is possible to restore normal B cell helper function of these cells by altering cell metabolism.

A better understanding of how the autoreactive lupus B cells become activated to produce lupus-related autoantibodies is needed in order to design therapies for lupus that are more effective and less globally immunosuppressive. We expect to collaborate with other ACEs that have similar scientific interests and complementary technologies; this should lead to synergistic interactions that will move us forward faster.

The principal project is focused on understanding the protective antibody response to vaccine in SLE patients and to the study of ABCs. Therefore there is no overlap.

GMS Diomaris Gonzalez Tel

Lupus Research Alliance (Mackay)

11/01/19 -10/30/22

0.4 cal mons

Investigators-Initiated Clinical Trials Co-PI Diamond

A novel Phase 2 double-blind, randomized, controlled clinical trial to evaluate the efficacy of centrally acting, non-toxic ACE inhibition in cognitive impairment associated with SLE

This is a clinical trial of centrally acting ACE inhibitors in NPSLE.

No overlap

Rationale

Tissue injury in SLE results from multiple and often redundant inflammatory pathways and mediators, therefore, a therapeutic approach that modulates multiple inflammatory mediators or pathways rather than ablating single pathways will be associated with greater clinical efficacy and less toxicity. We are also oriented towards the development of non-immunosuppressive treatments for SLE. To this end, the proposed clinical trial tests the efficacy of centrally acting Angiotensin Converting Enzyme Inhibitors (CA-ACEi), FDA-approved medication for hypertension with a highly favorable safety profile, compared to non-centrally acting ACEi (nonCA-ACEi) for improving abnormal resting brain metabolism and cognitive function in patients with SLE.

GMS Theresa Do Tel

NIH R01 AR074565

(Diamond/Kim)

07/05/19 –

0.96 cal mons

Alteration of function and specificity of T_{FH} in SLE 06/30/24

This proposal examines the T follicular helper cells in a mouse model of SLE.

No overlap

Specific Aims

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease with a complex pathogenesis 1. Recent studies show that genetic predisposition is involved in both the initiation and propagation of disease 2. While there is an insufficient understanding of disease mechanisms, it is clear that there is a breakdown in B cell tolerance as autoantibodies are present in all patients. These autoantibodies often appear to arise in a T cell dependent manner, suggesting the existence of autoreactive T cells 3, 4, 5. *PRDM1* (the gene encoding BLIMP1) was identified as a risk factor for SLE by genome wide association studies (GWAS) 6, 7, and

dendritic cells (DCs) of healthy individuals harboring the *PRDM1* SLE risk allele phenocopy murine BLIMP1-deficient DCs 8. Thus, mice with a conditional knockout of BLIMP1 in DCs (*Prdm1* CKO; *CD11c-CRE*; *Prdm1^{flox} / flox* on C57BL/6 background) were generated. These mice develop a lupus-like pathology in a T cell dependent fashion 9.

CD4⁺ follicular helper T (TFH) cells are pivotal for formation of the germinal center (GC), and an increased frequency of TFH has been observed in various autoinflammatory models 10, 11 and in active SLE patients 12, 13. DCs of *Prdm1* CKO mice skew to TFH differentiation, and the repertoire of TFH cells from these mice differs from the repertoire of TFH cells from control mice 14. The disease phenotype is observed only in female *Prdm1* CKO mice, suggesting gender dependent mechanisms are critical factors for this disease model. We have, Moreover, shown that autoimmunity does not develop in female *Prdm1* CKO mice that have been ovariectomized, but does develop when exogenous estrogen is administered to ovariectomized mice, demonstrating that hormone, rather than sex chromosome, regulates the autoimmunity. Based on these observations, we hypothesize that the fate and repertoire of self-reactive CD4⁺ T cells is established by aberrant antigen presentation by DCs in the thymus of *Prdm1* CKO mice. Estrogen and estrogen receptor signaling is critical to the altered repertoire of TFH cells, and to the skewing of effector T cells to the TFH subset. Finally, we propose that the fate of CD4⁺ T cells is epigenetically determined in the thymus and skewed to TFH differentiation in *Prdm1* CKO mice. We will test our hypotheses in three aims:

Aim 1: Determine the origin and function of CD4⁺ TFH cells in *Prdm1* CKO lupus prone mice. (A) We will compare the TCR repertoire of CD4⁺ T cells from the thymus of *Prdm1* CKO mice and control mice. If we observe a difference in the repertoire of CD4⁺ thymocytes from female *Prdm1* CKO mice compared to female control mice, we will determine if this is influenced by strength of TCR signaling, using TCR signaling reporter mice, *Nur77*-GFP. In vitro co-culture of thymocytes from *Nur77*-GFP with thymic DCs isolated from female control or female *Prdm1* CKO mice will tell us whether BLIMP1-deficient DCs induce a different TCR signal strength in thymocytes. **(B)** We will determine the B cell activation capacity of TFH cells from female *Prdm1* CKO mice compared to female control mice, and if TFH cells from *Prdm1* CKO mice will induce autoreactive B cells to become antibody secreting cells. Finally, we will compare the gene expression profile of TFH cells in spleens of female *Prdm1* CKO and female control mice.

Aim 2: Determine the mechanism of estradiol regulation of T cell selection and differentiation in female *Prdm1* CKO mice. (A) We will investigate the functional changes induced by estrogen in DCs and the responsible estrogen receptor. We will focus on alterations in the expression of genes that are involved in antigen processing and antigen presentation in DCs, especially *Ctss* and *Ii6*. We will further investigate how these changes contribute to the preferential differentiation of TFH in female *Prdm1* CKO mice. We will assess whether the estrogen receptor (ER) signaling skews T cells to TFH differentiation in vivo and in vitro by modulation of ER1 or ER2. **(B)** We will investigate hormonal regulation of repertoire and function of TFH cells in *Prdm1* CKO mice.

Aim 3: Investigate epigenetic modification of CD4⁺ T cells by DCs in the thymus. We will test the hypothesis that the epigenetic marks in CD4⁺ T cells are regulated by DCs in the thymus, and influence effector T cell differentiation in the periphery. To do this, we will **(a)** characterize epigenetic marks of CD4⁺ thymocytes, and **(b)** investigate how epigenetic marks influence T cell polarization.

GMS Roberta D Wolcott Tel:

NIH U19AI144306-03S1

(Diamond)

05/19/2020-04/30/2022

1.2 cal. mons

Etiology and outcome of MIS-C

This is a study of Multisystem Inflammatory Syndrome in children and young adults with Covid-19 infection.

The goal is to understand why some children get an acute disease and others MIS-C which is a delayed onset disease.

No overlap

Specific Aims

Multisystem Inflammatory Syndrome in Children (MIS-C) has recently been recognized as a late sequela of Covid-19 in the pediatric population. The syndrome arises late in disease when antibodies are present, can be catastrophic and has some similarities to Kawasaki Disease (KD). Northwell Health has cared for

approximately 40 patients with this diagnosis. Forty percent required ICU care; none have died. There is detailed annotation of the clinical course of all these children and stored plasma from some. More hospitalizations for acute disease and MIS-C are expected as the pandemic continues into the new school year.

It is our hypothesis that children with MIS-C will have a TLR-mediated immune complex disease while those with acute Covid-19 disease will have pathology mediated through the Renin- Angiotensin System (RAS). Which of these mechanisms dominates is expected to determine the anti-viral antibody response in these children. We posit that those patients with severe acute disease will have a germinal center-derived response with long lived immunity and neutralizing antibody and those with MIS-C will have an extrafollicular response with short term immunity and poor neutralizing titers. This model is supported by the observations that those with acute disease exhibit low interferon levels, that pediatric cases of acute Covid-19 are relatively rare which is consistent with higher ACE2 levels in children being protective, and that patients with KD, a syndrome presumed to be similar to MIS-C have upregulation of genes in the interferon pathway. We propose the following hypotheses and aims:

Aim 1: We hypothesize that those children with MIS-C will harbor alleles of genes in the interferon pathway that lead to high interferon levels. We will assess this through assigning an interferon risk score to each patient in each group that is based on the number of high interferon risk alleles.

Aim 2: We hypothesize that children with MIS-C will have an array of soluble inflammatory mediators consistent with TLR- mediated immune activation while those with acute COVID-19 infection will have an array of cytokines consistent with activation of the RAS. We will test serum of children with acute disease and children with MIS-C for levels of 54 analytes using an MSD platform.

Aim 3: We hypothesize that children with MIS-C will have higher IgM levels, and a clonally more diverse, low affinity IgG antibody to COVID-19 spike protein, likely associated with lower neutralization titer. Children who have recovered from acute infection will have higher affinity serum antibodies and a less diverse antibody repertoire dominated by clones to the receptor binding domain (RBD) and more likely to be neutralizing. We expect that the serum antibodies from the two groups will have differential ability to engage the activating Fc receptors on neutrophils and macrophages.

Aim 4: We hypothesize that the plasma metabolomic profile will differ between acute infection and MIS-C and will predict longterm outcome of MIS-C. We will assess this by HPLC mass spectrometry.

We will enroll all new pediatric patients with acute Covid-19 infection or with MIS-C and will obtain blood for DNA and blood samples longitudinally throughout a patient's hospital stay and for 1 year beyond. We will also analyze DNA and serology on all previously hospitalized pediatric patients with either acute Covid-19 infection or MIS-C. We will have a control group of healthy children who are sero-negative. As testing becomes more prevalent, we will add an asymptomatic sero-positive cohort. These comparisons will allow us to determine the features that are specific to children with MIS-C, the reasons that such different conditions can be triggered by the same virus and the implications for long term immunity and cardiac function.

GMS Teresa M Parker-Reeserm Tel:

DOD Lupus – Impact Award (Diamond)

09/15/2020 – 09/14/2023

1.2 cal mons

Understanding plasma cell differentiation in SLE

This is a study to distinguish plasma cells of germinal center origin from plasma cells of extrafollicular origin. No overlap

Impact statement

This proposal will address the focus area “Understanding lupus disease heterogeneity. We have developed a novel method to isolate and characterize ANA+ B cells and plasma cells in SLE. This method revealed two distinct clusters of SLE patients, based on the relative frequencies of ANA+ memory B cells and IgM and IgG ANA+ plasma cells. In the proposed research, we aim to develop this strategy to subtype patients and to further

increase our understanding of whether this stratification scheme reflects different plasma cell differentiation pathways. We will address whether the clustering relates to a dominant extrafollicular or germinal center pathway for differentiation of ANA+ plasma cells. In addition, we will study the expression profiles of ANA+ plasma cells from both groups of patients and will determine what governs the differentiation of ANA+ plasma cells in SLE.

We believe that with the proposed research we will develop a stratification system that will facilitate the assessment of therapeutic agents, and we will learn much more about B cell biology in SLE, with the possibility to discover new therapeutic targets. No current therapies for SLE specifically target the autoreactive plasma cells. As most current therapies are immunosuppressive, thereby leading to increased susceptibility to infection, more specifically targeted therapies would be highly beneficial.

Our research will address this in two ways: First of all, our RNA-sequencing studies combined with repertoire studies will reveal if the stratification of patients based on their pattern of ANA+ antigen-experienced cells reflects their plasma cell differentiation pathway and will also reveal differential expression of therapeutic targets by the plasma cells. In this case, our research can be used to develop personalized medicine with therapeutics that can specifically alter one of these pathways. This will likely result in fewer side effects compared to targeting the entire plasma cell or B cell compartment, which is associated with immunosuppression; leaving one plasma cell differentiation pathways intact may result in reduced immunosuppression. We will also learn the triggers for plasma cell differentiation in SLE. This will lead to a better understanding of the tolerance failure that leads to the emergence of ANA+ plasma cells, and will reveal how to prevent plasma cell differentiation.

The short-term impact of our research project is that it will provide us with insight into the mechanisms that lead to differentiation of ANA+ plasma cells in SLE. The results of these studies may enable us to stratify patients for clinical trials. In the short-term, our research can be the foundation to study the efficiency of existing therapies in the two subgroups of SLE patients, such as Belimumab, which may be more efficacious in targeting extrafollicular plasma cell differentiation, or Mycophenolate, which may be more efficacious in targeting T cells and a germinal center path to plasma cell differentiation. In addition, our research may reveal targets in either differentiation pathway for which drugs already exist and have been proven safe in other diseases. In both of these cases our research will allow for development of stratification methods to more specifically target the plasma cell differentiation pathways in a precision manner. In the long-term, we may learn how to block the enhanced plasma cell differentiation seen in SLE patients.

GMS Heather Sewell Tel: Leidos Biomedical
Research, Inc. (Gregersen) **Serological
Sciences Network**

10/01/2020 – 09/30/2021 1.2 cal mons

This is a study of Covid convalescent serum.
No overlap

Background:

In light of the emergence of the novel coronavirus, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), and the urgent need to mitigate the pathogen's spread, the National Cancer Institute (NCI) and the National Institute of Allergy and Infectious Disease (NIAID) are establishing a Serological Sciences Network of Excellence to mobilize collaborative efforts to develop serological assays of high specificity and high sensitivity for deployment to test for SARS-CoV-2 induced immune responses and to rapidly expand national serological testing capacity. The proposed Serological Sciences Network for SARS-CoV-2 (SeroNet) will contain several components. The Serological Capacity Building Centers are a critical component of the SeroNet. The Capacity Building Centers (CBC) shall focus on the rapid deployment and expansion of SARS-CoV-2 serological testing capacity and practice. In addition to the CBCs, the SeroNet includes the Centers of Excellence, Research Projects, and the Frederick National Laboratory for Cancer Research (FNLCR) Serology Laboratory. Serological Sciences Centers of Excellence and Serological Sciences Research Projects will be grant funded to understand the mechanisms driving serological, humoral, and cellular immune responses to SARS-CoV-2 viral infection to inform the development of novel serological tests. Each SeroNet component is expected to interact

closely, collaborate, share data, resources, and samples. The Serological Sciences Network Coordinating Center (SSNCC) at FNLCC will manage and coordinate data and communications across SeroNet, and a SeroNet Steering Committee (SSC) comprised of members from each component, including NCI and NIAID, will provide scientific oversight and guidance.

GMS

NIH P01AI148102-01A (Co-Investigator- Diamond)(Brenner) 06/15/2021 – 03/31/2026

1.2 cal mons

Differentiation of immune cells and fibroblasts in inflamed tissue in RA and SLE

Role: Co-investigator Project 1

My role in

this proposal is to perform cell isolations and single cell analyses of SLE tissue and blood, focusing on B cells.

No overlap

Project 1: Origin and function of atypical lymphoid populations in inflamed tissue in SLE and RA

Clinicians have considered systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) to be related diseases for decades as the two diseases are often present in the same family and, indeed, some patients appear to have both diseases simultaneously in a syndrome termed Rhupus (1, 2). Lymphocyte populations expanded in these chronic autoimmune diseases can possess phenotypes and functions that diverge from those described in established immunologic paradigms (3, 4). In particular, SLE and RA patients exhibit an expanded population of CD11c+, CD21, CXCR5- B cells termed age- or autoimmunity-associated B cells (ABCs).

Studies of ABCs in SLE kidney and RA synovium suggest functional differences from blood ABCs. Several challenges remain to understand the derivation and function of ABCs. First, different definitions have been used to characterize ABC-like cells, resulting in analyses of partially overlapping cell populations. A clear delineation of the heterogeneity in phenotype and transcriptome among putative ABC populations is critical to establish their functional heterogeneity.

Second, an understanding of how ABCs accumulate in tissues is required to design strategies to interfere with this process. Transcriptomic analyses indicate that ABCs may differentiate *in situ* in nephritic kidneys and in rheumatoid synovium. Third, the cellular interactions that promote ABC development and function within tissues remain unclear.

Mass cytometric analysis of circulating lymphocytes reveals a strong and specific correlation between the frequency of circulating ABCs and the frequency of T peripheral helper (Tph) cells, a tissue-homing B cell-helper T cell population, similar to but distinct T follicular helper (Tfh) cells suggesting potential interactions between these 2 lymphocyte populations, both of which are present in inflamed tissue (5).

Here we propose 3 complementary aims to evaluate ABC phenotypes and function, differentiation pathways, cellular interactions, and localization in SLE and RA tissue. Aims 1 and 2 will be performed in SLE kidney and blood and RA synovium synovial fluid and blood. Aim 3 will be performed in both RA and SLE tissue.

Aim 1 Define the heterogeneity, developmental pathway, and antigenic specificity of tissue ABCs.

We hypothesize that ABC populations differentiate locally within inflamed tissues and produce disease-associated autoantibodies. We will define the heterogeneity of tissue ABCs and compare tissue to blood ABCs. Using high

throughput repertoire sequencing, we will compare B cell receptor (BCR) repertoires of tissue ABCs with other tissue B cells, blood ABCs, blood plasmablasts and potential B cell precursor populations, naïve and memory B cells, to determine differentiation pathways. We will also evaluate which disease-associated antigens are recognized by blood and tissue ABCs and if plasma cells and ABCs in tissue recognize the same antigenic targets.

Aim 2 Determine the functional interactions between blood ABCs and T cells.

We hypothesize that ABCs and Tph cells interact with ABCs functioning as antigen presenting cells to drive Tph differentiation. We will evaluate the ability of ABCs to promote differentiation of naïve CD4+ T cells into IL-21 producing Tfh or Tph cells, or to activate established Tfh/Tph cells. We will evaluate the ability of Tph and

Tfh cells to enhance ABC survival, antibody secretion, and cytokine production and to induce differentiation of naïve B cells into ABCs.

Aim 3 Determine the major cellular partners interacting with ABCs in inflamed tissues in SLE and RA.

We hypothesize that ABCs and Tph cells will cluster near tissue DCs within inflamed tissues. We will map the localization of ABCs in SLE kidneys and RA synovium. We will specifically address the co-localization of ABCs with Tph cells and Tfh cells, as well as with NK cells, CD8+ T cells, myeloid cells, and also with proinflammatory stromal cells such as CD90+ fibroblasts. We will then evaluate expression of chemokine/chemokine receptor pairs in ABCs and closely co-localizing cell populations to identify candidate mediators of co-localization.

Impact: These studies derive from recent observations on tissue infiltrating cells in RA and SLE. They extend our knowledge of a recently identified B cell population and determine how they interact with Tfh and Tph cells, lymphocyte populations that are expanded in autoimmune disease, and will inform how these cells can be targeted therapeutically.

Synergy with other projects: All projects in this PPG are designed to understand the derivation and function of cell states unique to or highly enriched in inflamed tissue.

Use of cores: We will work closely with the Computational Systems Immunology Core for aims 1 and 3.

Pending

None

Current and Pending Support

GEORGIU, GEORGE

Current

Funding is listed in Total Direct Costs to UT Austin for current year.

Title: North Carolina Seronet Center for Excellence: Research Core C

Effort: 8.3%

Supporting Agency: NIH 5U54CA260543 (Co-I, PI: Baric)

Address: 37 Convent Dr., Bethesda, MD 20814

Contracting/Grants Officer: Elizabeth Lee Read-connoles, bconnoles@mail.nih.gov, Performance

Period: 10/01/2020-09/30/2025

Funding Amount:

Project Goals: The major goals of this project are to 1) characterize the immune responses elicited to SARS-CoV2 infection, 2) understand the mechanisms driving the serological, humoral and cellular immune responses, 3) determine modifiers of the serologic memory and 4) determine the serological correlates of disease pathogenesis, and protection against future infection. Research Core C will be integral to the examination of serological antibody repertoires in COVID-19 study subjects as well as in convalescent plasmas obtained from survivors.

Overlap: None

Title: Etiology and outcome of MIS-C

Effort: 4.2%

Supporting Agency: NIAID 3U19AI144306-02S1 (Co-I, PI: Diamond)

Address: 37 Convent Dr., Bethesda, MD 20814

Contracting/Grants Officer: David R. Johnson, drjohnson@niaid.nih.gov, Performance Period:

08/27/2020-08/26/2025

Funding Amount:

Project Goals: The major goal of this project is to test two hypotheses regarding the Multisystem Inflammatory Syndrome (MIS-C) recently identified in children and young adults infected with SARS-CoV-2.

Overlap: None

Title: Antibody Landscape following Human Norovirus Infection and Vaccination

Effort: 2%

Supporting Agency: NIH 1R01AI148260-01 (Co-I, PI: Baric)

Address: 37 Convent Dr., Bethesda, MD 20814

Contracting/Grants Officer: Rodolfo M. Alarcon, Rodolfo.alarcon@nih.gov, Performance Period:

03/05/2020-02/28/2025

Funding Amount:

Project Goals: The major goals of this project are to critically inform the development of broadly effective HuNoV vaccine.

Overlap: None

Title: Systematic, molecular level analysis of the Fc receptor ligation on antibody effector functions.

Effort: 8.3%

Supporting Agency: NIH U01AI148118 (PI: Georgiou)

Address: 37 Convent Dr., Bethesda, MD 20814

Contracting/Grants Officer: Kevin Roy Heath, kevin.heath@nih.gov,

Performance Period: 12/10/2019-11/30/2024

Funding Amount:

Project Goals: The major goal of this project is to delineate, in unprecedented detail, the cytotoxic mechanisms and signaling events triggered by each receptor on myeloid cells and thus provide a rational framework for the engineering of therapeutic antibodies having more potent effector functions and improved clinical efficacy.

Overlap: None

Title: Center for Influenza Vaccine Research in High-Risk Populations

Effort: 8.3%

Supporting Agency: NIH 75N93019C00052 (Co-I, PI: Ross)

Address: 37 Convent Dr., Bethesda, MD 20814

Contracting/Grants Officer: Erin Felent, Erin.Felent@nih.gov,

Performance Period: 09/10/2019 – 09/09/2026

Funding Amount:

Project Goals: The major goal for UT Austin's subaward on this proposal is to delineate the B-cell and serological antibody repertoires elicited by influenza virus, including a molecular-level assessment of influenza vaccine candidates through preclinical animal studies, early phase clinical trials, and healthy volunteer human challenge studies.

Overlap: None

Title: CIVICs A

Effort: 4.2%

Supporting Agency: NIH 75N93019C00050 (Co-I, PI: Moody)

Address: 37 Convent Dr., Bethesda, MD 20814

Contracting/Grants Officer: Erin Felent, Erin.Felent@nih.gov,

Performance Period: 09/10/2019-09/09/2026

Funding Amount:

Project Goals: The major goal for UT Austin's subaward on this proposal is to delineate the B-cell and serological antibody repertoires elicited by influenza virus, including a molecular-level assessment of influenza vaccine candidates through preclinical animal studies, early phase clinical trials, and healthy volunteer human challenge studies.

Overlap: None

Title: Molecular and Immunologic Analysis of Influenza Vaccine Response

Effort: 4.2%

Supporting Agency: CDC 75D30119C06088 (Co-I, PI: Kennedy)

Address: 1600 Clifton Road, NE, Atlanta, GA

Contracting/Grants Officer: Ronnie Williams, oga3@cdc.gov,

Performance Period: 09/01/2019-08/04/2021

Funding Amount:

Project Goals: The major goal for UT Austin's subaward on this proposal is the comprehensive analyses of the human humoral anti-HA repertoire elicited by three seasonal influenza vaccines.

Overlap: None

Title: Alteration of function and specificity of TFH in SLE

Effort: 4.2%

Supporting Agency: NIH R01 AR074565-01 (Co-I, PI: Diamond)

Address: 37 Convent Dr., Bethesda, MD 20814

Contracting/Grants Officer: Marie Mancini, mancini2@mail.nih.gov

Performance Period: 09/01/2019 – 08/31/2024

Funding Amount:

Project Goals: The major goals of this project are to study how BLIMP1-deficient DCs allow self-reactive T cells to escape central tolerance mechanisms in the thymus.

Overlap: None

Title: Development and Mechanistic Studies of an Engineered Human Therapeutic to Abrogate Immune Suppression due to Elevated Methylothioadenosine (MTA) by MTAP null/low Tumors.

Effort: 1%

Supporting Agency: NIH/NCI 1R01CA240700 (Co-I, PI: Stone)

Address: 37 Convent Dr., Bethesda, MD 20814

Contracting/Grants Officer: Leslie Hickman

Performance Period: 07/01/2019-06/30/2024

Funding Amount:

Project Goals: The major goals of this project are development of a novel biologic cancer therapeutic, to reverse the immunosuppressive effects of MTA and aid in the treatment of patients with MTAPnull/low tumors as a key biomarker

Overlap: None

Title: Heterogeneous pathways to autoantibody production: implications for prognosis and therapeutic targeting

Effort: 4.2%

Supporting Agency: NIH/NIAID 1 U19AI144306 (Co-I, PI: Diamond)

Address: 5601 Fishers Ln, Rockville, MD 20852

Contracting/Grants Officer: Regina Kitsoulis

Performance Period: 04/01/2019-03/31/2024

Funding Amount:

Project Goals: The major goals of this project are to compare vaccine responses in SLE patients and healthy subjects with respect to clonality and cross-reactivity with autoantigen.

Overlap: None

Title: Mechanism of DNA-specific Autoimmunity in Systemic Lupus Erythematosus

Effort: 1%

Supporting Agency: NIH/NIAMS 2 R01AR071703 (Co-I, PI: Reizis)

Contracting/Grants Officer: Marina Mancini

Performance Period: 04/01/2018-03/31/2024

Funding Amount:

Project Goals: The major goals of this project are to delineate the B-cell antibody repertoire in autoimmune DNASE1L3-deficient mice.

Overlap: None

Title: Antibody Discovery and Optimization using the Eclonal Technology

Effort: 8.3%

Supporting Agency: Clayton Foundation for Research (PI)

Grants Officer: Jarred Sloan, 1 River Way, Houston, TX 77056, 713-626-8646

Performance Period: 1/1/2013-12/31/2020(renewed annually)

Funding Amount: Total Direct/yr

Project Goals: The major goal of this project is the development of new technologies for IgG discovery and production for cancer therapy.

Overlap: None

Pending

None

Previous (Project Period Ending within the past 5 years)

Title: Human-Enzyme Mediated, Systemic Depletion of Cystine for Cancer Treatment

Effort: 3%

Supporting Agency: NIH: R01CA189623 (MPI: Georgiou, DiGiovanni, Stone)

Grants Officer: Michael C. Alley, 9000 Rockville Pike, Bethesda, MD
Performance Period: 08/01/2014-07/31/2020 NCE
Funding Amount: Total Direct/yr NCE- no additional funds
Project Goals: The major goal of this project is to determine that prolonged, systemic depletion of the serum L-Cys/CSSC pool using engineered human enzymes may constitute a potent cancer therapeutic avenue either as a monotherapy or as a sensitizer (due to the ensuing redox balance perturbation) for combination therapy with minimal toxicity.
Overlap: None

Title: Molecular, Functional and Structural Analyses of Anti-PAD Antibodies in Rheumatoid Arthritis
Effort: 1%
Supporting Agency: 1 R56AI143994-01 (Co-I, PI: Bridges)
Grants Officer: John Peyman, peymanj@niaid.nih.gov
Performance Period: 04/12/2019-03/31/2020
Funding Amount: UT Total Costs
Project Goals: The major goal of UT Austin's portion of this project is molecular identification and structure determination of the monoclonal antibodies that comprise serum IgG antibody repertoires.
Overlap: None

Title: HIV bNAbs
Effort: 1%
Supporting Agency: Leidos Biomedical Research Inc.: 15X219 (PI)
Grants Officer: Chris Case, PO Box B, Frederick, MD 21702-1201
Performance Period: 11/1/2015-03/15/2020
Funding Amount: Total Direct Costs
Project Goals: The major goal of this project is to adapt novel technologies for the analysis of humoral immune responses.
Overlap: None

Title: RSV IgSEQ
Effort: 1%
Supporting Agency: Sanofi Pasteur Biologics (Co-I, PI: Ippolito)
Address: 38 Cambridge, MA 02139
Contracting/Grants Officer: Joshua DiNapoli
Performance Period: 12/01/2018-06/30/2020
Funding Amount:
Project Goals: The major goals of this project are a molecular-level comparison of baseline repertoires (cellular and serological) in RSV-immune study subjects with their activated repertoires elicited by RSV.
Overlap: None

Title: Development of an engineered & pharmacologically optimized human methionine-gamma-lyase drug candidate for the treatment of prostate cancer and glioblastoma.
Effort: 2%
Supporting Agency: CPRIT RP180590 (Co-I, PI: Stone)
Performance Period: 03/01/2018-08/31/2021 (NCE)
Funding Amount:
Project Goals: The major goals of this project are to make further improvements in the potency of this drug, understand the mechanism of action and identify biomarkers for selecting patients most likely to benefit from this therapy.
Overlap: None

Title: Molecular Analysis of Serum Antibody Constituents in Zika Virus Infection
Effort: 1%

Supporting Agency: NIH: R21AI135682 (MPI: Georgiou, Baric)
Contracting/Grants Officer: Donna R Sullivan
Performance Period: 09/01/2017-01/31/2020
Funding Amount: Total Direct Costs
Project Goals: The major goal of this project is to assess and quantify the persistence of specific and protective antibodies as well as cross-reactive (possible pathogenic) antibodies in Zika-infected patient blood.
Overlap: None

Title: Duke DARPA Pandemic Prevention Platform (P3)
Effort: 16.6%
Supporting Agency: DOD/DARPA (Co-PI: Georgiou, PI: Sempowski)
Grants Officer: Colonel Matt Hepburn, 675 North Randolph Street, Arlington, VA 22203, Matt.Hepburn@darpa.mil
Performance Period: 10/01/2017-09/25/2019
Funding Amount: Total Project Costs
Project Goals: The major goal of this project is to develop an integrated technology platform that gives public health officials the capability to halt the spread of any viral disease outbreak before it can escalate to pandemic status.
Overlap: None

Title: Neutralizing Antibody Evolution Following Seasonal Flu Vaccination or Infection
The major goal of this project is to study the neutralizing antibody evolution following seasonal flu vaccination.
Effort: 1%
Supporting Agency: Harvard/Children's Hospital
Grants Officer: Karen Lee, 250 Longwood Ave, Boston, MA
Performance Period: 8/1/2014-07/31/2018
Funding Amount: /yr Total Direct Costs
Project Goals: Neutralizing Antibody Evolution Following Seasonal Flu Vaccination or Infection
The major goal of this project is to study the neutralizing antibody evolution following seasonal flu vaccination.
Overlap: None

Title: Preclinical Development of a Therapeutic Enzyme for Immune Checkpoint Inhibition in Cancer.
Effort: 8.3%
Supporting Agency: CPRIT: DP150061
Address: 1701 North Congress Ave, Austin, TX 78701
Contracting/Grants Officer: Wilfredo Ruiz
Performance Period: 12/1/2014-05/31/2018
Funding Amount: Total Direct Costs *NCE No addtl funds
Project Goals: The major goal of this project is to expand and execute the product development and preclinical studies needed to deliver a powerful immune checkpoint inhibitor into the clinic for cancer therapy.
Overlap: None

Title: Therapeutic Enzyme Development
Effort: 1%
Supporting Agency: KYN Therapeutics, LLC: Sponsored Research Agreement
Address: 400 Technology Square, 10th floor, Cambridge, MA 02139
Grants Officer: Mark Manfredi
Performance Period: 04/01/2016-05/31/2018 renewed annually
Funding Amount: Total Direct Costs

Project Goals: The major goal of this project is the development of a therapeutic enzyme that can reverse immunosuppression caused by metabolites produced by tumors.
Overlap: None

Title: Engineering, Optimization, and Validation of Novel Human Therapeutics

Effort: 8.3%

Supporting Agency: Aeglea BioTherapeutics, LLC: Sponsored Research Agreement

Address: 815-A Brazos, Suite 101, Austin, TX

Grants Officer: David Lowe

Performance Period: 12/01/2013-08/31/2018

Funding Amount: Total Costs

Project Goals: The major goal of this project is to engineer and characterize therapeutic arginine & methionine degrading enzymes and their initial preclinical testing in vitro and in vivo animal models.

Overlap: None

Title: High-throughput sequencing of the human paired T cell receptor α - β repertoire in identical twin pairs

Effort: 2.1%

Supporting Agency: NIH Cooperative Centers for Translational Research in Human Immunology (CCHI)

Address: 3172 Porter Drive, Palo Alto, CA, 650-498-8197

Grants Officer: Bach-Hong Tran

Performance Period: 04/01/2017-03/31/2018

Funding Amount:

Project Goals: Development and validation of the high throughput TCR paired sequencing technology, carry out the paired repertoire sequencing of twins and perform the data analysis.

Overlap: None

Title: Anaphylaxis and IgE heterogeneity

Effort: 1%

Supporting Agency: NIH Pass-through from La Jolla Inst. For Allergy and Immunology: R21AI115534

Address: 9420 Athena Circle, La Jolla, CA

Grants Officer: Jen Keyes

Performance Period: 12/01/2014-11/31/2017

Funding Amount: Total Direct Costs

Project Goals: The major goal of this project is to perform sequence analysis of IgE molecules from severely and mildly anaphylactic mice.

Overlap: None

Title: Serological and B cell repertoire analysis of the response to the Sanofi-Pasteur Vaccines Seasonal Flu Vaccine

Effort: 8.3%

Supporting Agency: Sanofi Pasteur: Sponsored Research Agreement

Address: 38 Sidney Street, Cambridge, MA

Grants Officer: Joshua DiNapoli

Performance Period: 1/1/2015-10/31/2017

Funding Amount: Total Direct Costs

Project Goals: The major goal of this project is B cell repertoire analysis of the response to seasonal flu vaccine

Overlap: None

Title: Genetic Factors and Molecular Metrics of Vaccine Efficacy via the Deconvolution of Humoral and Cellular Immune Responses

Effort: 8.3%

Supporting Agency: DOD/DTRA: HDTRA1-12-C-0105
Grants Officer: Amanda Horstman-Smith, 703-767-3372, amanda.l.horstmansmith.civ@mail.mil
Performance Period: 09/01/201-08/30/2017
Funding Amount: Total Direct Costs
Project Goals: The major goal of this project is to analyze the B cell and serum response in 25 volunteers vaccinated with the killed polio vaccine and also in the respective in vitro stimulated PBMCs and compare the in vivo and in vitro responses.
Overlap: None

Title: Development of therapeutic antibodies having both Fc[gamma] and Fc[alpha] effector functions and displaying potent cancer cell killing.
Effort: 3%
Supporting Agency: CPRIT: RP140664
Grants Officer: Wilfredo Ruiz, 1701 North Congress Ave, Austin, TX 78701
Performance Period: 8/1/2014-02/28/2017
Funding Amount: Total Direct Costs
Project Goals: The major goal of this project is to develop a "first in class" Fc antibody domain recently engineered to combine the mechanisms of cancer cell elimination displayed by both the IgG and IgA antibody isotypes.
Overlap: None

Title: Mechanisms of Norovirus Protective Immunity
Effort: 8.3%
Supporting Agency: NIH: R01CA106006
Grants Officer: 9000 Rockville Pike, Bethesda, MD
Performance Period: 09/01/2014-08/31/2016
Funding Amount: Total Direct Costs
Project Goals: The major goal of this project is to dissect in humans the mechanisms leading to antibody-mediated protection elicited by an experimental norovirus vaccine.
Overlap: None



Award Log Number: LR190120

Title: Understanding Plasma Cell Differentiation in SLE Award

PI: Betty Diamond, Feinstein Institutes, NY

Budget: 525,000

Topic Area: Lupus Research Program

Mechanism: W81XWH-19-LRP-IA

Research Area(s): SCS Coding (0500, 0505)

Award Status: Period of Performance Dates

9/15/2020 – 09/14/2021

Study Goals:

We have previously shown that a major breach to B cell tolerance in patients with systemic lupus erythematosus (SLE) is the enhanced differentiation of IgG plasma cells. Based on this observation, the goal of this study is to understand the induction of plasma cells in SLE. We propose to determine if there is a difference in plasma cell differentiation when they arise through an extrafollicular vs a germinal center pathway, and whether there is a difference in the differentiation of plasma cells directed to nuclear antigens (ANA+) and those without this auto-specificity. We also propose to develop approaches to blocking IgG plasma cell differentiation.

Specific Aims:

Specific Aim 1:

We have been studying whether we can determine through molecular analyses of the B cell receptor (BCR) the pathway for the generation of plasma cells in SLE patients. We have isolated ANA+ and ANA- naïve, memory and age/autoimmunity associated B cells and plasmablasts from SLE patients and subjected these to DNA sequencing of the B cell receptor.

We identify two groups of patients. In one group, plasma cells are most clonally related to naïve B cells; we believe these are patients in whom plasma cells are generated through an extrafollicular pathway. In the second group, plasma cells are clonally most related to memory B cells; we believe plasma cells in these individuals arise through a germinal center pathway. We further observe an increased frequency of somatic mutation in BCRs of those plasmablasts we believe arise from the germinal center response.

Specific Aim 2:

We have begun the transcriptional analysis of ANA+ and ANA- plasmablasts. We obtained these cell populations from 5 healthy and 5 SLE individuals. We are currently applying quality control metrics to the data and analyzing the results.

Specific Aim 3:

We have optimized protocols for the generation of plasma cells from naïve B cells to mimic the extrafollicular pathway and from memory B cells to mimic the germinal center pathways. We have begun to explore potential mechanisms to block plasma cell differentiation. We have found that metformin can prevent plasma cell differentiation. This does not appear to be through metabolic alteration, based on our preliminary data, but rather through cross-linking RAGE and LAIR-1 and preventing the entry of CpG, a factor used in our plasma cell differentiation cocktail, into the cell to engage endosomal toll-like receptors.

Key Accomplishments and Outcomes:

Publications: none to date

Patents: none to date

Funding Obtained: none to date