

AWARD NUMBER: CDMRPL-20-0-PR191209P1

TITLE: Contribution of the Human Gut Microbiome to the Development and Severity of Guillain-Barré Syndrome

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14. ABSTRACT Campylobacter jejuni is a prolific gastrointestinal pathogen, accounting for a significant proportion of bacterial diarrheal disease and dysentery worldwide, and particularly afflicts military travelers leading to substantial duty days lost as well as a myriad of chronic health consequences. It is also estimated that C. jejuni infection in 1 of 1000 cases subsequently results in neurological sequelae and paralysis, referred to as Guillain-Barré Syndrome (GBS). Development of GBS is due to an aberrant autoimmune response directed against GM1 ganglioside structures located on host nerve cells. A significant proportion of GBS cases are attributed to antecedent infection by C. jejuni due to its well-established mimicry of ganglioside structures by cell surface lipooligosaccharides (LOS). Our preliminary research has identified other bacteria in the chicken gut microbiota that also mimic GM1-gangliosides. These organisms may play an important role in GBS development, causing immune-mediated tolerance or training toward the ganglioside antigen. Studies are required to establish the prevalence of these bacteria in the human gut and whether gut microbial signatures exist that are associated with GBS development. This project aims to determine whether GM1-expressing bacteria exist in human stools. If so, they will be identified, and we will determine if they are more common among people in Bangladesh and US military personnel that developed GBS than in family members or study participants that were previously afflicted with C. jejuni-induced diarrhea only.										
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1. Introduction

This is a case-control study to examine the contribution of the human gut microbiota and carbohydrate-mediated immune responses to the development and severity of GBS. The study falls under an overarching project that also includes a case-control study of adults in Bangladesh as well as a GBS mouse model system that will allow us to examine the immune system cell types and molecular mechanisms responsible for the pathology of GBS and provide an opportunity to develop precision medicine prevention and treatment approaches for GBS at-risk populations.

2. Keywords

Gut Microbiome, Guillain-Barré Syndrome, GBS

3. Accomplishments:

What were the major goals of the project?

The primary objective of the project is to examine the contribution of the human gut microbiota and carbohydrate-mediated immune responses to the development and severity of GBS. Specific Aim 1 is to 'Establish U.S. and Bangladesh case-control studies. Collect human sera and fecal samples from former GBS patients as well as control populations located in Bangladesh and within the U.S. military, both populations where GBS incidence is higher than average.' This report covers the work for the U.S. military portion of the project.

What was accomplished under these goals?

The aim is to estimate the incidence of GBS in the military population and also to describe the clinical outcomes of GBS. The task of collecting the human sera and the fecal samples from GBS patients and the control populations within the U.S military to estimate the cost burden. Over the past year, reviews from the Scientific Review Board were received. The aim for the investigative team is to work towards addressing the Board's feedback and incorporate any necessary changes into the current protocol. At the time of this report, the task for the team is to submit the response back to the Board for further review and approval. The team has worked with collaborators at the University of Georgia, Michigan State University, and the

Naval Medical Research Center to finalize the specimen collection and laboratory testing plan.

Resubmission to the SRB will occur in October 2022. The regulatory documents required for IRB submission have been developed.

DoD cohort case-control study (data and specimen collection)	Achieved %	Months
Protocol development and local IRB approval (also DHA data sharing agreement)	50%	2-3
Milestone Achieved: HRPO Approval		
Contact GBS patients (N = 100) and control (N= 200) groups	0%	12-18
Retrieve blood from DoD Serum Repository (GBS cases) and from separate IRB-approved diarrhea case-cohort study (controls) and collect fecal samples (both cases and controls)	0%	16-24
Milestone(s) Achieved: Enrollment met target group numbers	0%	
Analysis of subject microbiota		
Extraction of gDNA and 16S rRNA analysis	0%	22-28
Extraction of GM1 bacteria from fecal samples	0%	22-27
α GM1 antibody/Protein G pull-down experiments	0%	23-28
Isolation/characterization of GM1 isolate glycan structures by MS/NMR	0%	27-36
Case-control analysis addressing gut microbiome association with GBS	0%	28-36
Milestone(s) Achieved: Hypothesis #2 tested (microbes exist within the gut of GBS patients that increases their susceptibility to this autoimmune disease)	0%	
Milestone(s) Achieved: Determine relevant patterns and which samples to pool for mouse fecal transplant experiments	0%	
Development of GBS mouse model		
Establish GBS model with DoD samples	0%	24-36
Milestone(s) Achieved: Establishment of fecal transplant models	0%	
Analysis of serum and whole blood samples		Months
Screen sera from each human cohort on ganglioside glycan array	0%	26-34
Case-control analysis addressing pre-GBS circulating anti-ganglioside antibodies association with GBS	0%	34-36
Milestone(s) Achieved: Hypothesis #1 tested (GBS patients have baseline levels of anti-ganglioside responses that are subsequently stimulated by <i>C. jejuni</i> infection to induce an autoimmune neuropathy)	0%	
Screen sera from mouse experiments	0%	24-36
Determine proportions of B-cell populations by FACS sorting	0%	
Stimulation of B-cell and cytokine measurement	0%	
Milestone(s) Achieved: Determine relevant patterns and which samples to pool for mouse fecal transplant experiments	0%	

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

Nothing to report.

How were the results disseminated to communities of interest?

Nothing to report.

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

Over the next quarter, we plan to receive approval from the Scientific Review Board, submit to the USU IRB, and begin creation of the data management system.

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

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

Protocol development and was delayed due to shifting priorities in response to the continuing SARS-CoV2 pandemic. Quarterly reports for 2022 have not been submitted due to shifting priorities in response to the COVID pandemic and delays in protocol development. The funds have been received by The Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc. The team is exploring other means of stool sample collection that are less cumbersome for participants, but still capture enough stool required for laboratory testing. IDCRP is working with our Michigan State University collaborators to identify potential stool specimen collection kits.

6. Products

Nothing to report.

7. Participants & Other Collaborating Organizations

What individuals have worked on the project?

Name:	David Tribble, M.D, DrPH	IDCRP Team
Project Role:	Principal Investigator	Study coordination
Research Identifier (e.g. ORCID ID):	N/A	N/A
Nearest person month worked:	2	3
Funding Support:	USU	HJF via CA with USU

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

Nothing to report.

What other organizations were involved as partners?

- a. University of Georgia
 - i. Organization Name: University of Georgia
 - ii. Location of Organization: Athens, Georgia, USA
 - iii. Partner's Contribution to the Project: Collaboration
- b. Michigan State University
 - i. Organization Name: Michigan State University
 - ii. Location of Organization: East Lansing, Michigan, USA
 - iii. Partner's Contribution to the Project: Collaboration
- c. Naval Medical Research Center
 - i. Organization Name: Naval Medical Research Center
 - ii. Location of Organization: Silver Spring, Maryland, USA
 - iii. Partner's Contribution to the Project: Collaboration

8. Special Reporting Requirements

None.

9. Appendices

None.