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14. ABSTRACT

Antiphospholipid syndrome (APS) is a potentially life-threatening complication of systemic lupus erythrematosus (SLE) that leads to venous/arterial blood clots and pregnancy loss. The most severe form of APS is known as catastrophic antiphospholipid antibody syndrome (CAPS) which is fatal in up to 50% of patients. How antiphospholipid antibodies (aPLs) cause blood clots and pregnancy loss is unknown. More importantly, there are no good treatments to reliably prevent blood clots and pregnancy loss in patients with severe APS and CAPS. We, and others, have shown that aPLs activate a portion of the immune system known as complement and that failure to regulate complement on the lining of blood vessels contributes to developing blood clots and pregnancy loss. Thus, drugs that inhibit complement are being proposed to prevent complications from APS/CAPS. The principal investigators (PIs) of this grant are at the forefront of studying complement biology and are authorities on the clinical development and use of a variety of approved drugs to treat complement-mediated disorders. The overarching goal of this study is to identify patients with lupus and aPL who are at highest risk of a first or recurrent thrombosis, and to elucidate the role of complement activation in these complications.

15. SUBJECT TERMS
antiphospholipid antibody syndrome; systemic lupus erythematosus; Beta-2-glycoprotein; catastrophic antiphospholipid syndrome; complement; modified Ham test.

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INTRODUCTION:

Antiphospholipid syndrome (APS) is the association of blood clots and/or pregnancy complications (e.g, fetal loss or severe pregnancy complications) with antiphospholipid antibodies (aPL). Up to 50% of systemic lupus erythematosus (SLE) patients test positive for aPL which can adversely impact SLE patients. Compared to SLE patients without aPL, those with aPL have more blood clots, pregnancy complications, heart valve disease, pulmonary hypertension, low platelet counts, kidney problems, stroke, and worsened quality of life. Catastrophic APS (CAPS), is the most severe form of APS and is characterized by widespread blood clots, multi-organ failure, and leads to death in up to 50% of patients. Current guidelines recommend anticoagulation for patients with APS; however, recurrent blood clots and pregnancy loss may occur in up to 25% of patients, despite anticoagulation. The mechanism whereby aPL cause blood clots and pregnancy complications is unknown; however, prior studies in mice suggest that heightened complement activation is responsible for the clotting, obstetrical complications, and other organ damage in APS. Recently, our group invented an assay (modified Ham, mHam) to probe how human cells reactive to complement the blood of patients with APS/CAPS and other complement-driven diseases. Using this assay, we demonstrated, for the first time, that aPL from APS patients directly activate complement on the surface of human cells resulting in cell death. We also demonstrated the patients with CAPS have genetic defects in complement regulatory genes that make them even more susceptible to complement killing.

Hypothesis: We hypothesize that: 1) anti-β2GPI that preferentially react with β2GPI domain 1, and of the IgG isotype induce more complement activation than those reactive with β2GPI domains 2-5 or those of IgM isotype, and are associated with thrombosis. 2) The modified Ham assay (as a measure of complement activation) is most positive around the time of thrombotic events. 3) That > 50% of CAPS patients will harbor rare germline variants in genes that regulate complement. 4) That C5 inhibition will block C5b-9 deposition induced by aPL.

Specific Aims: The overarching goal of this study is to identify patients with lupus and aPL who are at highest risk of a first or recurrent thrombosis, and to elucidate the role of complement activation in these complications. Specifically, we will:

1) **Test the hypothesis that anti-β2GPI that preferentially react with β2GPI domain 1, and are of the IgG isotype induce more complement activation than those reactive with β2GPI domains 2-5 or those of IgM isotype, and are associated with thrombosis (thrombosis).**
2) **Identify the temporal relationship between thrombotic events and complement activation in sera of patients with SLE-associated APS.**

KEYWORDS:

antiphospholipid antibody syndrome; systemic lupus erythematosus; Beta-2-glycoprotein; catastrophic antiphospholipid syndrome; complement; modified Ham test.

ACCOMPLISHMENTS:

What were the major goals of the project?

Major goals of this proposal seek to address the most important unanswered questions concerning APS: 1) how do we identify patients at highest risk of primary and/or recurrent thrombosis? 2) why do blood clots develop in some individuals with aPL and not others and 3) what is the best way to prevent acute and chronic complications related to APS.

What was accomplished under these goals?
Subtask 1 (Major task 1): We have collected serum for mHam and C5b-9 analysis from 39 patients with SLE and aPL. Goal is 150 in first 30 months. While we are slightly behind, this is due to hiring difficulties encountered during Omicron surge. We were unable to secure samples for almost 2 months but this has been rectified and we should be on pace to catch up in the next 6 months. All 39 samples have resulted.
Subtask 2: We continue to compare the ability of factor D inhibitors and C5 inhibitors to block C5b-9 deposition in APS serum. Similar to our preliminary data, it appears that C5 inhibition is much more effective in blocking C5b-9 deposition.
Subtask 3: We have collected serum from 39 patients (see above) to compare thrombosis risk of mHam positive versus mHam negative APS patients stratified by immunoglobulin subtype (IgG vs non- IgG).

Subtask 1 (Major task 2): We will run the first batch of ELISA for domain 1 autoantibodies and compare complement activation in mHam and flow cytometry assays once we have collected a total of 50 patients. This should by accomplished by the end of October, 2022. mHam results and flow cytometry data are already secured.
Subtask 2: See above. Serum collected from 39 subjects.

Subtask 1 (Major task 3): We now have extracted DNA and sequenced 19 patients with CAPS and over 50% harbor rare (< 1% VAF) using our 23 gene complement panel. Interestingly, 4 of these patients have variants involving CR1, an important transmembrane complement regulator. On separate funding we are studying the function of these variants in a cell model. Subject 20 has been collected but not yet sequenced. We will run another panel after collecting 15 more subjects with APS.

Subtask 1 (Major task 4): We have enrolled 6 patients with SLE and aPL presenting with and acute thrombotic event to follow prospectively.
Subtask 2: See above. 39 subjects enrolled and collecting serial samples.

What opportunities for training and professional development has the project provided?
Xiang-Zuo (Ryan) Pan: Funding on this proposal and applying to medical school. Ryan has great expertise in running complement-based assays.
Gloria Gerber, MD: Not funded on this proposal. She is funded on a grant from the HTRS and an NIH R56 to study how complement dysregulation leads to thrombosis. She was a former hematology fellow and is now an Instructor in the Division of Hematology.
Michael Cole MD/PhD: Not funded on this proposal. He is funded on an NIH T32 (PI, Brodsky). Dr. Cole is a second year hematology fellow in his first research year. He is studying how platelet microparticles become activated by complement on human platelets. He too is interested in studying this in patients with APS and CAPS.

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 fell slightly behind accrual goals in the first year due to hiring issues but this has been resolved and we are now collecting 5-6 new samples a month. This should get us even or near even for most tasks by the end of year 2.

IMPACT:

What was the impact on the development of the principal discipline(s) of the project?
Too soon for impact. Data still being collected and analyzed.

What was the impact on other disciplines?
None yet. Too early to analyze data.

What was the impact on technology transfer?
Nothing to Report

What was the impact on society beyond science and technology?
Too soon for impact. Data still being collected and analyzed.

CHANGES/PROBLEMS:

As stated above, we initially had some accrual problems due to the Omicron wave and hiring difficulties. This resulted in us being slightly behind goal for sample collection. This problem has been rectified and we are now running slightly above accrual so we should catch up by the end of year two. There have been no problems in executing the planned experiments on the samples we collected.

The major change in approach relates to our portion of the study to collect 50 patients with SLE and aPL presenting with an acute thrombotic event to follow prospectively. So far we have only collected 6 such patients. These patients have been less than anticipated; thus, we are now collecting patients with in 2 years (instead of just 1 year) in an effort to increase enrollment. There have been no changes that had a significant impact on expenditures. There are no significant changes in use or care of human subjects, vertebrate animals (none in project), biohazards and/or select agents.

PRODUCTS:

No publications to date but we anticipate publishing a manuscript on the relevance of CR1 rare variants associated with catastrophic antiphospholipid antibody syndrome (CAPS) in the next year. An abstract of this work will also likely be submitted to either ASH or one of the hemostasis and thrombosis meetings.

PARTICIPANTS
What individuals have worked on the project?

Robert Brodsky: no change
Michelle Petri: no change
Shruti Chaturvedi: no change

Name:	Xiang-Zuo (Ryan) Pan
Project Role:	Research Program Coordinator
Researcher Identifier(e.g. ORCID ID):	0000-0001-7339-5312
Nearest person month worked:	4
Contribution to Project:	Coordination in the lab, running assays
Funding Support:	W81XWH2110899 R56HL133113

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

R. Brodsky:

Previously active support now closed

- R01HL133113, NIH, *Complementopathies: Genotype and Phenotype.*
- Achillion Pharmaceuticals, *A Phase 2 Open-label study of ACH-0144471*

New award

- *this award W81XWH2110899, *Role of Complement in the Antiphospholipid Antibody Syndrome in Patients with SLE*, 09/2021-09/2024
- R56HL133113, NIH/NHLBI, *Complementopathies: biology, biomarkers, and targets* 09/2022-08/2023

M. Petri:

Previously active support now closed

- UH2/UH3, NIH, *Accelerating Medicines Partnership in RA and Lupus: Network Research Sites*
- Thermo Fisher Scientific, *Urine Biomarkers for Lupus Nephritis*
- GlaxoSmithKline, *The Long-Term Clinical Outcomes of Lupus Nephritis - Reanalysis of the Dataset*
- GlaxoSmithKline, *The link between SLE disease control, remission, LLDAS and long-term outcomes: Johns Hopkins Lupus cohort*
- Rheumatology Research Foundation, *Rediscovering R052 in systemic lupus erythematosus*

New Award

- Aurinia Pharmaceuticals, *Early renal remission status and its impact on long term renal outcomes*, 04/2021-04/2023
- NIH, *Transcription factor A mitochondria in SLE pathogenesis*, 02/2022-01/2021
- *this award W81XWH2110899 (PI: Brodsky), *Role of Complement in the Antiphospholipid Antibody Syndrome in Patients with SLE*, 09/2021-09/2024

S. Chaturvedi:

Previously active support now closed

- SHP655-201, Baxalta, *A Phase 2, multicenter, randomized, placebo-controlled, double blind study in patients with acquired thrombotic thrombocytopenic purpura (aTTP) to evaluate the pharmacokinetics, safety, and efficacy*

New award

- ASH Scholar Award, *Neurologic complications, and quality of life in TTP survivors* 07/2021-06/2024
- *this award W81XWH2110899 (PI: Brodsky), *Role of Complement in the Antiphospholipid Antibody Syndrome in Patients with SLE*, 09/2021-09/2024