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TITLE: Suppression of GWVI Toxin-Activated Microglia and Pathologies by DREADD

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Approximately one third of Veterans who served in the Gulf War later developed a chronic multi-symptom illness known as Gulf War Illness (GWI). While the exact cause is unknown, it is believed that persistent exposure to environmental toxins such as pesticides and chemical warfare agents may have interacted with combat-related stress to produce lasting neurological and psychiatric complications among this Veteran population. Neuroinflammation has been increasingly linked with psychiatric and neurological disorders and may play a role in GWI pathology. Microglia are a key mediator of neuroinflammation and the underlying goal of this project is to test the hypothesis that microglial activation acts as a causal factor to produce cognitive and psychiatric disturbances in a mouse model of GWI. In particular, this project will utilized novel Designer Receptors Exclusively Activated by Designer Drugs (DREADD) technology to inactivate microglia in our mouse model of GWI.

15. SUBJECT TERMS: NONE LISTED

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1. INTRODUCTION

Gulf War Illness represents The overall goal of this DOD funded research study is to develop a novel mouse model of Gulf War Illness (GWI) and test the hypothesis that microglia are a key mediator of GWI neuropathology. In pursuit of these goals, we are validating a Cx3Cr1-dependent designer receptor exclusively activated by a designer drug (DREADD) for the suppression of microglia in mice. In particular, we will use this mouse to test the hypothesis that microglial activation mediates neuroinflammation and behavioral abnormalities after exposure to permethrin and stress.

2. KEYWORDS

Gulf War Illness, microglia, permethrin, stress, pyrethroid, DREADD, learning and memory, depression, anxiety

3. ACCOMPLISHMENTS

a. Major Goals of the Project:

Finalize Breeding of DREADD Mice (Year 1)

-Generate a Breeding colony to produce sufficient numbers of DREADD mice necessary for all experiments.

Validate DREADD mediated microglial activation with LPS (Year 1)

-Use LPS administration to induce microglial activation

-Use inactivate microglia via administration of clozapine-n-oxide to validate technique.

Brain/Plasma Cytokine and FACS of Neuroimmune Cells (Year 1.5-2)

-Collect brains and plamsa from mice exposed to permethrin and stress to analyze immune cell populations via FACs

Immunohistochemistry of microglia (Year 2)

-Quantify microglial activation in mouse model of GWI and suppression.

Peripheral Leukocye Immunophenotyping (Year 2)

-Determine if microglial suppression in DREADD mice affects peripheral immune cell phenotypes.

Animal Behavior Assessments (cognitive) (Year 1)

-Determine if microglial suppression in DREADD mice prevents spatial memory impairments in GWI mouse model.

Animal Behavior Assesments (psychiatric) (Year 2)

-Determine if microglial suppression in DREADD mice prevents spatial memory impairments in GWI mouse model.

b. Accomplishments Under These goals

We proposed to expose mice to Gulf War toxins such as permethrin, and to test the hypothesis that behavioral impairments caused by permethrin were mediated by microglial activation. In pursuit of these goals we have successfully generated a

colony of Cx3Cr1-dependent DREADD mice for suppression of microglia. Additionally, we validated a novel mouse model of GWI by treating mice with 200mg/kg permethrin every day for 14 days followed by 7 days of unpredictable mild stress. Behavioral analysis of the treated mice showed a significant increase in depression like behavior as measured via forced swim test (fig.1). We also analyzed anxiety like behavior using the open field test, and found no significant difference between treatment groups (fig.1). We also collected fixed brains from our GWI mice and performed immunohistochemistry to detect changes in Iba1 and CD68 to measure changes in microglial activation. We are currently in the process of performing sholl analysis to quantify microglial activation on the images generated from these brains. Additionally, we are currently in the process of performing fluorescence activated cell sorting (FACS) using blood and brain tissue from GWI mice. Specifically, we are measuring changes in CD45, CD11b, TLR4, CD14, RAGE, CD86, MHC-II, or CD3, Ly6C, CD19.

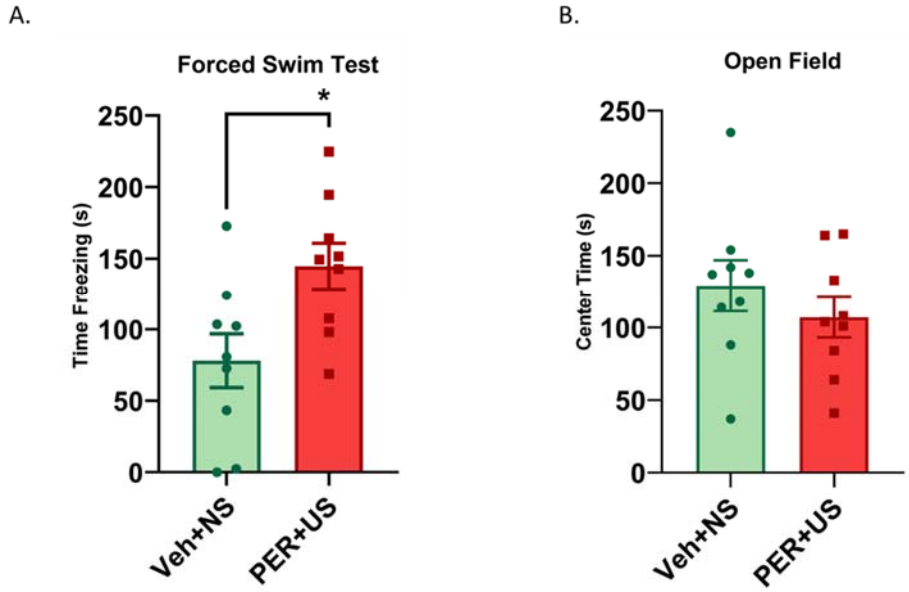

Figure 1. Behavioral changes in mouse model of GWI. Mice were exposed to permethrin (200mg/kg, i.p) for 14 days followed by 7 days of unpredictable stress. A) Mice displayed a significant increase in depressive behavior (time spent freezing) during forced swim test. B) No significant differences were detected in anxiety-like behavior as measured by time spent in the center during open field test.

c. Opportunities for Professional Development

Dr. Sean Naughton is a postdoctoral fellow working on the aforementioned research. Dr. Naughton has undergone professional development activities as a result of this work, by presenting the research at the Gulf War Illness State of the Science Virtual Conference 2020.

d. Dissemination of Information to Communities of Interest

Preliminary results were presented at the Gulf War Illness State of the Science Virtual Conference 2020.

The following poster was presented:

Naughton SX, Westfall S, Pasinetti GM "Immune Priming as A Driver of Gulf War Illness Pathology"
Abstract/Poster No. VA-28, Gulf War Illness State of the Science, Virtual Conference August 18-19, 2020

e. Plan for next reporting period

As we move into the next reporting period we plan to examine changes in the proportion of peripheral plasma T helper cells, then determine if suppression of microglia prevents such changes. Specifically, we will collect blood from GWI mice and isolate plasma to characterize immunophenotypes of blood lymphocyte cells using multiparametric FACS. This will allow for characterization of T effector cells and T helper populations in the plasma, respectively. Additionally, we will continue to perform behavioral and immunohistochemical analysis of additional cohorts of animals.

4. Impact

a. What was the impact on the development of the principal discipline(s) of the project?

Nothing to Report

b. What was the impact on other disciplines?

Nothing to Report

c. What was the impact on technology transfer?

Nothing to Report

d. What was the impact on society beyond science and technology?

Nothing to Report

5. CHANGES/PROBLEMS

a. Changes in approach and reasons for change

We initially planned to conduct mouse behavioral studies to assess spatial learning and memory during the first year and anxiety and depressive behaviors during the second year. We instead conducted anxiety and depressive behaviors during the first year and plan to measure spatial memory during the second year. This was because the behavioral tasks for depression and anxiety (forced swim and open field) were easier to perform while adhering to social distancing requirements during the COVID-19 pandemic.

b. Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to Report

c. Changes that had a significant impact on expenditures

Nothing to Report

Significant changes in use or care of human subjects
Nothing to Report

d. **Significant changes in use or care of vertebrate animals.**
Nothing to Report

e. **Significant changes in use of biohazards and/or select agents**
Nothing to Report

6. **PRODUCTS**
   a. **Journal Publications**
   Nothing to Report

   b. **Books or other non-periodical, one-time publications**
   Nothing to Report

   c. **Other publications, conference papers, and presentations**
   Naughton SX, Westfall S, Pasinetti GM "Immune Priming as a Driver of Gulf War Illness Pathology"Abstract/Poster No. VA-28, Gulf War Illness State of the Science, Virtual Conference August 18-19, 2020

   d. **Website(s) or other Internet site(s)**
   A recording for a virtual poster presentation based on this work can be found through the online conference proceedings available at : https://www.cvent.com/d/37q773 .

   e. **Technologies or techniques**
   Nothing to report

   f. **Inventions, patent applications, and/or licenses**
   Nothing to report
   .

   g. **Other Products**
   Nothing to Report

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**
   a. **Individuals who have worked on the project**
      i. **Dr. Sean X Naughton, PhD**
      Role: Post-Doctoral Fellow
      Contribution: Responsible for overall design of the project and execution of experiments.
      Funding Support: 10%

      ii. **Kyle Trageser, BS**
      Role: Research Assistant
      Contribution: Assisting in the execution of experiments.
      Funding Support: 5%

iii. **Dr. Chad Smith, PhD**
Role: Post-Doctoral Fellow
Contribution: Responsible for breeding and validating DREADD mice
Funding Support: 5%

iv. **Dr. Giulio Pasinetti, MD PhD**
Role: Principle Investigator
Contribution: Overall Experimental Design.
Funding Support: 15

b. **Changes in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period**
Nothing to report

c. **Other Organizations involved as partners**
Nothing to Report

8. **Special Reporting Requirements**
Nothing to Report

9. **Appendices**
Nothing to Report