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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.					
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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 A β correlates with increased numbers of IL-1 α 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?

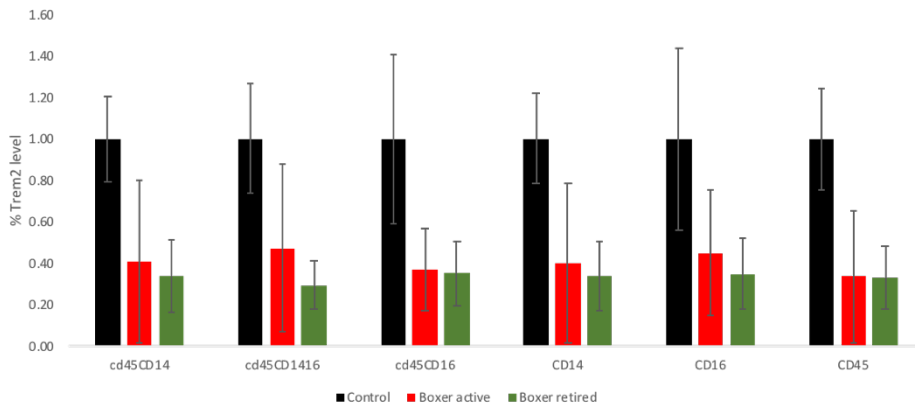
1. Approvals: HRPO and IRB approval for all studies; month 1-6; 100% complete
2. Staff training: Optimized procedures for sample collection, transfer, and processing; month 3-12; 100% complete
3. Preliminary study to detect biomarkers: Inflammatory biomarkers confirmed in peripheral blood; month 12-15; 100% complete
4. Blood sample collection: Collect blood from separate groups of active or retired profession fighters and age-matched controls; month 15-33; 100% 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; 30% complete
8. Manuscript publication: Submit manuscript for publication; month 33-36; 5% complete

What was accomplished under these goals?

During this past year reporting period, we finished collecting blood from separate groups of active or retired professional fighters and age-matched controls. For the inflammatory protein analysis, we collected 269 samples. All samples have been shipped to Indiana University School of Medicine where they have been aliquoted to perform 4 different assays. The plasma samples are currently being queried for proinflammatory (IFN γ , IL1 β , IL2, IL4, IL6, IL8, IL10, IL12p70, IL13, TNF α), cytokine (GM-CSF, IL1 α , IL5, IL7, IL12/IL23p40, IL15, IL16, IL17A, TNF β , VEGF-A), angiogenesis (FGF-basic, PIGF, Tie2, VEGFA, VEGFC, VEGFD, VEGFR1/Flt1), chemokine (IL8, CCL2, CCL13, CCL22, MIP1 β , CCL11), and vascular injury (CRP, ICAM1, SAA, VCAM1) biomarkers. We have completed optimization of each of these inflammatory protein analysis assays and have commenced querying the samples. Currently, we have completed about 20% of the samples. The organization of the samples and time to run the samples has taken considerably longer than anticipated. With optimization of the assay, we determined that an overnight incubation step was needed. This increased our run time by 100%.

Approximately, 85 of the plasma samples for the inflammatory protein analysis concurrently had flow cytometry analysis to determine TREM2 levels on circulating leukocytes. The flow cytometry analysis was performed by Dr. Kinney's group at UNLV. Their focus was on the boxer samples. They have determined that the proportion of TREM2-expressing monocytes is significantly reduced in both the active and retired boxers (**Fig. 1**). After the plasma samples have been queried and analyzed, comparisons will be made between the TREM2 levels and the matching inflammatory protein analysis.

Significant changes have occurred at the IU School of Medicine site. As reported in the last annual report, Dr. Bemiller, who helped set up technical procedures and organize communication between sites, left the University for another position in May 2018. Dr. Chopra took over management of the project. In November 2018, a faculty level researcher, Dr. Stephanie Bissel, was hired to oversee the Lamb lab projects. Unfortunately, Dr. Chopra left the lab on 01April 2019 in preparation for a new teaching position. Dr. Bissel has resumed the project management. On 02May 2019, Drs. Bissel, Kinney and Lamb held a video conference to present data updates and create a plan to resolve the setbacks that occurred due to researchers leaving the project. A plan was agreed upon to move the project forward for a timely completion and coordinate samples. Once the IU School of Medicine site has finished running their assays and analyzing the data, Drs. Bissel and Kinney will coordinate the comparisons and writing of the manuscript.



	MEAN						STANDARD ERROR					
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Control	1	1	1	1	1	1	0.20389	0.26405	0.40757	0.21849	0.43861	0.24537
Boxer active	0.406	0.4702	0.3697	0.4009	0.4495	0.3342	0.39417	0.40378	0.19718	0.38697	0.30435	0.31592
Boxer retired	0.3389	0.2948	0.3496	0.34	0.3478	0.3283	0.17251	0.11393	0.15157	0.16736	0.16983	0.1525

Figure 1. TREM2-expressing monocyte populations in active and retired boxers as proportion to control. Active and retired boxers show statistically significant less TREM2+ monocytes for all monocyte subsets.

Requests for additional items to be addressed for the review of protocol were received. Much effort was invested to satisfy these requests. IRB exemptions for IU and the UNLV sites were issued in late 2018. Subsequently, all requirements for the IU, UNLV, and CCF/Luo Ruvo site were met and HRPOs issued. The Cleveland Clinic site is still in the review process. Dr. Bissel has been in contact with Ms. Heather Rodney, our point of contact at the Federal Interagency Traumatic Brain Injury Research informatics system (FITBIR), to attend an Introduction to FITBIR training session. PseudoGUIDs have been requested for samples that did not have one assigned. A Biomarker form for FITBIR has been generated and the study is now an active study. Dr. Bissel will begin to deposit data to the database once it comes available.

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?

We will finish querying the plasma samples for the inflammatory protein concentration. This data will be analyzed and graphed. We will then perform correlation analyses with the flow cytometry analysis of TREM2 levels on leukocytes. Data will be uploaded to the FITBIR system, and then a manuscript will be completed.

4. **Impact**

Nothing to report.

5. **Changes/Problems**

We suffered several setbacks in our efforts to complete this study. First, significant changes have occurred at the IU School of Medicine site. Dr. Bemiller, who helped set up technical procedures and organize communication between sites, left the University for another position on 01May 2018. Dr. Chopra took over management of the project, but he left on 01April 2019. Dr. Bissel has resumed the project management. Even though significant improvement had been made in communication between sites and shipment of the samples, it took considerable time to coordinate information and to receive the samples. With optimization of the assays, we determined that an overnight incubation step was needed. This increased our assay run time by 100%. Thus, we have been unable to complete the analysis of the plasma samples before the end of the study period.

Attempts to coordinate shipment of CSF and to obtain the imaging data have been futile. For the CSF, it was very challenging to acquire samples due to the unwillingness of the recruited subjects to donate CSF. Thus, we were unable to collect sufficient n for analysis. For the imaging data, turn around and retirement of personnel have complicated collection of these data. Likewise, we do not have sufficient n for the image analysis. We will not be able to complete these aspects of the study. Both the CSF collection and imaging data were funded via other mechanisms and had been planned to be a collaboration, so no funds for these two facets of the study were used from this project.

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.

As stated above, we were unable to acquire sufficient number of samples for the CSF and imaging goals of this project. Thus, we will not complete these goals at this time.

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

We are on target to finish running the plasma samples inflammatory protein concentration analysis in a few months' time. We will request a no cost extension to complete the query, analyze the data, and finish writing the manuscript.

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**

Kinney JW, Bemiller SM, Murtishaw AS, Leisgang AM, Salazar AM, Lamb BT. Inflammation as a Central Mechanism in Alzheimer's Disease. *Alzheimers Dement (N Y)* (2018, September 6) 4:575-590. doi: 10.106/j.trci.2018.06.014. PMID: 30406177

7. Participants and collaborating organizations

What individuals have worked on this project?

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: Nipun Chopra
 Project Role: Postdoctoral Fellow
 Nearest person month worked: 4
 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.

Name: Stephanie Bissel
 Project Role: Research Assistant Professor
 Nearest person month worked: 4
 Contribution to Project: Dr. Bissel is the new project manager coordinating efforts between IU School of Medicine, UNLV, and CCF.

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