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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 the contribution of the human gut microbiota and carbohydrate-mediated immune responses to the development and severity of GBS. To determine the epidemiology and clinical outcomes of Guillain-Barré Syndrome (GBS) and its relationships with gastroenteritis in the military population.					
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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. This is a retrospective case-control study including all active-duty service members (ADSM) and Activated Guard/Reserve members meeting the following case and control definitions between 2005-2022 to examine epidemiological trends of GBS and the contribution of the human gut microbiota and carbohydrate-mediated immune responses to the development and severity of GBS. A total of 300 GBS cases will be analyzed in this study along with 600 controls (1:2 case control ratio). The control group will comprise of 300 subjects with documented *C. jejuni* but with no evidence of GBS following infection and 300 subjects with no documented evidence of *C. jejuni* infection and no medical history of GBS. To estimate incidence of GBS in the military population

The study will involve a HIPAA and a consent waiver that will allow study personnel to identify individuals for potential inclusion in the study and prioritize individuals who have samples available in the DoDSR. The HIPAA and the consent waiver will allow for creation of a PHI Limited Data Set (LDS) database of GBS cases to be used to describe the illness characteristics and impact within the military.

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. The specific aims are to examine the contribution of the human gut microbiota and carbohydrate-mediated immune responses to the development and severity of GBS. Also to determine the epidemiology and clinical outcomes of Guillain-Barré Syndrome (GBS) and its relationships with gastroenteritis in the military population.

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. 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 the significant changes into the current protocol. It was submitted back to the Scientific Review Board and obtained approval. The current protocol has been submitted to eIRB for approval.

The Team has worked with Kennell and Associates, Inc (Kennell) who are being subcontracted by IDCRP to provide data/analytical support for this study. After IRB/DSA approval, Kennell will apply to access the MDR/DaVINCI for this project. They will query the MDR for the GBS cohort and coordinate with Walter Reed and DOD Serum repository for additional data to be sent to/from the MDR/DaVINCI. They will also create PHI LDS file (encrypting Person and Medical IDs) before sending to the PI/study team at IDCRP.

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		
Retrieve blood from DoD Serum Repository (GBS cases) and from separate IRB-approved diarrhea case-cohort study (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%	
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 EIRB, submit the DSAA, 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 was delayed due to shifting priorities in response to the continuing SARS-CoV2 pandemic. Quarterly reports were submitted to update the reporting and was delayed 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. Currently in the process of submitting the NCE.

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

8. Special Reporting Requirements

None.

9. Appendices

None.