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TITLE: **The Role of Inflammation in development of Alzheimer's disease following repetitive head trauma**

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CONTRACTING ORGANIZATION: Indiana University School of Medicine

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14. ABSTRACT

Traumatic brain injury (TBI) affects approximately 3.8 million people annually and costs the US more than $48 million. Furthermore, TBI has become an increasingly common feature of modern military conflicts. It has been estimated that in the Iraq and Afghanistan conflicts following the terrorist attacks of September 11, 2001, the rate of TBI in military populations has dramatically increased to upwards of 10-20% of those serving, with over 250,000 soldiers exposed to some form of TBI (Source; DoD). The long-term consequences of TBI are multifaceted and include increased risk for AD. To date, mechanisms linking TBI to AD remain unclear. One of the earliest hallmark features of TBI is neuroinflammation, which is defined as the brain's innate immune response. Post-injury neuroinflammation includes activation of brain resident microglia, infiltration of peripheral monocytes due to disruption of the blood-brain barrier, and high level production of pro- and anti-inflammatory molecules. Although this initial response is thought to promote repair following TBI, exaggerated or persistent neuroinflammation can be detrimental. For example, TBI can trigger progressive neurodegeneration, brain atrophy, neuronal loss, and axonal degeneration for months to years after the initial insult and these events are often associated with neuroinflammation. We hypothesize that the TBI-induced neuroinflammatory response is critical in mediating AD-related pathology and specific inflammatory proteins can be used as post-injury biomarkers.

15. SUBJECT TERMS
Nothing Listed

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1. Introduction

Little is currently known regarding the role of inflammation in the progression of traumatic brain injury induced neurodegenerative disorders. Alzheimer's Disease is a common outcome of patients who have experienced mild to moderate brain injury, as well as those who have suffered from repeated concussive injury. There is a persuasive body of evidence favoring a significant inflammatory component in AD. A large number of inflammatory cells including microglia, astrocytes, and infiltrating peripheral immune cells and inflammatory molecules are present at elevated levels in the AD brain. potential role of neuroinflammation in regulating AD pathologies as a result of brain trauma is supported by several observations. First, a recent set of experiments demonstrated that MAPT pathology temporally co-exists with gliosis following mild repetitive TBI in the hTau mouse model of MAPT pathology. Our preliminary studies supported by a previous DoD grant (W81XWH-14-1-0265) have confirmed that even a single TBI enhances accumulation of activated macrophages and phosphorylated MAPT in the hTau mouse model. Similar findings have been reported in wild-type mice after blast induced brain injury, as well as in a triple transgenic mouse model of AD following a single moderate TBI. Second, numerous reports have demonstrated activated microglia near the injury release several pro-inflammatory cytokines and chemokines and that these inflammatory components in turn can exacerbate MAPT pathologies. Third, post-injury neuronal accumulation of Aβ correlates with increased numbers of IL-1α expressing microglia. Finally, our preliminary studies (DoD grant W81XWH-14-1-0265) show that a single moderate TBI induces expression of key pro-inflammatory cytokines at acute (3 days post-injury, DPI) time points in non-transgenic mice. Together, these studies suggest that brain injury induced neuroinflammation could be an initiating factor in AD-related pathologies and provides substantial rationale for studies aiming to identify and characterize post-injury inflammatory biomarkers associated with TBI and AD. The current study proposes to characterize inflammatory states in peripheral blood, plasma, and CSF of active and retired MMA fighters and boxers. We predict that our observations will add critical knowledge to the fields of neuroinflammation and traumatic brain injury as well as identify potential biomarkers of disease and novel therapeutic targets.

2. Keywords

Alzheimer's Disease, Inflammation, Microglia, Monocytes, Neurodegeneration, TREM2, immunity, Traumatic Brain Injury

3. ACCOMPLISHMENTS

What were the major goals of the project?

1. Approvals: HRPO and IRB approval for all studies; month 1-6; 100% complete
2. Staff training: Optimized procedures for sample collection, transfer, and processing; month 3-12; 100% complete
3. Preliminary study to detect biomarkers: Inflammatory biomarkers confirmed in peripheral blood; month 12-15; 40% complete
4. Blood sample collection: Collect blood from separate groups of active or retired profession fighters and age-matched controls; month 15-33; 30% complete
5. Complete imaging studies: Complete magnetic resonance imaging (MRI) and positron emission tomography (PET) amyloid imaging in a subset of retired fighters; month 18-33; 50% complete
6. Collect and process cerebral spinal fluid (CSF): Collect and process CSF for expression of inflammatory biomarkers in a subset of retired fighters; month 18-33; 30% complete
7. Data analysis: Complete data analysis and begin manuscript preparation; month 30-36; 10% complete
8. Manuscript publication: Submit manuscript for publication; month 33-36; 0% complete

What was accomplished under these goals?

During this past year reporting period, we restructured the project personnel to include Drs. Shane Bemiller and Nipun Chopra. We completed the required regulatory elements in order to process and analyze samples at the IU School of Medicine. This included IRB exemption and HRPO approval. Drs. Lamb, Bemiller and Chopra have established a relationship with Ms. Heather Rodney, who is our point of contact within the Federal Interagency Traumatic Brain Injury Research informatics system (FITBIR). We have gained access to the database, created personal FITBIR accounts and created an active study.

Our primary focus this year continued to be collecting patient samples for flow cytometry and banking plasma until we have recruited our full patient number. We established a pipeline for the processing, shipping, and analysis of plasma, CSF, and blood samples from CCF to the IU School of Medicine and from CCF to UNLV, which took time and effort to organize. Drs. Bemiller and Kinney organized a formal meeting among the DOD collaborating PIs across UNLV, IU School of Medicine and CF Lou Ruvo Center for Brain Health. The meeting took place in Las Vegas, Nevada on February 5-7th. Data updates and plans for moving the research forward were developed. Dr. Kinney's lab at UNLV established a flow cytometry protocol to examine TREM2 levels on circulating leukocytes. Dr. Kinney's lab has been processing and running the flow cytometry on the day of collection. For the plasma assays to examine the inflammatory protein analysis, we have made a plan to run the analysis as large batches of samples towards the end of the sample collection period. This will limit the confounding variables of running samples over long periods of time. The inflammatory protein analysis requires characterization of the relative abundance of proteins in order to determine the dilution factor for each assay. We have four different assays to optimize and we have completed characterization of two of the assays. Figure 1 demonstrates our proficiency running a small subset of 10 plasma samples each from AD patients, Parkinson's disease patients, and healthy control individuals. We have not found any significant differences in our current pilot study, however inclusion of multiple groups in combination with low *n* has resulted in variability and prevented us from doing meaningful statistical analysis.

CSF samples are actively being collected from a subset of fighters at the CCF Lou Ruvo Center for Brain Health in Las Vegas. There are several challenges to acquire these samples due to the willingness of recruited subject to donate CSF.

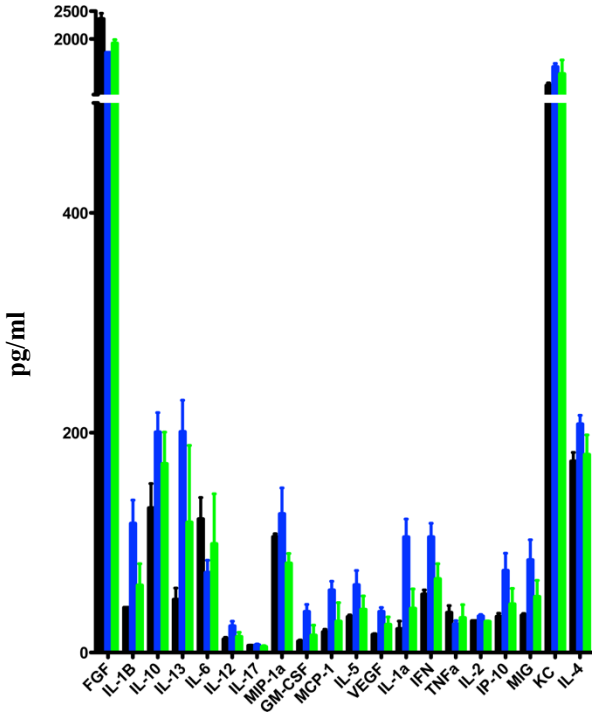

Figure 1. Analysis of 18 inflammatory chemokines and cytokines reveals trends towards increased IL1β and GM-CSF in plasma of AD patients compared to PD patients or healthy control groups. n=10 per group. **Black** – healthy controls; **Blue** – AD patients; **Green** – PD patients.

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

Funding from the current project has been utilized to enhance the professional development of two key members of the Lamb Lab, namely Drs. Shane Bemiller, and Nipun Chopra. Drs. Bemiller and Chopra were able to attend and present at the Society for Neurosciences Annual Meeting in Washington DC, November 11-15, 2017 where they presented posters.

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?

We have begun to receive large batches of samples from CCF Lou Ruvo Center for Brain Health. Samples are being aliquoted for their respective endpoints. Dr. Kinney and his group will continue their same day analysis of the leukocytes using flow cytometry, while the concurrent plasma samples will be processed and frozen until ready for a batch shipment. This way, we can correlate the inflammatory protein analysis with the leukocyte TREM2 analysis. We will complete characterization of the remaining two inflammatory protein analysis assays, so that we can commence running samples for project analysis.

4. Impact

Nothing to report.

5. Changes/Problems

Our new investigators have made significant advances in coordinating the study and communicating with FITBIR and the collaborating institutions. We have resolved the majority of the communication issues among institutions through the addition of key collaborators associated with related studies. Unfortunately, one setback to the study occurred in May. Dr. Shane Bemiller left IU School of Medicine to take a new position. We hope that the setbacks that accompany this change are minor. Dr. Chopra will take over Dr. Bemiller's responsibilities and assume the coordination of the study.

The CSF sample collection has been proceeding slowly. There are several challenges to acquire these samples due to the willingness of recruited subject to donate CSF.

Changes in approach and reasons for change:

In order to avoid confounds of collection of samples in real time, we have established a plan to bank plasma samples until we have recruited most of the patient participants. This way we can avoid batch effects.

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

With the departure of Dr. Bemiller, Dr. Chopra will assume the responsibility to coordinate logistics and to provide data and general project updates. He will coordinate the activity of the 3 participating institutions and continue to hold teleconferences with Dr. Kinney. There is also a plan to hire a faculty level individual to oversee the projects in Dr. Lamb's laboratory. This individual will assist Dr. Chopra.

Changes that had a significant impact on expenditures:

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents:

Nothing to report.

6. Products

Submitted manuscript: Kinney JW, Bemiller SM, Murtishaw AS, Leisgang AM, Salazar AM, Lamb BT. Inflammation as a Central Mechanism in Alzheimer's Disease.

7. Participants and collaborating organizations

What individuals have worked on this project?

Name:	Sarah Banks
Project Role:	PI
Nearest person month worked:	1
Contribution to Project:	Dr. Banks is working along with the Bernick Group to facilitate sample transfers and imaging.

Name:	Jefferson Kinney
Project Role:	PI
Nearest person month worked:	9
Contribution to Project:	Dr. Kinney and his group are isolating blood cells and performing analysis by flow cytometry.

Name:	Shane Bemiller
Project Role:	Postdoctoral Fellow
Nearest person month worked:	5
Contribution to Project:	Project manager coordinating efforts between IU School of Medicine and UNLV/CCF. Dr. Bemiller has completed all trainings required by the DOD and IU SOM.

Name:	Nipun Chopra
Project Role:	Postdoctoral Fellow
Nearest person month worked:	6
Contribution to Project:	Assisting with data collection and interpretation

Name:	Pamela Dino
Project Role:	Patient/Sample Coordinator
Nearest person month worked:	2
Contribution to Project:	Ms. Dino works with the Bernick Group to coordinate subject participation, data collection and sample distribution.

What other organizations were involved as partners?

Cleveland Clinic Lou Ruvo Center for Brain Health, Las Vegas, NV, USA

University of Nevada Las Vegas (UNLV)

8. Special Reporting Requirements

None

9. Appendices

None