SUMMARY STATEMENT
(Privileged Communication)

PROGRAM CONTACT: [redacted]

Release Date: **07/08/2023**

Revised Date:

Application Number: **1R01AI181321-01**

Principal Investigators (Listed Alphabetically):

CREECH, CLARENCE BUDDY
LIU, GEORGE Y (Contact)

Applicant Organization: UNIVERSITY OF CALIFORNIA, SAN DIEGO

Review Group:	**IHD** **Immunity and Host Defense Study Section**		
Meeting Date:	**06/22/2023**	***Opportunity Number:***	**PA-20-185**
Council:	**OCT 2023**	***PCC:***	**M30A BR**
Requested Start:	**09/01/2023**		

Project Title:	**Interrogating human anti-staphylococcal antibody responses for S. aureus vaccine insights**
SRG Action:	**Impact Score:[redacted] Percentile:1**
Next Steps:	**Visit https://grants.nih.gov/grants/next_steps.htm**
Human Subjects:	**30-Human subjects involved - Certified, no SRG concerns**
Animal Subjects:	**30-Vertebrate animals involved - no SRG concerns noted**
Gender:	**1A-Both genders, scientifically acceptable**
Minority:	**1A-Minorities and non-minorities, scientifically acceptable**
Age:	**1A-Children, Adults, Older Adults, scientifically acceptable**

Project Year	Direct Costs Requested	Estimated Total Cost
1		
2	[redacted]	[redacted]
3		
4		
5		
TOTAL	[redacted]	[redacted]

1R01AI181321-01 LIU, GEORGE

RESUME AND SUMMARY OF DISCUSSION: This R01 application proposes to characterize the protective components of the human antibody response to *Staphylococcus aureus*, and to distinguish these from non-protective or suppressive responses, in order to elucidate critical mechanisms of anti-staphylococcal antibody protection. The reviewers agreed that defining the mechanisms underlying failure of vaccines against *S. aureus*, and identifying antigens that may not be impacted by pre-existing immunity against *S. aureus*, could have very significant impact on vaccine development. The rigor of the prior research strongly supports the significance and experimental approach. The Principal Investigators (PIs) of this multi-PI (MPI) application have complementary expertise, and an excellent track record of productivity. The MPIs and collaborators are well-suited for undertaking the proposed work. The MPI plan is well laid out. The application is conceptually and technically innovative. The research plan is rigorous with adequate statistical analysis and experimental replicates. Sex as a biological variable is appropriately addressed. Anticipated results, pitfalls and alternative approaches are thoroughly considered. No score-driving weaknesses were identified. Overall, the review committee expressed an exceptional level of enthusiasm for this application.

DESCRIPTION (provided by applicant): Staphylococcus aureus is a leading cause of infection worldwide and a major driver of antibiotic resistance. Although many experimental staphylococcal vaccines have been reported, all vaccines tested to date in human trials have failed for unclear reasons. Unlike mice, humans are exposed to S. aureus beginning early in life, leading to generation of antibodies to S. aureus antigens. In preliminary experiments, we have shown that select human anti-S. aureus antibodies are protective, but many are not. In mice exposed to S. aureus, vaccination against protective antigens leads to immunity against S. aureus whereas vaccination against non-protective antigens induced recall of non-protective immunity which further interferes with protective antibodies by direct competition. Based on these findings, we generated a model of how pre-existing antibodies shape vaccine responses and how this predicts novel ways to develop effective vaccines against S. aureus. To query the validity of our model, in Aim 1, we will recruit children and old adults with invasive S. aureus infections, survey the anti-S. aureus antibody profile and define functionally protective antibody responses to S. aureus antigens. In Aim 2, we will identify and characterize protective and non-protective anti-S. aureus antibodies from candidate samples acquired in Aim 1, and assess structural and functional features of the specific antibodies that confer protection or non-protection. In Aim 3, we will study these antibodies and their target in the context of naïve mice and mice previously exposed to S. aureus. We will evaluate mechanisms whereby non-protective memory shapes vaccine efficacy and test strategies that circumvent interference. Overall, using the novel model systems, we aim to develop a more predictive framework for explaining staphylococcal vaccine failures and developing novel strategies for effective vaccination against S. aureus.

PUBLIC HEALTH RELEVANCE: Based on preliminary studies of S. aureus vaccine failures, we have generated a model of how pre- existing anti-staphylococcal humoral response shapes protective and non-protective immune responses to S. aureus. We will leverage new in vivo model systems to interrogate our hypothesis using protective and non-protective antibodies isolated from children and elderly subjects with invasive S. aureus disease.

CRITIQUE 1:

Significance: [redacted]
Investigator(s): [redacted]
Innovation: [redacted]

Approach: [redacted]
Environment: [redacted]

Overall Impact: The application by Dr. Liu and Dr. Creech is focused on characterizing the human antibody response to S. aureus infection with a goal of identifying antigens that result in protective antibody generation. To do this the PIs have developed a mouse model with human serum or antibody transfer to test the concept that pre-existing immunity can impact vaccine induced protective antibody. The PIs will screen human sera from infected patients for antibody repertoire and test the highest hits for protection in mice. They will then characterize top protective versus non-protective antibody hits for Ig class, opsonization, neutralization, glycosylation and perform BCR Seq for antigen mapping. Finally they will test protective antibodies in their pre-immune model to look for interference. They will also test subdominant antigens for the ability to escape interference. The application is conceptually important and could reveal better antigen targets for vaccine development. It also could explain the mechanisms by which pre-existing immunity impacts current vaccines that have worked in naïve mice, but not humans. There are many strengths focused on study impact and a clear approach that heavily outweigh minor weaknesses. Enthusiasm for the application is very high.

1. Significance:

Strengths

- There have been no successful S. aureus vaccines that have shown efficacy in clinical trials. This application advances a theory of pre-existing immunity against S. aureus as a cause of vaccine failure in humans. The goal is to identify mechanisms of interference and antigens that may not be impacted for vaccine development.
- The study is rigorously supported by two recent publications in Cell Host Microbe and JID.
- This study raises important issues with studying vaccines in naïve mice as a model for humans with lifelong exposure to certain pathogens.

Weaknesses

- A limitation is that the human sera tested are only shown to provide protection at 6 weeks after infection and this wanes completely by 6 months. This could limit future vaccine efficacy significantly. The PIs acknowledge this limitation.

2. Investigator(s):

Strengths

- Dr. Liu is an expert in S. aureus research and vaccine studies. Dr. Creech is an expert in clinical S. aureus infection. The MPI plan is acceptable.
- Dr. Slaughter provides biostatistical support.

Weaknesses

- Minor that Dr. Liu and Dr. Creech have not collaborated yet, but Dr. Thomsen will consult to establish the work.

3. Innovation:

Strengths

- Dr. Liu's group developed the human serum transfer model in mice to test pre-exisiting immunity.
- There is conceptual innovation in studying pre-existing antibody interference as a mechanism of S. aureus vaccine failure.
- Targeting subdominant S. aureus antigens for vaccine design is of interest.

Weaknesses

- The PI has published two papers in this model to date, slightly undercutting novelty.

4. Approach:

Strengths

- Overall study design is rigorous with adequate power, replication, and statistical testing.
- Preliminary and published data are strongly supportive of the application.
- Aim design is clear and interpretable. The PI has taken care to have alternative antigen target plans to avoid previous Aim dependence.
- Alternative studies of the role of IL-10 on antibody glycosylation are intriguing.
- Aim 3.3 has the potential to be highly impactful if antigen targets are identified that escape interference.

Weaknesses

- The source of healthy control or non-invasive S. aureus infected patient samples is not discussed.
- Minor grantsmanship issues with Figure layout and discussion not being linear.
- Minor the depth of immunology studies regarding memory recall of B cells is limited.

5. Environment:

Strengths

- The research environments at UCSD and Vanderbilt are excellent.
- All relevant cores are present.

Weaknesses

- Physical location of the two teams could produce some issues.

Protections for Human Subjects:

Acceptable Risks and/or Adequate Protections

- They will recruit 75 kids and 25 older adults with invasive S. aureus infections and sampel up to 20ml of blood at 3-4 time points post infection.

Inclusion Plans:

- Sex/Gender: Distribution justified scientifically.
- Race/Ethnicity: Distribution justified scientifically.
- Inclusion/Exclusion Based on Age: Distribution justified scientifically.
- They will sample kids for serum likely to have protective antibody and adults to examine lifelong S. aureus exposure and the impact on antibody response.

Vertebrate Animals:

NO, animal welfare concerns or incomplete

- The application lacks adequate discussion of how pain and distress will be monitored and criteria for euthanasia.

Biohazards:

Acceptable

- S. aureus and human blood are BSL2.

Resource Sharing Plans:

Acceptable

Authentication of Key Biological and/or Chemical Resources:

Acceptable

Budget and Period of Support:

Recommend as Requested

CRITIQUE 2:

Significance: [redacted]
Investigator(s): [redacted]
Innovation: [redacted]
Approach: [redacted]
Environment: [redacted]

Overall Impact: This new R01 proposal from established investigator George Liu seeks to understand why so many *Staphylococcus aureus* vaccines have failed and to identify new vaccine candidates. The focus of the project is to understand the features of a functionally protective SA antibody response and to use this to both guide vaccine design and to understand vaccine failure. This project is based on solid preliminary data demonstrating that previous infection with SA, and the antibodies that accompany that infection, interferes with vaccines that otherwise prevent infection in a naïve host. It addresses a highly significant topic, as SA is an important pathogen and there is an urgent need for an effective vaccine. The proposal is conceptually innovative on its focus on subdominant antigens as vaccine candidates and the mechanisms behind antibody interference of vaccine-induced responses, while it is

technically innovative in its animal model for testing protection in serum from naturally-acquired infections. The investigators have the necessary expertise and excellent environments in which to accomplish the stated goals of the proposal, and it is extremely clear and well-written proposal that thoroughly considers potential pitfalls and the many potential outcomes of each experiment. Overall, this proposal is likely to be successful and have a high impact.

1. Significance:

Strengths

- SA is the most common invasive bacterial pathogen in children in the US and thus important for public health.
- SA vaccines have an exceptionally high vaccine trial failure rate; thus, understanding the mechanisms of failure would be an important step forward towards developing a vaccine.
- Rigorous prior studies demonstrated that previous infection with SA, and the antibodies that accompany that infection, interferes with vaccines that otherwise prevent infection in a naïve host, providing a strong rationale for the project.

Weaknesses

- None noted

2. Investigator(s):

Strengths

- The study team has a strong record in this field and is well-suited to carry out the proposed work.

Weaknesses

- None noted

3. Innovation:

Strengths

- Using pre-infected mouse model as a readout for protection is a novel solution to a previously difficult problem.
- Hypothesis that OAS and antibody competition are the cause of vaccine failure is conceptually novel.

Weaknesses

- None noted

4. Approach:

Strengths

- Hypotheses are based on strong published and unpublished preliminary data.

- Aims are not interdependent, but are complementary such that the results of each aim can inform strategies and experimental design in other aims.
- Potential pitfalls and alternative approaches are well considered and discussed throughout all aims.
- Experimental logistics are well thought out (for example, the thorough planning of serum volumes needed for each experiment), an extremely important consideration when working with precious patient samples.
- There is a good discussion of statistics and relevant biological variables.

Weaknesses

- Minor - There is an assumption that there will be antigens predictive of protection in the pediatric cohort; the rationale for this assumption (versus the possibility that responses might be very variable among patients) is not totally clear.
- Minor Discussion of how age was expected to affect antibody responses, or how age would be incorporated into the analysis was somewhat limited, given the focus on this variable in the study design. Only pediatric sera are being used to generate the list of candidate antigens, even though protection in a non-naïve situation (older adults) might be more informative, considering their preliminary data.

5. Environment:

Strengths

- Vanderbilt and UCSD have all the necessary facilities to accomplish the proposed study.

Weaknesses

- None noted

Protections for Human Subjects:

Acceptable Risks and/or Adequate Protections

Inclusion Plans:

- Sex/Gender: Distribution justified scientifically.
- Race/Ethnicity: Distribution justified scientifically
- Inclusion/Exclusion Based on Age: Distribution justified scientifically.

Vertebrate Animals:

YES, all criteria addressed.

Biohazards:

Acceptable

Resource Sharing Plans:

Acceptable

Authentication of Key Biological and/or Chemical Resources:

Acceptable

Budget and Period of Support:

Recommend as Requested

CRITIQUE 3:

Significance: [redacted]
Investigator(s): [redacted]
Innovation: [redacted]
Approach: [redacted]
Environment: [redacted]

Overall Impact: This is a very well written proposal from a seasoned veteran and colleague of the *Staphylococcus aureus* (SA) and methicillin-resistant SA (MRSA) fields. The rigor of prior research with respect to both significance and approach is presented with inclusion of publications as well as a plethora of preliminary results. Scientific rigor is addressed with presentation of statistical analyses as well as defined cut-offs and the like throughout the aims. Sex as a biological variable is addressed with the use of female and male human samples and animals. This project deals with addressing the "original sin" of SA/MRSA. They have found with, at least IsdB, that a portion of the protein, while eliciting protection in naïve mice, causes immune evasion in pre-exposed mice. They intend to address this throughout the project as well as address this potential in other antigens. Anticipated results, pitfalls and alternative approaches are given throughout. This is an exciting project with a high potential of success. The potential impact of this project is high.

1. Significance:

Strengths

- SA and MRSA remains a highly significant public health issue.
- The rigor or prior research is strong with a number of publications noted as well as a plethora of preliminary results presented. Additionally, each aim is supported by these results.

Weaknesses

- No weaknesses noted.

2. Investigator(s):

Strengths

- The MPI plan is well laid out.

- Each PI is quite capable of completing this project as they are experts in their field with significant experience.

Weaknesses

- No weaknesses noted.

3. Innovation:

Strengths

- The innovation of this proposal lies in the combination of human samples and mouse samples combined to forward the cause of a vaccine that avoids the immune imprinting that is plaquing the SA field.

Weaknesses

- No weaknesses noted.

4. Approach:

Strengths

- The rigor of prior research is considered with preliminary results presented for each aim.
- Scientific rigor is addressed with by listing the statistical analysis that will be performed, by defining cut-off values throughout the proposal as well as an analysis plan for aim 1.
- Sex as a biological variable is addressed by the use of males and females.
- The project itself is very well laid out with experimental plans defined and anticipated results, pitfalls and alternative strategies listed. This was especially important to define on aims 2 and 3 with the inclusion of many antigens that have the potential to be protective antigens.
- Addressing the “original sin” of SA is an important feature of this project.

Weaknesses

- No weaknesses noted.

5. Environment:

Strengths

- UCSD and Vanderbilt facilities are sufficient to conduct this work.

Weaknesses

- No weaknesses noted.

Protections for Human Subjects:

Acceptable Risks and/or Adequate Protections

- All topics addressed.

Inclusion Plans:

- Sex/Gender: Distribution justified scientifically.
- Race/Ethnicity: Distribution justified scientifically.
- Inclusion/Exclusion Based on Age: Distribution justified scientifically.
- All topics addressed.

Vertebrate Animals:

YES, all criteria addressed

- All points are adequately addressed.

Biohazards:

Acceptable

- Facilities are defined for each location.

Resource Sharing Plans:

Acceptable

Authentication of Key Biological and/or Chemical Resources:

Acceptable

Budget and Period of Support:

Recommend as Requested

THE FOLLOWING SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE, OR REVIEWERS' WRITTEN CRITIQUES, ON THE FOLLOWING ISSUES:

PROTECTION OF HUMAN SUBJECTS: ACCEPTABLE

INCLUSION OF WOMEN PLAN: ACCEPTABLE

INCLUSION OF MINORITIES PLAN: ACCEPTABLE

INCLUSION ACROSS THE LIFESPAN: ACCEPTABLE

VERTEBRATE ANIMALS: ACCEPTABLE

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

Footnotes for 1R01AI181321-01; PI Name: Liu, George Y

NIH has modified its policy regarding the receipt of resubmissions (amended applications).See Guide Notice NOT-OD-18-197 at https://grants.nih.gov/grants/guide/notice-files/NOT-OD-18-197.html. The impact/priority score is calculated after discussion of an application by averaging the overall scores (1-9) given by all voting reviewers on the committee and multiplying by 10. The criterion scores are submitted prior to the meeting by the individual reviewers assigned to an application, and are not discussed specifically at the review meeting or calculated into the overall impact score. Some applications also receive a percentile ranking. For details on the review process, see http://grants.nih.gov/grants/peer_review_process.htm#scoring.