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14. ABSTRACT This study attempts to address a major knowledge gap, the role of inflammatory complement proteins, which are elevated during a prolonged asymptomatic period in mild repeated traumatic brain injury (rmTBI), in chronic traumatic encephalopathy (CTE). Current CTE models recapitulate some but not some of the key features, which may in part due to the lack of sulci in the mouse brain where in humans the pathology begins, specifically perivascular tau pathology. We predict that novel transgenics model overexpressing specific inflammatory proteins in mice subjected to repeat lateral mild, head trauma model may better recapitulate CTE. We used three transgenic models to examine responses to rmTBI, one that accumulates C1q (Serpin KO) and that accumulates C5a, a byproduct of the neurotoxic complement cascade and a model of human tau overexpression. Bigenic tau mice showed low viability, so we developed a htau bigenic that overexpressed the astrocyte protein MEGF10 responsible of removal of synapses including synaptic tau, and showed deficits. The outcomes of this study included identified rmTBI- dependent plasma biomarkers in brain derived vesicles (ER stress marker BIP/GRP78, GFAP, tau and Aqp4, an astrocytic endfeet protein on vessels). We showed tg-dependent locomotor disturbances associated with neuroinflammatory pathology and increased perivascular glial tau and abnormal distribution of AQP4 as well as demyelination. Despite poor viability of serpinKO-tau bigenic, the serpinKO alone appears to develop tau and warrant future investigation. This study should produce 4 publications and provide data to support new funding.

15. SUBJECT TERMS Glia, microglia, mild traumatic brain Injury, chronic traumatic encephalopathy, complement cascade, neuroinflammation, neurofibrillary tangles, tau, trans-synaptic, phagocytosis

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Table of Contents

1. **INTRODUCTION:** The purpose of this study is to use animal models to elucidate the mechanisms after repeated mild traumatic brain injury (mTBI), leading to neurodegenerative disease, such as chronic traumatic encephalopathy that occur after several asymptomatic months or years. This long asymptomatic period suggests that the brain has strong protective mechanisms against deleterious effects, but that eventually there is failure to compensate. The main pathology thought to cause onset of the disease is accumulation of abnormal aggregates of a protein called tau, which is a pathology common to many neurodegenerative diseases. Chronic aberrant neuroinflammation (dysregulation of astrocytes and microglia), during the asymptomatic period is known to drive tau pathogenesis through activating tau kinases, but the mechanisms remain elusive. We have identified an inflammatory pathway called the complement cascade involving the microglia, which plays an essential role in synaptic pruning, but no model to date has modeled its hyperactivation, known to occur after TBI. Since our preliminary data shows that C1q plays a prominent role in tau accumulation and that these effects are mediated by C5 convertase, we have obtained novel models that will for the first time allow study of these mechanisms. Our data also show that the complement cascade plays a role in tau accumulation that is distinctive and opposite from its role in amyloid accumulation. This proposal investigates the hypothesis that the dysregulation of glia plays a critical role in tau spreading leading to cognitive loss.

2. **KEYWORDS:** Microglia, Astrocytes, tau, complement 5a, serpin, C1 esterase inhibitor, tau kinases, chronic traumatic encephalopathy, post-traumatic brain injury, trans-synaptic, phagocytosis.

3. **ACCOMPLISHMENTS:**
   - **What were the major goals of the project during this Period (Years 1-4)?**

   - *Specific Aim 1: To determine the role of the proinflammatory complement factor 5a on modulation of tau pathogenesis after repeated mild concussion*
   - *Specific Aim 2: Determine the role of the complement activation factor C1q on modulation of tau pathogenesis after repeated mild concussion*
   - *Specific Aim 3 To determine the effect of C5a and C1q in TBI associated tau pathogenesis on microglia, astrocyte and oligodendrocytes and synaptosomal-glial interaction*

   1) In the first year we received ACURO and Institutional approval for the animal studies. We obtained the C5a and Serpin embryos from UC Irvine and Washington University as required for the study. We also recruited a post-doctoral fellow (Dr. Denver) from Ireland and a technician from UCLA (Peter Kim) and trained undergraduate students to work on the project. Due to the initial inability of the UCLA Core to obtain viable mice from the pathogen-free embryos for the investigator-derived strains as required for housing in UCLA's barrier-maintained vivarium, we had to repeatedly apply more unto re-derivation of viable mice and final UCLA approval to house and use them. While overcoming these setbacks, we focused on developing techniques that would be useful in characterizing the new models and to create an alternative to studying C1q should the transgenic model failed. Despite these issues, we were still able to conduct experiments in non-transgenic mice using an i.c.v.

infusion approach that yielded important data, contributing to an insight into the overarching goals:

a) First, we infused C1q with, and without Abeta, i.c.v., i.e., directly into the brain, which showed that: (1) C1q is synaptotoxic, and (2) robustly proinflammatory, and (3) in the presence of Abeta, C1q stimulates tau accumulation, and (4) that this effect is mediated by C5. These results are significant because elevated C1q and complement factor pathway component expression are activated by multiple factors previously implicated in tauopathy, viz., aging, Alzheimer disease and TBI. Complement factors are found by bio-informatic analysis of transcriptomics in AD and tauopathy patients and tauopathy model mice, but to date there has been little or no direct evidence of complement pathway factor roles and their interaction with Abeta in tau accumulation, a process central to TBI induced chronic traumatic encephalopathy (CTE). Of course, for modeling CTE our infusion of C1q that requires cannular implantation cannot easily substitute for a transgenic sensitive to C1q activation (e.g., by knocking out serpin inhibitor) with no CNS cannulation required. Nevertheless, our infusion model results are significant and relevant results because CNS injury increases Abeta, C1q and C5 and we show that the combination of these factors are sufficient to increase in ptau accumulation. Further, while data from our ultimate serpin KO-tau bigenic model is still delayed, we now know that we can go ahead and infuse C1q (or vehicle control) pre or post-mTBI to directly examine the impact of elevated C1q on TBI induced injury and tau accumulation.

b) We conducted pilot TBI studies to refine the method and developed a helmet to minimize variation and direct impact to the intended target brain region for our specific studies. We believe that more generally, standardized 3D printed helmets directing impact injury to specific Bregma points for different regions could be developed as an invention for more controlled non-invasive repeated mild TBI studies.

c) The pilot studies showed that after the fourth mTBI hit, mice consistently suffered acute Class I seizure-like symptoms and endured longer recoveries from anesthesia (compared to sham). In other words, these two outcomes appeared to serve as good internal controls reflecting the intensity of the impact injury which, despite identical impactor settings and due to unknown factors, typically produce wide within group variance. Monitoring seizures guides us to investigate the balance of excitotoxic and inhibitory markers implicated in both risk for TBI-induced seizures and excitotoxicity associated with both TBI and AD. Our initial data suggests increased levels of PSD95 an NMDA scaffolding protein in response to chronic TBI in both C57bl and htau, which corresponds to the seizures. Increases above baseline (as we saw in our studies), can reflect aberrant sprouting, while deficits can indicate loss of vulnerable excitatory synapses, an early event in MCI . Over the four years, we have continued

to observe the single acute seizure, specifically after the fourth hit which implies the previous hits resulted in the emergence of an excitatory/ inhibitory network imbalance.

2) In the second year, since we obtained and bred the required novel complement models, we did not expect to have more setbacks with breeding. Unfortunately, new problems arose, breeding out the tau deletion background took more time than expected and new issues arose with bigenic survival. First the yield was low for both the C5a-tau and Serpin-tau, then the mice that survived died early. We thought that the problem might be due to a potential lethality caused by SerpinKO x tau interactions, as we found that if we deleted a copy of mouse tau then the yield of viable mice increased but this did not prevent early mortality. These problems can occur with some breeding pairs of viable strains (like ApoE3 or ApoE4) and could be related to unique features of our breeders and not the transgenes per se, so we are not ready to definitively say that the coexistence of Serpin KO with increased C1q activation and several fold human tau overexpression is lethal before euthanizing the surviving mice early enough to determine if they have increased neurotoxicity and or tau pathology. We have new VA Merit funding for a TBI project that will permit us to examine these mice and which also enables us to use an alternative tetracycline transactivator regulated human tau transgene model where the tau transgene can be turned off during development. This should ensure viability and enable us to observe the interactions of tau and complement factors in adults with tau expression before or after repeat mild TBI. Despite these issues and caveats, our second year yielded several fruitful results:
   a) The initial C1inhKO (serpinKO) tau bigenic survivors showed increased tau and pyknotic neurons as well as activation of tau kinases associated with tau hyperphosphorylation, and increased microglial neuroinflammation supporting a pathogenic role of C1q in tau-dependent injury after TBI. These bigenics also had elevated activated microglia . Now that we have accumulated more SerpinKO bigenic mice and have new VA funding for studies of TBI and tau, we will be able to determine whether or not we can reproduced the initially observed negative synergism between Serpin KO and tau in TBI. This confirming data would significantly support efforts to target the complement pathway to limit tauopathy- regardless of whether or not our bigenic model can become more practical to breed enough viable mice to prove useful to others in the TBI field.
   b) We conducted pilot experiments in the parent strains with hemizygous tau deletion (used to develop the model). With or without mouse tau deletion, mice overexpressing human tau show apathy, as we and others have previously reported as a trait of tau overexpression). In contrast, when they were subjected to rmTBI, they displayed excessive contacts with both objects (novel and familiar) suggesting hyperactivity. However, in the htau, C57b, and serpin KO lines, rmTBI causes reduced speed in the open field test independent on transgene status. Therefore we observe rmTBI-

dependent traits that are transgene dependent and several other responses to rmTBI which occur in all transgenes (e.g. seizures, PSD95)

c) Also in Year 2, we used blood plasma samples from clinical TBI patients and htau mice subjected to mTBI to identify promising glial markers in brain derived plasma exosomes (aquaporin IV, GFAP and C1q). These appeared relevant to AD and ApoE as well reflecting neuroinflammation. Drs Frautschy and Cole submitted an invention report based on his biomarkers associated with Alzheimer's, with Dr. Frautschy as a co inventor because results dependend on samples from mice generated by the DOD project, which were necessary for the invention. Subsequently, the DOD grant was added to the UCLA/VA invention.

3) **In the third year** we continued to breed mice for the study, and continued to have problems with low bigenic yields which limited experiments with bigenics. A major focus became C5a-TBI effects that were independent of human tau transgene but present six weeks after TBI. This was based on the rationale that six weeks post-injury is usually sufficient for subsidence of acute mild TBI-induced neuroinflammation and recovery after behavioral deficits.

Inury- and transgene-dependent locomotor disturbances were associated with neuroinflammatory pathology. Further TBI increased blood GFAP and ER stress biomarkers (Grp7/BIP) as candidate brain-derived plasma biomarkers that will be helpful in dissecting the dynamics of neuroinflammation in the complex prodromal sequalae of TBI leading to chronic epilepsy or CTE. These changes were most pronounced in the htau mice, but were also present in the serpin KO expressing only mouse tau. Interesting they were lacking in the TauKO suggesting an important role for mouse tau in the persistent injury response leading to rmTBI effects on plasma GFAP and ER stress biomarkers.

Finally, we also observed increased actin and calpain-cleaved actin fragments in the brain as a response to rmTBI in the htau mouse and to a lesser extent in the Serpin mouse but not in the tau KO mouse. These fragments are consistent with persistent calpain activation, possibly linked to increased excitotoxic or complement-mediated calcium influx. During the third year we also observed that mTBI increased soluble ptau (normal bands and large aggregates) measured by CP13 and led to total tau measured by DAKO tau in SDS extracts of lysis buffer insoluble pellets which was greater in C5a mice subjected to rmTBI compared with wildtype mice subjected to the same TBI regimen.These results support an important role for complement in responses to TBI relevant to risk of tauopathy in CTE, the central premise of our project . We also provided new behavior data showing that rmTBI-associated hypoactivity in the htau mice was not present in C57bl or serpin KO.

4) **We requested a no cost extension to continue work on this project In a final Fourth year.**

a) During the fourth year, we demonstrated that in response to rmTBI, C5a but not C57bl showed a thinning of the corpus callosum and clustering of

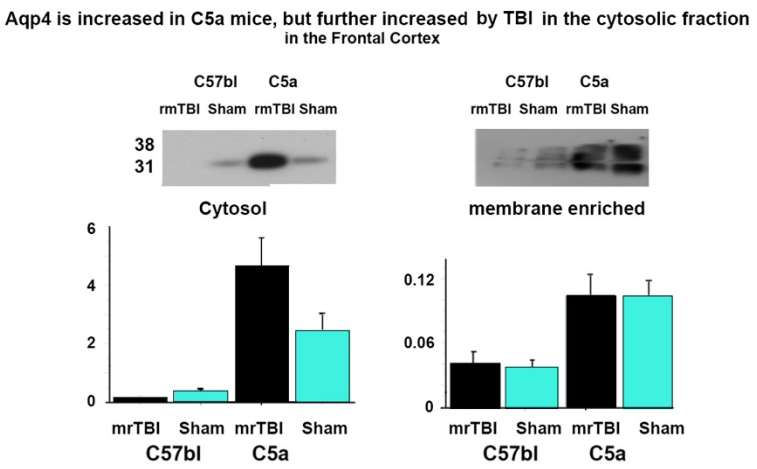
myelin basic protein suggestive of demyelination. This corresponded to loss of total MBP in by Western in that group. Interestingly independent of transgene or rmTBI, males are less MBP. There was a trend for rmTBI to reduce microtubule stability in the frontal cortex, as measured by reduced beta tubulin. While we will continue to evaluate this variable with larger sample sizes and in other regions, we now have strong data arguing that increased complement C5a expression causes more repeat mild TBI-induced white matter/ axon loss as well as increased ptau accumulation- two key aspects of the CTE phenotype .

b) Additional behavior analysis showed that poor performance in the Y maze in the C5a line was independent of rmTBI effects and instead related to slower slower speeds. . The Barnes maze was useful in identifying rmTBI effects. rmTBI impaired performance and this impairment was more severe in the C5a mice. Again, this new behavior data support a role for the complement pathway and its C5a component in the response to repeat mild TBI.

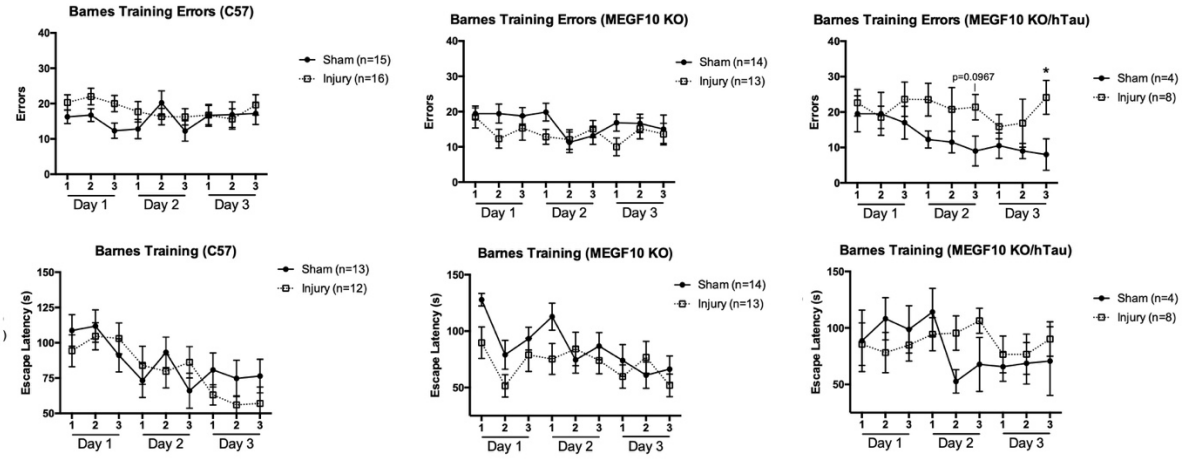
c) We have continued to quantify the histological neuroinflammatory changes using image analysis of Iba1 (microglia) and GFAP (astrocytes). The rmTBI effects were difficult to isolate from the transgene effects due to large transgene effects on both iba1 and GFAP. However, an analysis of frequency distribution of class size of the labeling showed a distinct morphological phenotype at baseline in the C5a transgenics, which was further altered by rmTBI. Essentially the C5a astrocytes have different cell body morphology and process morphology with thinner processes and larger cell bodies, than in C57bl. rmTBI increases the size of the cell body in C57bl while in C5a rmTBI increases the fragments of processes in the rmTBI, suggestive of glial dysfunction, possibly caused by increased calcium influx and calpain activation.

d) Recently, we have also acquired new data on the astrocyte water channel protein, Aqp4 which has been identified as a factor in Ann McKee's human CTE cases. We found significant Aqp4 changes in response to rmTBI in the frontal cortex. More specifically, our Western data showed that C5a transgenics displayed increased Aqp4 levels in both membrane enriched fraction and cytosolic fraction (see below). For the cytosolic fractions, we found a significant transgene effect ($F(3,38)=9.479$, $p<0.0001$) and interactions with group and traumatic brain injury ($F(1,38)=51.53$, $p=0.04$). Further, post hoc analysis showed more Aqp4 in rmTBI and sham within C5a. In lysis fraction there was only a transgene effect ($F(3,39)=7.6$, $p=0.0005$) and no rmTBI effect or interaction between rmTBI and transgene. Immunocytochemical evaluation of Aqp4 revealed that one region with alterations similar to our Western blot results was the globus pallidus. We can speculate that this may indicate that GP vessels or axons are more vulnerable to our paradigm. Whatever the causes, striatal vulernability may be related to the risk of Parkinson's disease after repeat TBI, most famously in boxers.

e) We were very recently able to acquire (via an MTA) the cis-tau antibody produced by Dr. Kun Lu (Harvard). Dr. Lu's antibody to cis-proline isomer modified tau was Dr. Goldstein has used to demonstrate major TBI effects in his blast TBI model that was designed to model the blast wave injuries received by many combat veterans. We have only stained one fraction and one region but both showed a trend for increased soluble cis-tau in the frontal cortex in the C5a animals subjected to mTBI (p=0.06). Further evaluation across models will hopefully provide additional evidence that like blast tube TBI in the Goldstein model, repeat mild TBI also induces substantial cis-tau.

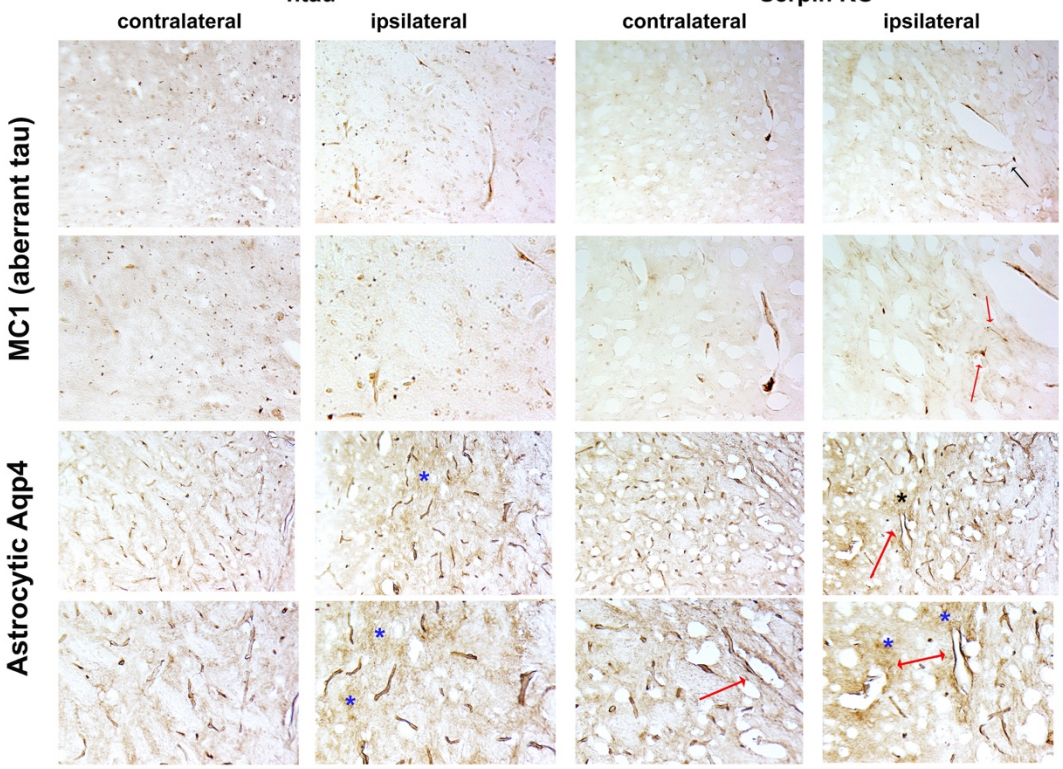


f) Finally, we will be subjecting our recently created bigenic MEGF10 -tau model to mTBI. Perivascular, astroctyic tauopathy is a distinguishing hallmaker of some tauopathies, in particular CTE. It's signicance is unknown but since it is accompanied by tau accumulation, it may reflect failure of a normal tau clearance pathway. MEGF10 is an astrocyte protein that mediates astrocytic phagocytosis of synapses which may include synaptic tau. We hypothesized that by MEGF10 deletion would impede normal clearance of tau that were further be impeded by the tau transgene. Although we have not finished the entire cohort, MEGF10/tau bigenic mice display increased errors in the Barnes Maze and acquisition deficits compared to wildtype or MEGF10KO. as shown by repeated ANOVA. We are looking forward to evaluating the tissue when we receive new funding.

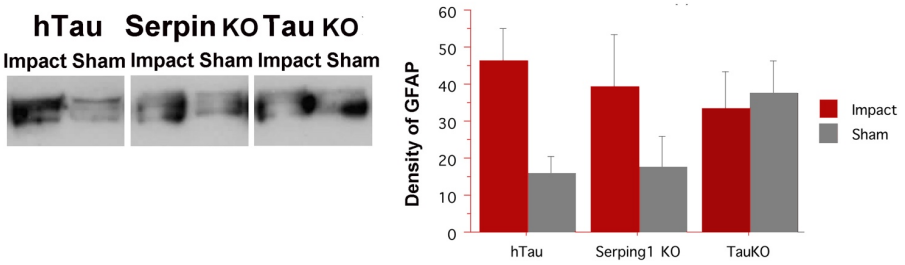


New data support the possibility that the Serpin KO alone may provide a useful model without the need for human tau. We have for the first time demonstrated that this model shows enlarged cerebral vessels in adulthood. Prior to this these mice were only used at young ages to study viral immunology. Examples of these findings are shown in the next figure. First, a noticeable increase in MC1 , a conformational tau antibody is observed and localized to vessels (red arrows), in Serpin KO and in response to injury or vessel associated glia. At first we thought this may be an artefact, but staining of other mice such as htau with or without injury did not reveal such staining. Then we determine whether the staining of astroglial Aqp4, which stains end feet of glia on vessels paralleled effects we observed with MC1, and it did, supporting that the tau was not in pericytes, but astrocytes, In both htau and Serpin KO, there was a aberrant distribution of Aqp4 after TBI leading to intense background staining in the neuropil (not seen in healthy mice). There were slight differences. In htau, after injury, there were clear glial fibers in the neuropil while the neuropil AqP4 in the serpin KO, is more patchy and diffuse. Thus we will continue to pursue the monogenic SerpinKO to determine if it may be a useful model without human tau, which would make the model more feasible, economic and efficient.

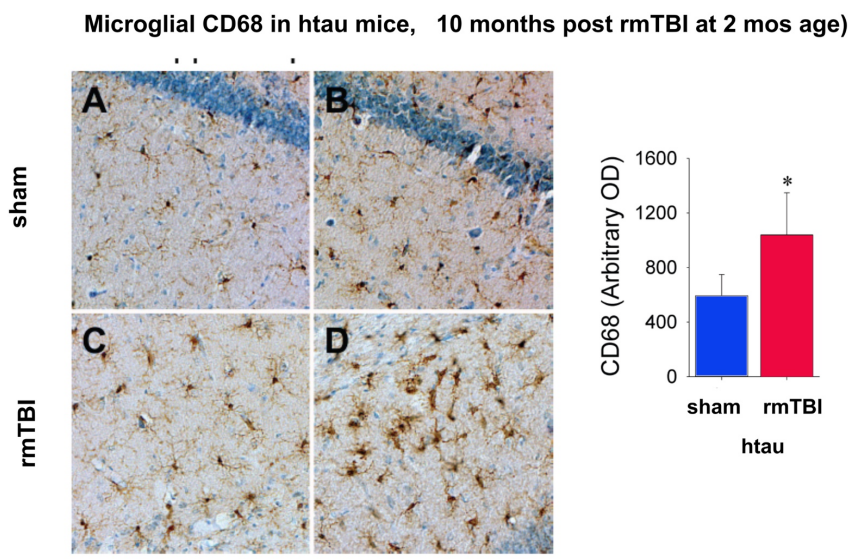
Unlike in htau mice, SerpinKO exhibit MC1-ir and Aqp4-ir swollen vessels (red arrows) consistent with astrocytic colocalization. Aqp4 is normally distributed in vessels but after rmTBI appears to redistribute in neuropil (blue asterisks) in both htau and Serpin KO suggestive of astrocytic damage



The Serpin KO also show elevated GFAP in plasma exosomes in response to injury.



Finally for a practical model of CTE one has to determine the minimum time post rmTBI and simplest repeat TBI paradigm. We have determined that at least 6 weeks post rmTBI is needed to establish an aberrant inflammatory milieu. However, waiting for 10 months can produce a robust neuroinflammatory response such as in the stratum lacunosum of the hippocampus, where there was an increase in number and size of microglia.



Collectively, our data from this proposal provide strong support for complement pathway or astrocytic phagocytic proteins in involvement in TBI-induced injury and tauopathy. Pending anticipated confirming data, we expect to publish the specific results discussed above over the next year. This DOD provides substantial data for applying for and obtaining new funding to complete analysis.

- Paper 1: review article in Special issue of Frontiers Immunology on C5a
- Paper 2: Plasma neural -derived exosome biomarkers in response to rmTBI (ER stress marker BIP/GRP78, GFAP, Aqp4, tau, C1q). We may add neurogranin to this panel which we see in AD models, but need to measure this.
- Paper 3: aged C5a mice, rmTBI, effect of demyelination, Barnes deficits, Aqp4 tau and aberrant glial responses.
- Paper 4: Serpin KO mouse and C1q-Abeta infusion, effect on tau pathogenesis
- Paper 5: MEGF10-tau bigenic

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

- Several undergraduate students receiving course credit to perform research in this program during this program. They learned rmTBI and behavior, analysis and presentation of findings at laboratory meetings. They learned Western blot to analyze biochemical changes and assisting in sectioning brains and immunostaining for pathological protein changes. They include Carmen Leal, Marisa Mekkittikul and Danielle Tran.
- In addition to undergraduate students we have two skilled visiting scholars who are neuroscientists skilled in neurosurgery and behavior: Dr. Cansheng Zhu and Dr. Katsuya Sakimura. They have their own projects, but are assisting in euthanasia, tissue collection and biomarker studies, as the assessment of

biomarkers and establishment of new methods on their projects in AD (ApoE4, PS1/APP, human AD) will be beneficial to our assessment in TBI.

- Finally two post-graduate students who were previously on the SRP199 course program: Trevor Nguyen and Nisha Choothakan, worked on Western blot analysis and biochemical processing of tissue. They have also left to go to medical school (both UC San Francisco).

- **How were the results disseminated to communities of interest?**

I presented the data in a lecture entitled "Role of complement proteins after TBI on tau accumulation" (May 17-18, 2018) at the a TBI conference at University of South Florida, which was organized via the Veteran's administration (Dr. Mark Kindy). The conference was entitled "New perspectives on central and peripheral inflammation in traumatic brain injury: Program to study the impact of inflammatory state in TBI in the VA population and establish collaborative research programs". The purpose was two-fold: first to bring together major laboratories researching brain pathology from postmortem tissue of people with head trauma (Ann McKee) and different models of TBI (blast injury) , CTE (Fiona Crawford), blast injury (Dr. Zezong Gu) and several other laboratories, all affiliated with the VA and the associated universities to discuss where the field is and where it has to go. The second purpose was to plan collaborations that will result in funding to extend our current projects and accelerate advancements in the field. As a result we have worked together to produce and submit, a program with four linked VA projects with Dr. Ann McKee, Weimin Xia, Karen Ashe and Zezong Gu. Our group (Cole PI ,Frautschy CoPI) is responsible for CTE model and biomarkers for all groups, Dr. Ashe is providing a new tau transgenic mouse model that will be used by all groups, and Dr. Gu for the blast injury model. the title of our program and project is "BX004332 CTBI: Tauopathy in mice and human: Surrogate Plasma Biomarkers for Brain Trauma-Initiated Neurodegenerative Disease", and the data in this DOD project accumulated has been important for this proposal as well as Dr. Cole's biomarker data in TBI human subjects. During this year the proposal has gone through one review and we have already submitted a revised stronger program and addressed concerns and will hear the outcome of the resubmission shortly.

- **What do you plan to do during the next reporting period to accomplish the goals?**
  - No plans , Final report, Data will be used to obtain New funding to complete studies via NIH or VA .

4. **IMPACT:**
   - **What was the impact on the development of the principal discipline(s) of the project?**
     - The impact on the principle discipline is unknown. The role of these proteins known to be present in humans after TBI is unproven and poorly studied at the basic level. The lack of the field's focus on investigating the complement mechanisms in animal models, despite these complement proteins showing up in nanostring and RNA seq data in human TBI is surprising.. Our data supporting a role in C5a exacerbating pathology in a CTE-like model, suggesting that this study is likely to have an important impact .
   - **What was the impact on other disciplines?**
     - *This study may have an impact on understanding mechanisms of inflammation in other tauopathies (FTD, or Alzheimer's), particularly in overlap on biomarkers and role of glia. It may also intersect with mixed*

dementia risk in cardiovascular diseas, as it will allow investigation if this milieu contributes to vulnerability to poor outcomes in CTE and also explain the mechanisms of accumulation of vascular tau.

- **What was the impact on technology transfer?**

- *This study may identify new biomarkers (AQP4, BIP GPR78 and GFAP) for TBI, We are working on developing a kit to detect neuroinflammation in plasma samples using the biomarkers identified in this project.*

- **What was the impact on society beyond science and technology?** *NOTHING TO REPORT*
- *T*

5. **CHANGES/PROBLEMS:**
   - **Changes in approach and reasons for change**. *NOTHING TO REPORT*
   - **Actual or anticipated problems or delays and actions or plans to resolve them**
   - *Development of the project and publications have been slowed this year due to insufficient funds and understaffing.*
   - **Changes that had a significant impact on expenditures.** Inability to fully characterize all the models.
   - **Significant changes in biohazards or select agents. N/A/**
   - **Significant changes in use or care of human subjects. N/A**
   - **Significant changes in use or care of vertebrate animals. NO**
   - **Significant changes in use of biohazards and/or select agents. N/A**
6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*
   - **Publications, conference papers, and presentations** *Presented this work at the University of South Florida Conference on TBI. May 17-18, 2018, Marissa Mekkitutal : UCLA undergraduate student, Poster Session: Best Poster Neuroscience.*
   - **Journal publications.** *NONE*
   - **Books or other non-periodical, one-time publications.** *NONE*
   - **Other publications, conference papers, and presentations.** *NONE*
   - **Website(s) or other Internet site(s)** *NONE*
- **Technologies or techniques:** We are planning on refining the plastic mouse helmut used for rmTBI but CAD 3D print with holes according to desired bregma target, which would facilitate the research community having a rmTBI modeling carefully controlled for desired brain region/ Bregma target for lateral head trauma.

- **Inventions, patent applications, and/or licenses** *We are developing techniques to assess Plasma Extracellular Vesicles derived from the brain that may monitor inflammation related to TBI. Currently we are using human samples from another grant, and we can apply this new technology to the mouse models in this study. We have submitted an invention report (Dr. Cole; inventor, Dr. Frautschy, co-inventor, Dr. Xiaohong Zuo, co-inventor.)*

Other Products N/A

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name:	*Paul Denver*
Project Role:	*Post Doctoral Fellow*
Researcher Identifier (e.g. ORCID ID):	*n/a*
Nearest person month worked:	*12*
Contribution to Project:	*Dr. Denver is involved in participating in all aspects of analysis and working with the PI to supervise the completion of the studies and writing of the papers..*
Funding Support:	*N/A*

Name:	*Marissa Mekkikutul*
Project Role:	*Undergraduate Student*
Researcher Identifier (e.g. ORCID ID):	*n/a*
Nearest person month worked:	*3*
Contribution to Project:	*Ms Mekkitkutul was an undergraduate honors neuroscience students who worked on several aspects of the project: rmTBI, behavior and histology. She is now working in our lab as an SRA2 and will go to USC medical school next FALL.*
Funding Support:	*N/A*

Name:	*Peter Kim*
Project Role:	*Senior Research Associate 2*
Researcher Identifier (e.g. ORCID ID):	*n/a*
Nearest person month worked:	*12*
Contribution to Project:	*Mr. Kim manages the colony and breeding and works with the PI to conduct the TBI. He genotypes mice and ensures that the appropriate number are bred for the DOD project and communicates weekly about progress. He is also responsible for overseeing the work of undergraduate students.*
Funding Support:	*N/A*

Name:	*Carmen Leal*
Project Role:	*Senior Research Associate 1*
Researcher Identifier (e.g. ORCID ID):	*n/a*
Nearest person month worked:	*4*
Contribution to Project:	*Ms. Leal is assisting in behavioral analysis and animal care, and receiving academic credit for this research.*
Funding Support:	*N/A*

Name:	*Mychica Jones*
Project Role:	*SRA3*
Researcher Identifier (e.g. ORCID ID):	*n/a*
Nearest person month worked:	*3*
Contribution to Project:	*Ms.Jones is the laboratory manager and histologist. She performs histology and image analysis and trains and supervises students in histology/*
Funding Support:	*N/A*

Name:	*Andrea Tenner*
Project Role:	*Director of MIND institute UC Irvine*
Researcher Identifier (e.g. ORCID ID):	*andreatenner*
Nearest person month worked:	*1 (no cost)*
Contribution to Project:	*Provided C5a Tg mice and assisting in recovering embryos and troubleshooting rederivation of the colony at UCLA*
Funding Support:	*T32 AG000096 "Training in the Neurobiology of Aging"* NIH NIA (PI, C.W. Cotman, Project Leader - A.J. Tenner) 5-01-14 through 4-30-19 $250,000 P01 AG 00538 "Behavioral and Neural Plasticity in the Aged", Project Neuroprotection and neuroinflammation induced by complement proteins and receptors $800,000 5-01-14 through 4-30-19

▪

Name:	Scott Barnum
Project Role:	*Professor University of Alabama*
Researcher Identifier (e.g. ORCID ID):	*barnum*
Nearest person month worked:	*1 (no cost)*
Contribution to Project:	*Developed C5a Tg mice*
Funding Support:	*R24 AI067039* *P30 AI027767* Impact Fund of Children's of Alabama

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
  No
- **What other organizations were involved as partners?**
  N/A
- **Personnel exchanges**
  N/A

8. **SPECIAL REPORTING REQUIREMENTS**
   - **COLLABORATIVE AWARDS:** *N/A*
   - **QUAD CHARTS:** *N/A*
9. **APPENDICES:**
   - *Introduction to C5a Manuscript*
   - *Poster : Honors Undergraduate Student Marisa Mekkikutul ; Best Poster UCLA Neurosciences*

APPENDIX I

Introduction to Review on C5a for Special Issue of Frontiers in

Immunology : Neuroinflammtion in Neurodegeneration:

Editor: Maya Koronyo and Sally Frautschy

Overexpression of C5a in the central nervous system exacerbates neuroinflammation, neuropathology and cognitive impairments in aged mice subjected to repeated mild traumatic brain injury

Denver P, Kim P, Castro D, Hu S, Jones M, Gu X, Mekkittikul M, Leal C, Xiaohong Zuo, Andrea Tenner, Cole GM, Frautschy SA

Introduction

Traumatic brain injury (TBI) is a leading cause of mortality and morbidity globally, with 2.8 million emergency room visits and 56,000 deaths related to TBI reported in the USA in 2013 (Taylor et al., 2017). Overall incidence of TBI was shown to be highest in the USA (1299 per 100,000), followed by Europe (1012 per 100,000) (Taylor et al., 2015, Dewan et al., 2018) and global annual incidence of mild TBI (mTBI) is thought to be around 600 per 100,000 million (42 million per year) (Gardner and Yaffe, 2015). Mild TBI (mTBI), characterized by transient disturbance of neurological function following a physically traumatic event (Young et al., 2016, Hobbs et al., 2016), comprises a significant proportion of TBI cases (Thompson et al., 2006, Mak et al., 2012). Recent appreciation for persistent pathologies and functional deficits associated with mTBI has primarily focused on sports-related injuries in adolescents and young adults (Hunter et al., 2019). However, the incidence of mTBI, particularly repetitive mTBI, is also high in geriatric cohorts (Papa et al., 2012).

As the world's population ages and elderly people remain mobile for longer, the median age of TBI patients has increased from 1984 to 2004, as has the proportion of TBI patients aged over 50 (Roozenbeek et al., 2013). Roughly 39 million adults over the age of 65 were evaluated at emergency departments in the US between 2009 and 2010 (Peters, 2016) and individuals aged 65 or older who had previously reported a concussive TBI are at an increased risk of subsequent re-injury and hospitalization (Dams-O'Connor et al., 2013). Everyday falls constitute a large proportion of mTBI cases (Cassidy et al., 2004, Thompson et al., 2006) and increased rates of emergency room visits in 2013 compared to 2007 has been attributed to fall-related TBIs in patients aged 75 or older (Taylor et al., 2017). One recent study suggested that the average age of patients admitted to hospital for fall-related TBI was 80 years old (Teo et al., 2018). Furthermore, 45.1% of these patients had visited an emergency department within the last 12 months and 20.9% within the last 3 months, a

significant proportion of which were related to TBI (Teo et al., 2018). Interestingly, patients with a milder head injury were found to have experienced two or more falls previously, while those with a more serious TBI-related subdural hemorrhage reported fewer previous falls (Teo et al., 2018). This has relevance in the context of repeated mTBI (rmTBI), which is known to compound the effects of a single mTBI and dramatically worsens outcomes (Fehily and Fitzgerald, 2017). **More about rmTBI**

A significant proportion of older adults admitted for a fall-related TBI subsequently received a formal diagnosis of mild cognitive impairment or dementia (Teo et al., 2018). Exposure to mTBI has been associated with 22-26% increased risk of dementia in the 5 to 7 years following injury, but only in individuals aged 65 or older when the mTBI occurred (Gardner et al., 2014). Activities of daily living decline post-injury and almost half of those admitted for fall-related TBI were readmitted within a year (Teo et al., 2018), suggesting that repeated head injuries are common in older adults, predisposing them to declining quality of life and further burdening healthcare resources. Biswas et al. (2017) found that consequences of TBI were more severe and extensive in elderly patients, particularly women, compared with younger individuals. Moreover, cognitive performance is worse in older individuals that suffered a TBI compared to younger TBI cases (Senathi-Raja et al., 2010, Spitz et al., 2012). Mild TBIs may not produce appreciable gross injuries and as a result individuals may not seek medical attention, potentially going without evaluation or treatment and allowing any underlying neuropathology to progress unchecked. In fact, it has been shown that a significant proportion of people that suffer a TBI do not seek medical care (42% of 1381 study participants) and that older individuals and mTBI patients were less likely to do so following injury (Setnik and Bazarian, 2007). Early identification of biomarkers or behavioral patterns that can predict the development of dementia or other neurodegenerative cascades post-mTBI will allow for timely intervention in at risk populations.

Neuropathological outcomes following mTBI are also affected by aging. One study compared gene expression changes and neuroimaging results between young (20-35 years old) and old (60-89 years old) subjects following mTBI. They found that computed tomography (CT) scans from young individuals showed less evidence of TBI-related damage than scans from old patients at 48 hours post-injury and although both groups of patients had similar MRI results at 48 hours, young patients'

aberrations but not those of old subjects, had resolved when scanned again at 1 week post-injury (Cho et al., 2016). Furthermore, suffering a recent TBI over the age of 65 is associated with increased risk of mortality (Dams-O'Connor et al., 2013), suggesting that elderly people that suffer a TBI are more likely to repeatedly present at emergency departments with worse, more persistent symptoms than younger individuals, a cycle of injury and hospitalization that not only burdens health care resources, but also exacerbates TBI-related pathologies and increases the risk of dying. The factors influencing the increased risk of neurodegeneration, neurological dysfunction and dementia following rmTBI in the elderly are not fully understood.

Experiments in animals have shown that persistent neuroinflammation is a consistent outcome following mTBI.

Inflammation is an important TBI effect

rmTBI causes more inflammation than mTBI

Inflammation is also enhanced in the aged brain

Complement in TBI

Complement in aging

Tie complement to vascular dysfunction and white matter damage

Expression of genes related to inflammation was altered in blood from old, but not young, TBI patients in a manner that suggests a relatively unrestrained and pro-inflammatory response to mTBI in old subjects, at least in the periphery (Cho et al., 2016).

Others have shown that the post-TBI neuropathological sequelae is worse in aged rats and that treatment with human adipose-derived stem cells, which is effective in young adult rats, was not so in aged animals (Tajiri et al., 2014), suggesting that aged rats that suffered a TBI were resistant to treatment, while younger injured rats were not.

It was recently shown that aged hTau mice that experienced 5 mild closed head TBI injuries over 9 days demonstrated impaired learning in the Barnes maze when tested 21 days post-TBI (Morin et al., 2018). Additionally, gliosis was elevated in the corpus callosum of aged hTau mice, compared to sham animals, but not in the hippocampus (Morin et al., 2018).

Multiple mild closed head injuries in C57Bl/6 mice has been shown to induce white matter damage, characterized by atrophic corpus callosum and reduced myelin basic protein (MBP) staining, along with persistent gliosis along white matter tracts (Gold et al., 2018).

Complement components (C4d and iC3b) were found to be elevated in brains of human AD patients, compared to age-matched non-AD controls, but were also elevated in aged non-AD brain, compared to young subjects without AD (Loeffler et al., 2004), suggestive of age-related augmentation of various complement pathways in the brain. Using microarray technology, Cribbs et al. (2012) found that of 759 genes related to immunity/inflammation, 6% were significantly altered in the brains of aged AD patients, compared to age-matched non-AD cases, whereas 40% of the 759 genes were altered in brains of aged non-AD subjects, when compared to young non-AD controls. This surprising finding suggests that aging *per se* is associated with robust modulation of immune/inflammatory signaling. Interestingly, normal aging was associated with upregulation of a multitude of complement proteins, including C5 and C5aR1, in the brain (Cribbs et al., 2012).

Upon upstream activation of the alternative complement cascade, C5 convertase cleaves a C5 molecule into the anaphylatoxin C5a and bioactive fragment C5b (Merle et al., 2015). C5b interacts with several further complement components, culminating with the formation of the membrane attack complex C5b-9 (MAC), which induces membranous pore formation and lysis of the target cell (Merle et al., 2015, Hammad et al., 2018). It is understood that MAC formation exacerbates neuropathology and neurological dysfunction following TBI (Fluiter et al., 2014), implicating C5b as a critical down-stream component of the cascade that leads to MAC formation subsequent to TBI (Merle et al., 2015, Hammad et al., 2018). The role of C5a in the brain following injury however is less well characterized.

The C5a receptor (CD88) is expressed at low levels in normal brain, but is robustly upregulated upon brain injury, with elevated expression detected in astrocytes, microglia and, to a lesser extent, endothelial cells (Gasque et al., 1997). C5a is involved in recruitment of neutrophils to the site of traumatic brain cryo-injury with subsequent exacerbation of injury size (Sewell et al., 2004), possibly via Ras/Raf/MAPK signaling (Buhl et al., 1994). Moreover, suppression of the alternative complement cascade and reduction of serum C5a has been found to attenuate

neuropathology following TBI (Leinhase et al., 2007, Leinhase et al., 2006). However, since the treatment in these studies was an inhibitor of factor B, an early component of the alternative complement cascade, these results likely reflect consequences of generally suppressing the alternative cascade, including terminal MAC formation, as opposed to the effects of suppressing C5a *per se*. In fact, astrocyte-specific overexpression of C5a does not exacerbate pathology in the experimental autoimmune encephalitis (EAE) model of multiple sclerosis (Reiman et al., 2005). Additionally, C5a has been shown to increase expression of glutamate transporter GLT-1 in microglia (Persson et al., 2009) and GluR2 in neurons (Mukherjee et al., 2008). C5a can also protect neurons against glutamate-mediated excitotoxicity and neuronal apoptosis (Mukherjee et al., 2008, Osaka et al., 1999, Mukherjee and Pasinetti, 2001), possibly via modulation of glutamate transporters on glial cells and neurons. C5aR signaling in T cells suppresses programmed cell death through a cascade that involves up-regulation of anti-apoptotic Bcl-2 and down-regulation of pro-apoptotic Fas (Lalli et al., 2008). This suggests that another mechanism by which C5a could mediate neuroprotection is modulation of survival signals, in cells within the brain, immune or otherwise.

Contrary lines of evidence have developed around the issue of C5aR signaling in AD brain. A recent study suggests a deleterious role for C5aR1 signaling in Arctic AD mice, whereby deficiency of C5aR1 resulted in restoration of cognitive function and reductions of neuropathologies associated with an AD transgene (Hernandez et al., 2017a). Findings from the same group further suggest that C5aR1 signaling enhances toxicity of fibrillar Aβ in neurons (Hernandez et al., 2017b) and that microglial C5aR expression associates with Aβ deposition and glial recruitment in transgenic AD mice (Ager et al., 2010). Others have also demonstrated a pathogenic role for C5a signaling in brains of mice with experimentally-induced CNS lupus (Jacob et al., 2010), findings that have been subsequently ascribed, at least partially, to pro-apoptotic effects in brain vascular endothelial cells (Mahajan et al., 2016) and disruption of blood-brain barrier integrity (Mahajan et al., 2015).

Other groups, however, have found that mice deficient for C5aR are cognitively impaired, concurrent with down-regulated CREB/CEBP signaling in brain (Gong et al., 2013). Furthermore, restoration of normal brain levels of C5a in AD mice augmented synaptic long-term potentiation and restored cognitive function

through C5a-mediated induction of CREB/CEBP signaling (Gong et al., 2013, Gong et al., 2014), suggesting potential positive effects of C5a on synaptic plasticity. C5a may also protect neurons from Aβ-mediated toxicity (O'Barr et al., 2001), contrary to findings mentioned above.

How these C5a-mediated neuroprotective effects are mediated is not fully understood. Studies have shown that exposure of astrocytes and neuronal cells to C5a increased expression of nerve growth factor (NGF), effects that were enhanced by co-stimulation with IL-1β (Jauneau et al., 2006). C5a also has a role in stimulating secretion of NGF from dental pulp fibroblasts and may be involved in neurite outgrowth (Chmilewsky et al., 2016). In tubule epithelial kidney cells, C5a stimulates secretion of transforming growth factor β (TGF-β), through PI3K/Akt signaling (Yiu et al., 2017). Others have found that C5aR signaling in neurons and glia is associated with anti-inflammatory effects (Gavrilyuk et al., 2005) and it has been found that C5a promotes proliferation, migration and angiogenic vessel formation by endothelial cells (Kurihara et al., 2010).

In addition, it is known that C5a increases intracellular calcium concentration in neutrophils (Fujita et al., 2004), macrophages (Roach et al., 2008) and microglia (Moller et al., 1997, Hoffmann et al., 2003), an action that is important for microglial proliferation, migration, ramification, release of cytokines and brain-derived growth factor (BDNF) (Kettenmann et al., 2011). Elevated C5a in the brain of C5a-GFAP transgenic mice may prime microglia, such that these cells respond more rapidly and efficiently to damage caused by repetitive mild TBI in our experiments.

Following TBI, it is likely that C5a is involved with exacerbation of secondary brain injury, as a result of chronic neuroinflammation. However, C5a may also support neuroprotection at certain time-points post-TBI. This could occur via multiple possible mechanisms, including modulation of glutamate homeostasis, anti-apoptotic and pro-survival effects, enhanced efficiency of debris clearance and secretion of growth factors. This study addressed the hypothesis that selective overexpression of C5a in the brain exacerbates neuropathology, white matter damage, neuroinflammation and cognitive dysfunction following repeated mTBI in aged mice.

Materials and methods

Animals

All animals were housed at the UCLA School of Medicine vivarium facility under a 12-hour light/dark cycle with access to food and water *ad libitum*. The study was approved by the UCLA Chancellor's Animal Research Committee and all animal experiments were performed in compliance with guidelines. In order to investigate the role of C5a in TBI-induced neuropathology and cognitive dysfunction in aging, we utilized the C5a/GFAP mouse model, in which selective C5a overexpression in astrocytes is driven by the GFAP promoter, as described previously (Reiman et al., 2005). (**Where did we get the +/+ C5as? Were they a gift from Reiman et al? Or were they purchased? If so, where from?**) A total of 25 C57Bl/6 and 24 homozygous C5a/GFAP mice aged 16-18 months were sex and age-matched and divided into sham and injury groups. Within the C57Bl/6 control group, there were 13 injury animals (6 x male, 7 x female) and 12 sham animals (5 x male, 7 x female), while the C5a/GFAP group consisted of 12 injury mice (6 x male, 6 x female) and 12 sham mice (6 x male, 6 x female).

Experimental design and repeated mild traumatic brain injury model

Wild-type C57Bl/6 and homozygous C5a/GFAP mice were aged to 16-18 months and then divided into sham and injury groups. Mice in the injury group were anesthetized and mounted onto a stereotaxic frame upon which anesthesia was maintained with 2.5 L/min of oxygen and 2.5% isoflurane flowing into a nose cone, while sham animals remained unconscious in the anesthesia chamber for the duration of the procedure. Once anesthetized, the heads of the mice in the injury group were shaved, before mounting the animals onto the stereotaxic frame. The piston was attached to the stereotaxic frame and zeroed to the scalp of the mouse, 4 mm posterior and lateral to bregma on the right side of the head and at an angle of approximately 113° from the level of the top of the animal's head. This meant that the injury was delivered to the skull approximately at the level of (http://mouse.brain-map.org/experiment/siv?id=100142143&imageId=102162147&imageType=atlas&initImage=atlas&showSubImage=y&contrast=0.5,0.5,0,255,4). For our repeated injury model, we used a 5 mm flat-tip piston connected to an electromagnetic stereotaxic impactor (Leica Biosystems, Buffalo Grove IL) and the injury was delivered at a velocity of 3.5 m/s, with a dwell time of 200 milliseconds to a depth of 0.1 mm (**Check this is right**). This procedure was repeated on three subsequent days, for a

total of 4 x mTBIs, spaced 24 hours apart (D0-D4), with appropriate sham controls. After each mTBI was delivered, anesthesia was removed from sham and injury mice, which were then allowed to recover on a heating pad under a cage top. Mice were considered "recovered" when they regained consciousness and were able to walk adequately with no staggering or dragging of the hind legs. Behavioral testing began on D14 and animals were sacrificed on D24.

Behavioral testing

Open field task

On D14 of the study, mice were assessed in the open field task (OFT) to assess locomotor function and anxiety. Mice were placed into a white box (**dimensions?**) and allowed to explore freely for 15 minutes. The box was dimly illuminated from above with a 60W light bulb and the animal's movements were tracked and recorded with overhead camera and Anymaze software. Speed and path length were measured to assess locomotor function, while rearing, grooming and defecation were measured to indicate anxiety levels.

Novel object recognition task

Twenty-four hours following the OFT (D15), mice were placed into the same box along with two identical objects, Lego bricks or sand-filled cell culture flasks, that were secured to the floor of the box with Blu-tack adhesive gum. The mice were again allowed to explore the box, including the objects, freely for 15 minutes before being returned to their home cage. Another 24 hours later (D16), mice were returned to the box with the two identical objects again and allowed to explore for 10 minutes. After this time they were placed back in their home cage for 1 hour and then returned to the box for the test phase of the novel object recognition (NOR) task. This involved replacing one of the identical objects with a previously un-encountered novel object, which the mouse was allowed to explore, along with the familiar object, for 10 minutes. Time (t) spent exploring the familiar (F) and novel (N) objects was measured and recognition indices (RI) for both objects were calculated (tF or fN / (tF + tN)). Statistical comparisons were made between the RIs for the novel and familiar objects in the test phase of the NOR task and significant preference for the novel object, compared to the familiar object was considered indicative of intact NOR memory,

while an insignificant difference between RIs for novel and familiar objects signified impaired NOR memory.

Y maze

On D17, mice were placed into a white Y maze (**dimensions**) and allowed to explore freely for 8 minutes. The maze was dimly lit from above and the animal's movements were tracked and recorded using Anymaze software. Correct arm entries were defined as an entry into an arm that was not entered immediately before (eg. A > B > C), whereas an error was defined as an entry into an arm the was entered immediately before (eg. A > B > A). Spontaneous alternation was calculated as the percentage of total arm entries, minus the first two arm entries, that were "correct" (correct arm entries / (total arm entries-2)).

Barnes maze

Assessment of spatial learning and memory in the Barnes maze took place on D20-23 of the study. The apparatus consisted of a circular, white wooden platform (**Diameter?**) with 20 evenly spaced holes (**dimensions?**) positioned along the outside of the platform. Divider boards were positioned around the maze, on which visual cues were attached. An escape box was placed under one of the twenty holes, designated as the target or escape hole, which was positioned in the center of one of the quadrants that divided the maze. The maze was lit from above with a 60W bulb and the animals' movements were tracked and recorded with an overhead camera and Anymaze software.

On the initial habituation day (D20) mice were placed under a transparent beaker in the center of the maze for 30 seconds, while an aversive air horn sound was played through computer speakers within the room. After 30 seconds, the mouse was slowly guided to the escape hole using the beaker. The mouse was allowed to enter the escape hole, at which time the aversive air horn sound was stopped. The mouse was allowed to remain in the hole in silence for a total of 1 minute. If the mouse left the escape hole during that time, the timer was stopped and the air horn played again until the mouse returned to the hole. On the first training day (D21) mice were placed in the center of the maze under an opaque bucket for 15 seconds, after which time the air horn sound was switched on and the bucket was removed. The mouse was allowed to explore freely until it found and entered the escape hole, at which point the air horn

sound was stopped and the mouse was allowed to remain in the hole in silence for 1 minute. If the mouse did not find the escape hole within 2 minutes, it was guided to the hole using a transparent beaker. The first training day involved 3 of these trials spaced 20 minutes apart, while the second training (D22) day consisted of 2 trials. On D23, the probe test was performed, which involved a single trial in the absence of the escape box. The animal was allowed to explore the maze for 2 minutes, while its movements were tracked and recorded. Time spent in the "target quadrant" and the exact target hole were measured as an indication of spatial memory retention. Errors, speed and path length were also measured.

Results

Recovery time from anesthesia after mild traumatic brain injury is elevated in aged C5a/GFAP mice

After each mTBI, recovery from anesthesia was recorded for both injury and sham animals. Two-way ANOVA detected a significant effect of injury/genotype ($p<0.001$) and *post hoc* analysis determined that recovery time post-TBI was significantly higher in C5a/GFAP-injury mice, compared to C5a/GFAP-shams on day 1 ($p<0.05$) and day 4 ($p<0.01$). Recovery time was also significantly elevated in C5a/GFAP-injury animals on day 4, compared to C57-sham ($p<0.001$) and C57-injury ($p<0.05$) mice. No significant differences were detected when comparing recovery time in C57-sham, C57-injury or C5a/GFAP-sham mice.

Repeated mild traumatic brain injury causes hypolocomotion in aged C5a/GFAP, but not C57Bl/6 mice

Performance in the OFT was assessed at D14 of the study, ten days following the last mTBI. While there were no significant effects of rmTBI in C57Bl/6 mice, speed ($p<0.05$) and path length ($p<0.05$) were significantly reduced in C5a/GFAP-injury mice, compared to C5a/GFAP-shams. There was a reduction in the number of rearing events and fecal pellets in C57-injury, compared to C57-sham mice, although two-way ANOVA showed that this was not significant. Number of grooming events did not differ significantly among the four groups.

Repeated mild traumatic brain injury corrects novel object recognition memory deficits in aged C57Bl/6, but not C5a/GFAP mice

As shown in **Fig.** , at D16 of the study, time spent exploring the novel object in the test phase of the NOR task did not differ significantly from the familiar object in sham C57Bl/6 and C5a/GFAP mice, suggesting an impairment of NOR memory in aged sham animals. Conversely, C57-injury mice spent significantly more time exploring the novel object, compared with the familiar ($p<0.001$) in the test phase of the NOR task, while the time spent exploring the novel object by C5a/GFAP-injury animals did not differ significantly from the familiar object. This implies that there was a NOR memory deficit in aged C57Bl/6 and C5a/GFAP mice that was corrected following rmTBI in C57-injury, but not C5a/GFAP-injury mice. Similarly, the discrimination index (DI) was significantly greater in C57-injury animals, compared with C57-shams in the test phase of the NOR task. Although the DI was increased in C5a/GFAP-injury mice, compared to C5a/GFAP-sham, this difference was not significant; nor were there significant differences in the NOR test phase DI between C5a/GFAP-injury animals, compared to sham or injury C57Bl/6 mice.

Performance in the Y maze was comparable in sham and injury C57Bl/6 and C5a/GFAP mice

Mice were assessed in the Y maze at D17, 13 days after the last mTBI. Spontaneous alternation, correct arm entries, number of errors, speed and distance did not differ significantly between C57-sham, C57-injury, C5a/GFAP-sham and C5a/GFAP-injury groups.

Repeated mild traumatic brain injury impairs spatial learning and memory in C5a/GFAP, but not C57Bl/6 mice

Performance in the Barnes maze was assessed at D20-22, 16-18 days following the last mTBI. Escape latency during the training phase of the Barnes maze task was significantly impacted by injury/genotype ($p<0.05$). *Post hoc* analysis showed that escape latency was significantly elevated in C5a/GFAP-injury mice, compared to C57-injury on trials 1 and 2 of training day 1 and compared to C57-shams on trial 3 of day 1 and trials 1 and 2 of day 2. Escape latency was also significantly elevated in C5a/GFAP-injury mice, compared to C57-shams on trial 2 of the first training day.

The Barnes probe trial took place on D23, 19 days after the last mTBI. Entries into the target hole during the Barnes probe trial were significantly reduced in C5a/GFAP-injury mice, compared to C57-shams ($p<0.05$). A trend ($p=0.0512$) towards a reduction in probe trial target hole entries was also evident in C5a/GFAP-injury mice, when compared with C5a/GFAP-sham animals.

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APPENDIX 2

Undergraduate Poster for Annual Poster Presentation in Neuroscience:

Marisa Mekkikutul: Neurosciences Major

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Undergraduate Poster for Annual Poster Presentation in Neuroscience:

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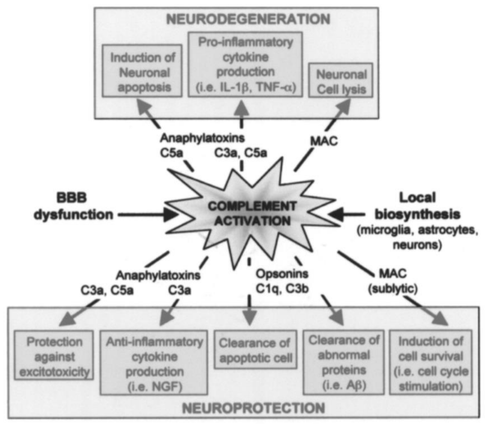
INTRODUCTION

Background

- C5a is part of complement immune system. Upregulation of C5a and its receptor (CD88) is correlated with normal aging.
- Binding of C5a to CD88 results in immune response such as: neutrophil mobilization, histamine release, increased vascular permeability, and tissue factor production.
- Expression of CD88 is upregulated upon infliction of traumatic brain injury. The resulting neuroinflammation can lead to more pronounced secondary brain injury. However, previous studies provide support that C5a can also play a neuroprotective role, in a time-dependent fashion post-TBI.



Neuroprotective and neurodegenerative effects of complement system activation (Van Beek et al., Annals of the New York Academy of Sciences, 2003).

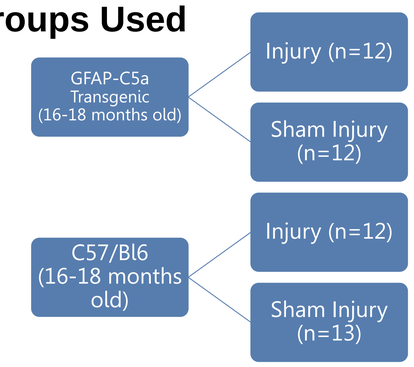
Aims

- Determine if c5a overexpression can cause behavioral deficits in mouse model.
- Compare whether anesthesia recovery time is affected by overexpression of C5a and traumatic brain injury

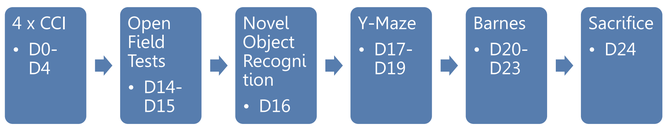
HYPOTHESIS: Mice that are homogenous for GFAP promoted C5a overexpression in the central nervous system will show exacerbated cognitive and behavioral deficits after acute traumatic brain injury.

METHODS

Animal Groups Used

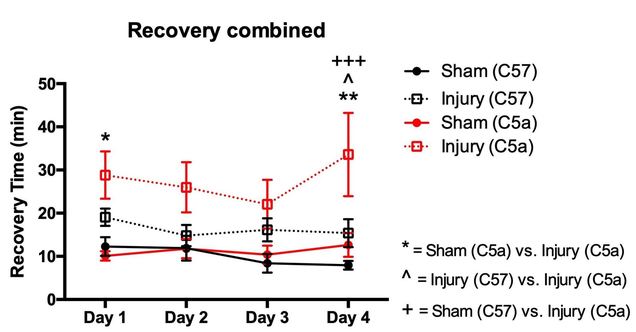


Timeline of Acute TBI Study



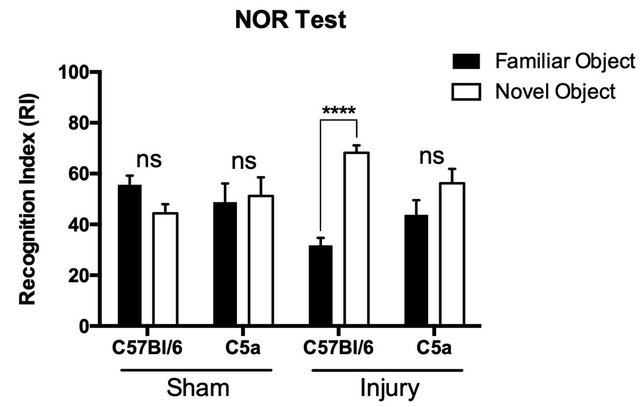
RESULTS

Recovery time from isoflurane is significantly longer for injured GFAP-C5a mice compared to all other groups.



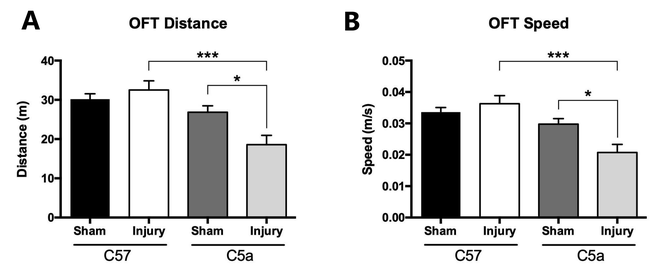
Recovery time was measured between moment when the controlled cortical impact (CCI) was delivered and animal was fully recovered. Full recovery was noted when mice cease dragging at the hind legs and walk normally. Injured GFAP/C5a mice took more time to recover across all 4 days of CCI, with the effect most pronounced on the final day of traumatic brain injury.

Significant improvement in Novel Object Recognition performance between injury and sham group is only seen in C57/Bl6 mice.



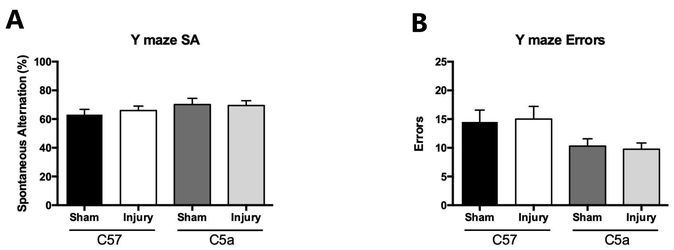
Percent of spontaneous alternation were similar across all animal groups. (A) Spontaneous alternation is measured as number of correct responses (triads)/total arm entries. The mouse must enter 3 different arms consecutively for the triplet to be considered correct, while errors occur when the mouse returns to a previously visited arm out of 3 arm entries (B).

Injured GFAP-C5a mice travel the least distance and at the lowest speed in open field tests.



(a) Injured GFAP-C5a animals traveled significantly smaller distance compared to both GFAP-C5a animals with sham injuries and injured C57/Bl6 animals. (b) GFAP-C5a mice traveled at a significantly slower speed compared to both GFAP-C5a animals with sham injuries and injured C57/Bl6 animals. Data was combined from both days of open field tests.

Performance on Y-Maze is comparatively similar across all animal groups.



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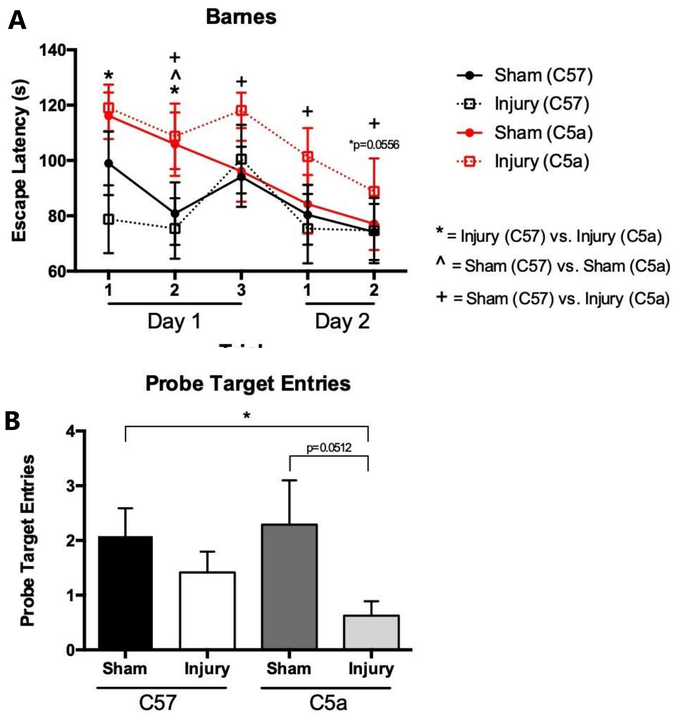
Injured GFAP-C5a mice have the lowest performance in Barnes Mazes throughout training phase and probe trials.



(A) Escape latency is defined as the amount it takes the mouse to access the target hole during the first 2 days of Barnes maze training. Escape latency was highest in injured GFAP-C5a mice across all 3 trials of Day 1, and both trials of Day 2. (B) During probe trial on the third day of Barnes maze testing, injured GFAP-C5a mice entered the target area fewer times than both control C57 mice and sham GFAP-C5a mice. Probe target entries are counted when the animal enters the region where the target hole once was.

1. Open field tests exacerbates def function upon t
2. Increased neuro overexpression animals.
3. Injury appears t recognition me impede restorat
4. Spatial learning affected in injur
5. Selective overex system affects b brain injury and when compared

FUT

- Perform immun associated with GFAP, IBA1, Aqu
- Further explore
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Separation of reco reveals more pron recognition memo mice.

ACK

Thank you Stella K started in the lab, a Castro and Mychica assistance and pat experiment would

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