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TITLE: Identifying Immune Drivers of Gulf War Illness Using a Novel Daily Sampling Approach

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14. ABSTRACT Analyses were run on serum samples provided from 22 individuals who provided blood draws over 25 consecutive days. We examined 21 secreted serum cytokines and chemokines in this analysis. There are 8 individuals with GWI, 8 healthy veterans who served in the 1991 Persian Gulf War, and 6 non-veteran men with fibromyalgia (FM). These simple analyses were run on the entire group. We hypothesized that the GWI group would demonstrate greater variability of analytes than the healthy controls. We indeed found that GWI participants showed greater fluctuations (as measured by the coefficient of variation) of Eotaxin-1 and IL-1beta than did the healthy group. Interestingly, the fibromyalgia group ALSO showed elevated variability in those exact same two analytes when contrasted against the healthy controls. There were no differences between the GWI and FM groups. Those results suggest that GWI and FM may involve a common immunological dysregulation. We also hypothesized that GWI individuals would have elevated levels of proinflammatory markers. We see what previous reports have shown. IL-1beta, in addition to having greater variability in GWI and FM, is also higher than in healthy controls. Again, we see that there are no differences between the GWI and FM groups. Based on the serum cytokines results so far, GWI and FM are indistinguishable from each other. Third, we hypothesized that proinflammatory markers would predict day-to-day changes in symptom severity. We found that IL-1beta, MMP3, MMP9, MCP1, MIP1B, IL12p40, IL18, and IL23 changes are associated with symptom changes in GWI (some are anti-correlated). The same list also predicted symptoms in fibromyalgia individuals, with the exception of MCP1 and IL23. The results of our preliminary analyses suggest that men with GWI look very similar to men with FM in terms of cytokine levels, day-to-day variability, and relationship between cytokines and symptom fluctuations. The two disorders may share the same pathophysiological mechanism.		

15. SUBJECT TERMS Gulf War Illness, cytokines, microglia, daily, immune, phlebotomy, fibromyalgia					
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1. INTRODUCTION:

The major aim of this research project is to identify aspects of the immune system that are dysregulated in veterans with Gulf War Illness. A second aim is to determine whether identified immune system dysregulations are similar to those found in men with fibromyalgia. To accomplish those aims, we are recruiting 40 male veterans diagnosed with Gulf War Illness, as well as 10 healthy veteran controls, and 10 males with fibromyalgia. Participants will complete 25 consecutive days of blood draws and provide daily reports of symptom severity. Analyses will then be conducted to identify immune system factors that correlate with day-to-day symptom fluctuations in the participants. Ultimately, this information may be used to develop new treatments that specifically target the pathophysiological mechanisms of Gulf War Illness.

2. KEYWORDS:

Gulf War Illness, cytokines, microglia, daily, immune, phlebotomy, fibromyalgia

3. OVERALL PROJECT SUMMARY:

(Note: Listed accomplishments are based on the originally approved SOW that was active during the reporting period. We have submitted a revised SOW based on the move from Stanford University to UAB).

Current Objectives:

- Complete regulatory approvals for transferring grant funds to UAB
- Resume recruitment and data collection at UAB.
- Complete the protocol on 35 participants.

Results, Progress and Accomplishments

Task 1: Team review and progress meetings

50% Completed. Three more major meetings are planned in 2015.

Task 2: Submission of Documents for Regulatory Approvals

100% Completed.

Task 3: Start up Machine/Personnel

100% Completed

Task 4: Advertisement

50% Completed. Will launch new recruitment tools in January 2015 in Alabama.

Task 5: Screen GWI Participants for Study

40% Completed. Will resume recruitment in January 2015.

Task 6: Recruit control groups

40% Complete. Will resume recruitment in January 2015.

Task 7: Collection of blood samples and self-reported symptom data

40% Completed. Further progress is dependent on completing Tasks 5 and 6.

Task 8: Quantification of biochemicals in blood samples

40% Completed. Further progress is dependent on completing Task 7.

Task 9: Analyses

25% Completed. Further progress is dependent on completing Tasks 7 and 8.

Task 10: Preparation of final report and publications

0% Completed. This task is contingent on completion of Tasks 7, 8, and 9.

Key methodology:

We have used the methodology outlined in other submitted documents. As stated in the SOW, we have recruited participants from a variety of sources, including local advertisements and referrals from the local VA Hospital. We have performed the study protocol exactly as described in the SOW. Twenty-five individuals have completed the protocol.

Research conclusions:

Because we have not completed the data collection, we have performed only preliminary analyses. The results and conclusions from those analyses are reported in Section 4 (Key Research Accomplishments).

Actual or anticipated problems or delays:

We encountered a significant delay associated with moving the project from Stanford to UAB. Delays included the physical process of moving, identifying new collaborators and resources, transferring the IRB, and transferring the grant funding. We have completed almost all of the preparation, and should be able to resume data collection in January of 2015, pending the DoD approval of the transferred funds. We do not anticipate delays in the future.

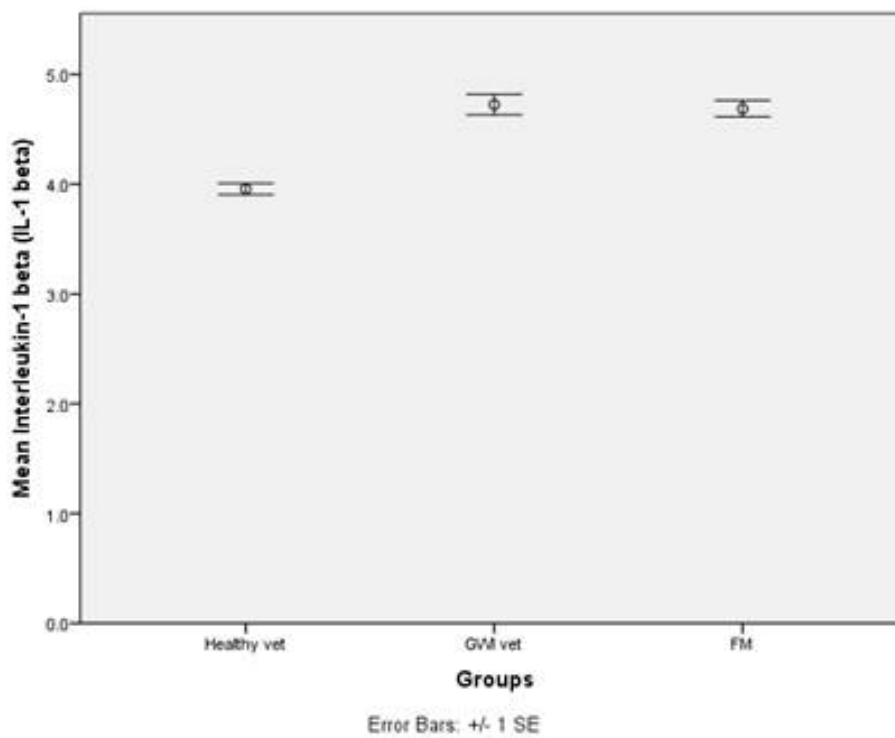
Changes to approach:

We are not proposing any changes to the approach. We have changed institutions from Stanford University to the University of Alabama at Birmingham (UAB), so some performance locations have changed. We will conduct the study protocol at UAB's clinical research unit (CRU). The CRU is identical to the CTRU that we used at Stanford, and has all the same expertise and equipment. We will maintain the same recruitment methods. However, we will utilize a difference VA (Birmingham VA Medical Center) to recruit participants.

4. KEY RESEARCH ACCOMPLISHMENTS:

(Note: results are from interim analyses and are preliminary)

- GWI individuals show greater day-to-day fluctuations in Eotaxin-1 and IL-1beta than healthy individuals.
- Fibromyalgia individuals also show greater day-to-day fluctuations in Eotaxin-1 and IL-1beta than do healthy individuals.
- There is no difference in Exotaxin-1 or IL-1beta fluctuations between GWI and fibromyalgia men.
- Both GWI and fibromyalgia men show elevated serum levels of IL-1beta when contrasted with healthy controls.



- In GWI individuals, day-to-day fluctuations in symptom severity is associated with changes in IL-1beta, MMP3, MMP9, MCP1, MIP1B, IL12p40, IL18, and IL23.

- With the exception of MCP1 and IL23, the same cytokine/chemokines also predict day-to-day disease severity in men with fibromyalgia.

5. CONCLUSION:

We have only performed preliminary analyses at this time because the entire sample for each group has not yet been collected. Therefore, the conclusions are tentative at this time. When the complete sample has been collected, we will re-run all analyses for the final conclusions. However, we can tentatively say that we have identified very interested relationships between the immune system and GWI symptoms. First, GWI individuals show abnormally large day-to-day swings in inflammatory cytokines. These cytokines (Eotaxin-1 and IL-1beta) are closely associated with microglia activation and may therefore be a proxy measure of microglia activity. The data suggest that GWI symptoms are being driven by inflammatory factors. Also, the GWI group showed elevated levels of certain cytokines when contrasted with healthy controls. Interestingly, the immune profile of the GWI individuals and fibromyalgia individuals were indistinguishable. It is possible, then, that GWI and fibromyalgia share common pathophysiological mechanisms. The link between GWI and fibromyalgia would be exciting because it would mean advances and treatments in one group could be generalized to the other. There are several advances being made in the field of fibromyalgia that may now be applied to GWI. If these findings continue to be supported as we collect additional data, we will suggest a new view of GWI that involves focusing on novel inflammatory mechanisms. Our goal from this point is to finish our target recruitment numbers and perform the final analyses.

6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:

Nothing to report.

7. INVENTIONS, PATENTS AND LICENSES:

Nothing to report.

8. REPORTABLE OUTCOMES:

Nothing to report.

9. OTHER ACHIEVEMENTS:

Nothing to report.

10. REFERENCES:

No references.

11. APPENDICES:

No appendices.