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EFFECTS OF HYPOBARIA ON SYSTEMIC IMMUNE FUNCTION FOLLOWING BRAIN TRAUMA

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14. ABSTRACT

In these preclinical studies we examined systemic and central immune function following experimental traumatic brain injury (TBI; controlled cortical impact) plus secondary hypobaria exposure in adult male C57Bl/6J mice. The experimental animals were also subjected to a battery of neurobehavioral tests to probe posttraumatic motor and cognitive deficits. The studies were designed to test the hypothesis that exposure to hypobaria after experimental TBI will result in an amplification of neuroinflammation and increased neurological dysfunctions compared to animals subjected to TBI alone.Our data have shown only minor evidence of hypobaria-induced increases in inflammation responses after TBI and no evidence of worsening of neurological deficits. The current findings differ from our previous work using *rat lateral fluid percussion model of TBI* in which hypobaria amplified posttraumatic secondary injury and neurological dysfunctions. We conclude that hypobaria effects on TBI outcomes is species and brain trauma model specific.

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1.0 SUMMARY

In these preclinical studies we examined systemic and central immune function following experimental traumatic brain injury (TBI; controlled cortical impact) plus secondary hypobaria exposure in adult male C57Bl/6J mice. The experimental animals were also subjected to a battery of neurobehavioral tests to probe posttraumatic motor and cognitive deficits. The studies were designed to test the hypothesis that exposure to hypobaria after experimental TBI will result in an amplification of neuroinflammation and increased neurological dysfunctions compared to animals subjected to TBI alone. Our data have shown only minor evidence of hypobaria-induced increases in inflammation responses after TBI and no evidence of worsening of neurological deficits. The current findings differ from our previous work using *rat lateral fluid percussion model of TBI* in which hypobaria amplified posttraumatic secondary injury and neurological dysfunctions. We conclude that hypobaria effects on TBI outcomes is species and brain trauma model specific.

2.0 INTRODUCTION

TBI is a serious global health problem and a major cause of mortality and long-term disability. Injured Active Duty Service Members (ADSMs) moved from theater are exposed to decreased atmospheric pressure (hypobaria) during Aeromedical Evacuation (AE), but effects of such pressure changes on TBI are unknown[1-3]. Recent studies supported by the Air Force (FA86650-11-12-6D04; PIs: Drs. Gary Fiskum and Alan Faden, University of Maryland School of Medicine) demonstrated that hypobaric exposure following experimental TBI in a rat lateral fluid percussion injury (FPI) model significantly worsens long-term TBI outcomes[4]. Specifically, simulating AE conditions (8000 feet (ft); 6 hour of hypobaria exposure), but maintaining oxygenation, hypobaric exposure between 6-72 hours after moderate TBI markedly worsens cognitive function and neuronal cell death as compared to TBI alone, which appears to reflect enhanced activation of neurotoxic microglia that can drive secondary neurodegeneration[4,5]. Furthermore, additional data demonstrate that disruption of the blood brain barrier (BBB) following TBI is prolonged following hypobaric exposure, thereby potentially allowing for greater systemic influences (e.g. infiltrating immune cells/factors) on TBI neuropathology and functional outcomes.

3.0 BACKGROUND

Multi-organ failure induced by systemic inflammation remains the most frequent cause of late death in combat casualty trauma patients who survive initial resuscitation and stabilization. Outcomes following TBI are strongly related to the post-traumatic occurrence of non-neurologic organ dysfunction, with up to 89 percent (%) of patients with severe TBI developing dysfunction of at least one non-neurologic organ, and 35% progressing to organ failure[6]. Post-TBI inflammation and infection are the primary contributors to the initiation of organ dysfunction[7].

Thus, the systemic and cerebral inflammatory responses to TBI may prime the patient for an exaggerated inflammatory response to altitude exposure and hypobaric hypoxemia, thereby creating a ‘‘two hit’’ injury paradigm that places acutely AE patients at additional risk for multi-organ dysfunction[8].

We have shown using a rat model of TBI (FPI) that hypobaria during simulated AE worsens cognitive function and TBI neuropathology, and appears to exacerbate post-traumatic cerebral inflammatory responses[4]. Currently, there is a paucity of available data on the effects of AE on systemic immune function following TBI, and how it may interact with cerebral immune responses to worsen neurological outcomes or how it contributes to secondary multi-organ dysfunction.

The goal of this study is to investigate the impact of simulated AE hypobaria on systemic immune function following moderate-level TBI in mice.

4.0 METHODS

Moderate level controlled cortical impact (CCI) was induced in adult male C57Bl6J mice and was followed by hypobaric exposure (568 millimeters of mercury (mmHg)for 6 hours) using the protocol described by Skovira et al.,[4]. Briefly, mice were anesthetized with isoflurane (4% induction, 2% maintenance), and a 5-millimeter (mm) craniotomy was made over the left parietal cortex midway between the lambda and bregma. Using a custom designed pneumatic device, a moderate level CCI was delivered (CCI = 6m/sec, 2mm depth[9]) to produce the TBI.

Hypobaria was induced using a chamber equipped with internal oxygen, carbon dioxide, and pressure gauges and connected to a vacuum pump. Animals were placed into the chamber in their home cages with access to water and food to reduce stress from acclimation to the HB chamber. Multiple animals in various groups were randomly exposed simultaneously. The chamber was de-pressurized over 30 minutes (min) to reach 568 mmHg (=8000 ft. altitude)—approximating the cabin pressure during military AE with cruising altitudes of 30,000–40,000 ft. To account for the mean oxygen saturation decrease of 5.5% experienced at this pressure, 28% Oxygen (O_2) was continuously delivered to the chamber to maintain partial pressure of oxygen (pO_2) at sea level despite the drop in atmospheric pressure. Chamber gases were continuously monitored to validate concentration of O_2 delivered, as well as to verify that CO_2 was not accumulating in the chamber. At 5.5 hours of “flight,” the chamber was re-pressurized over 30 min to 1 atmospheric pressure (765 mmHg), and the animals were then removed. Sham controls were represented by naive animals that did not receive CCI. At the indicated timepoints mice were euthanized and the brain, blood, spleen tissues were collected for flow cytometry analysis.

For flow cytometry analysis blood, spleen, and brain samples were processed into single cell suspensions as described[10]. For all multicolor experiments, controls included both single-color and FMO. The gating strategy for systemic immune cell analysis is shown in **Figure 1**. Immediately following isolation, immune cells were assessed *ex vivo* for functional changes in phagocytosis (Red latex beads), respiratory burst (ROS; DHR123), and cytokine production, tumor necrosis factor (TNF) using intracellular staining protocols[10].

Long-term neurological recovery through 28 days post-injury was assessed using the following tests: a) beam walk test was fine motor coordination, b) morris water maze for hippocampal dependent spatial learning and memory, and c) novel object recognition for declarative memory. Mice (n=12/group) were tested according to the experimental protocol by an investigator blinded to treatment and group.

5.0 RESULTS

5.1. Effect of TBI on lymphocyte populations in the blood and spleen

To investigate the effects of focal TBI coupled with hypobaria (568 mmHg for 6 hours) on systemic immune function, we induced moderate-level CCI in adult male C57Bl/6J mice and moved them to a hypobaria chamber set at 568 mmHg for 6 hours. Control sham and TBI mice remained in their home cage environment and were not exposed to hypobaria. All animals were euthanized at 3 days post-injury for systemic immune function analyses by flow cytometry. We used an established gating strategy to identify cellular subsets of myeloid and lymphocytic origin using flow cytometry (**Figure 1**).

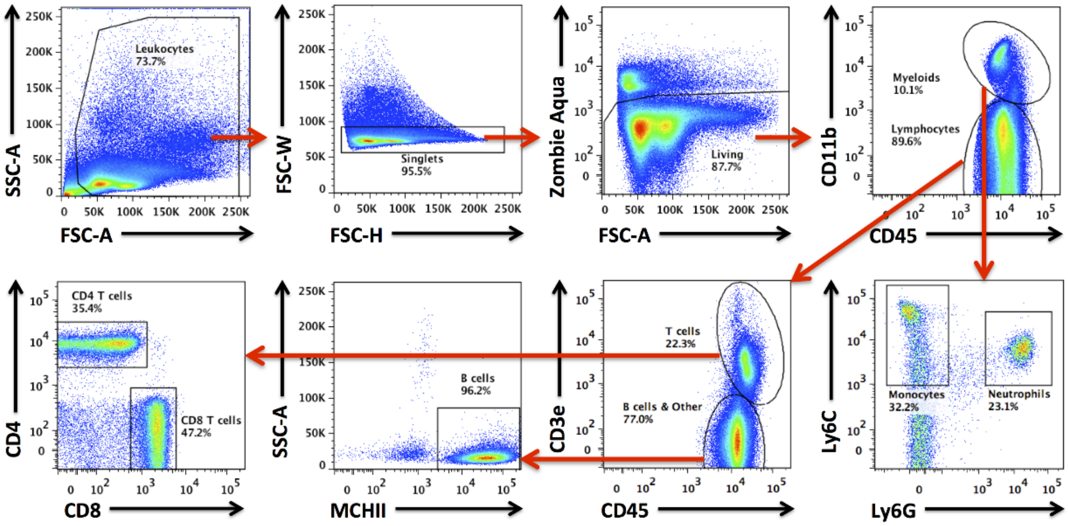

Figure 1. Gating strategy to identify immune cells in systemic immune compartments
Representative flow plots show the sequential gating strategy in which leukocyte populations were identified. Leukocytes were first identified by side scatter and forward scatter properties based on a splenocyte reference. Forward scatter width and height measures were used to determine singlets. Exclusion of the viability dye Zombie Aqua identified living cells. CD45-positive leukocytes were gated on CD11b+ to identify myeloid cells that were further subdivided into Ly6C+Ly6G- monocytes and Ly6C-Ly6G+ neutrophil subsets. CD45-positive CD11b-negative cells were subsequently gated on CD3+ to identify T lymphocytes that were further subdivided into CD4+ and CD8+ T cell subsets. The remaining CD45+CD11b-CD3e- cells were then gated on MHCII+ to identify B lymphocytes.

Analysis of blood revealed no significant changes in the percentage of circulating lymphocytes (MHCII$^+$ B cells and CD3$^+$ T cells) (**Figure 2A**) at 3 days post-injury. However, there was a modest but non-significant increase in the number of circulating lymphocytes in TBI + Hypo mice compared to TBI only mice. The percentage of CD11b$^+$ myeloid cells in blood was increased in Sham + Hypo mice compared to Sham only mice, but this change did not reach statistical significance (**Figure 2A**). TBI only and TBI + Hypo did not alter the percentage of CD11b$^+$ myeloid cells in blood at 3 days post-injury. Further analysis of myeloid cell subset determined that the percentage Ly6G+ neutrophils was significantly increased in in Sham + Hypo mice compared to Sham only mice ($p<0.05$ versus Sham only; **Figure 2B**). TBI only and TBI + Hypo mice had similar levels of Ly6G+ neutrophils that were reduced compared to levels in Sham + Hypo mice ($p<0.05$ versus Sham + Hypo). Deeper analysis of Ly6C+ monocytes revealed minor and non-significant changes in percentages of monocytes at 3 days post-injury (**Figure 2B**).

We then performed functional assessment of the systemic inflammatory response to TBI by evaluating reactive oxygen species (ROS) levels in myeloid cells in blood using the dye DHR123. When compared to Sham levels there were minor but non-significant reductions in ROS levels (as determined by MFI of DHR123) in Sham + Hypo, TBI only, and TBI + Hypo mice in both the Ly6C+Ly6G- monocyte and Ly6C-Ly6G+ neutrophil populations (**Figure 2C**). We also performed a Red latex bead uptake assay to determine phagocytic capabilities in myeloid cells. In the Ly6C+ monocytes there was a significant reduction in phagocytosis in Ly6C+ monocytes in Sham + Hypo, TBI only, and TBI + Hypo mice when compared to levels in Sham only mice ($p<0.05$ and $p<0.001$ versus Sham; **Figure 2D**). Similar defects in phagocytosis were observed in Ly6G+ neutrophils in Sham + Hypo, TBI only, and TBI + Hypo mice (**Figure 2D**), but reductions in phagocytic capacity failed to reach statistical significance (compared to Sham only levels).

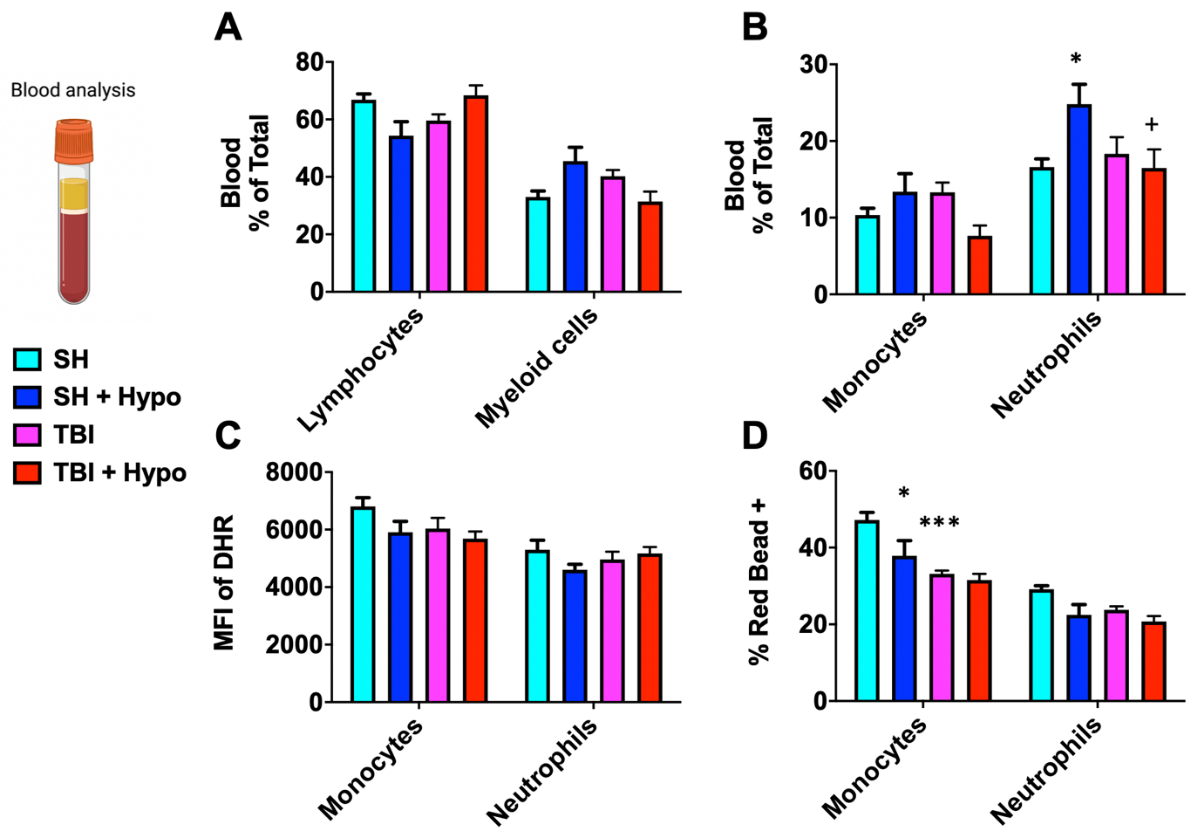

Figure 2. TBI + hypobaria (576 mmHg for 6 hours; 6-12 hours post-injury) had minor effects of systemic immune function in blood at 3 days post-injury

*Analysis of lymphocytes and myeloid cells (monocytes and neutrophils) in blood at 3 days post-injury (A and B). Ex vivo functional assessments of oxidative burst (DHR123; C) and phagocytosis (red latex bead engulfment; D) performed using flow cytometry in blood at 3 days post-injury. SH = sham; SH + Hypo = sham + hypobaria treatment; TBI = controlled cortical impact; TBI + Hypo = controlled cortical impact + hypobaria treatment. Statistical analysis: Two way ANOVA (injury x treatment (hypo)): Panel (B) Post-hoc analysis: Neutrophils: *p<0.05 versus Sham only group; +p<0.05 versus Sham+Hypo group. Panel (D) Post-hoc analysis: Monocytes: *p<0.05 versus Sham only group; ***p<0.001 versus Sham group. N = 6-8/group. Error bars show mean SEM.*

We next examined the effect of TBI and hypobaria exposure in altering leukocyte dynamics in spleen. TBI resulted in a significant increase in spleen weight at day 3 post-injury when compared to sham spleen weights (p<0.05 versus Sham; **Figure 3A**). There was not a significant increase in spleen weight in TBI + Hypo mice when compared to spleen weight in Sham + Hypo mice. We used flow cytometry to quantify leukocyte subpopulations in spleen and determined that there was a ~two-fold increase in splenic monocyte and neutrophil numbers in spleen at day 3 post-injury (p<0.001 versus Sham; **Figure 3B**). The levels of monocyte and neutrophil cells in TBI + Hypo mice were similar to TBI only mice meaning that hypobaria exposure did not further increase cell accumulation in the spleen.

Using the Red latex bead uptake we determined that there was a reduction in phagocytosis in Ly6G+ neutrophils in spleen from Sham + Hypo, TBI only, and TBI + Hypo mice when compared to levels in Sham only mice (**Figure 3C**); these changes did not reach statistical significance. There was also a non-significant trend to reduced phagocytic capacity in Ly6C+ monocytes from Sham + Hypo, TBI only, and TBI + Hypo mice (**Figure 3C**). Thus, there were minor reductions in myeloid cell phagocytic activity in spleen following TBI with subsequent hypobaria exposure. Next, oxidative stress levels in lymphocytes and myeloid cells in spleen was assessed using DHR123. There was no change in ROS levels with TBI or hypobaria exposure in lymphocytes (MHCII$^+$ B cells and CD3$^+$ T cells), neutrophils or monocytes at 3 days post-injury (**Figure 3D**). Finally, cytokine levels were assessed in myeloid cells in spleen using intracellular staining for TNF, and there was an increase expression of TNF in monocytes and neutrophils in spleen at 3 days post-injury injury (**Figure 3E**), but hypobaria exposure did not alter these levels significantly.

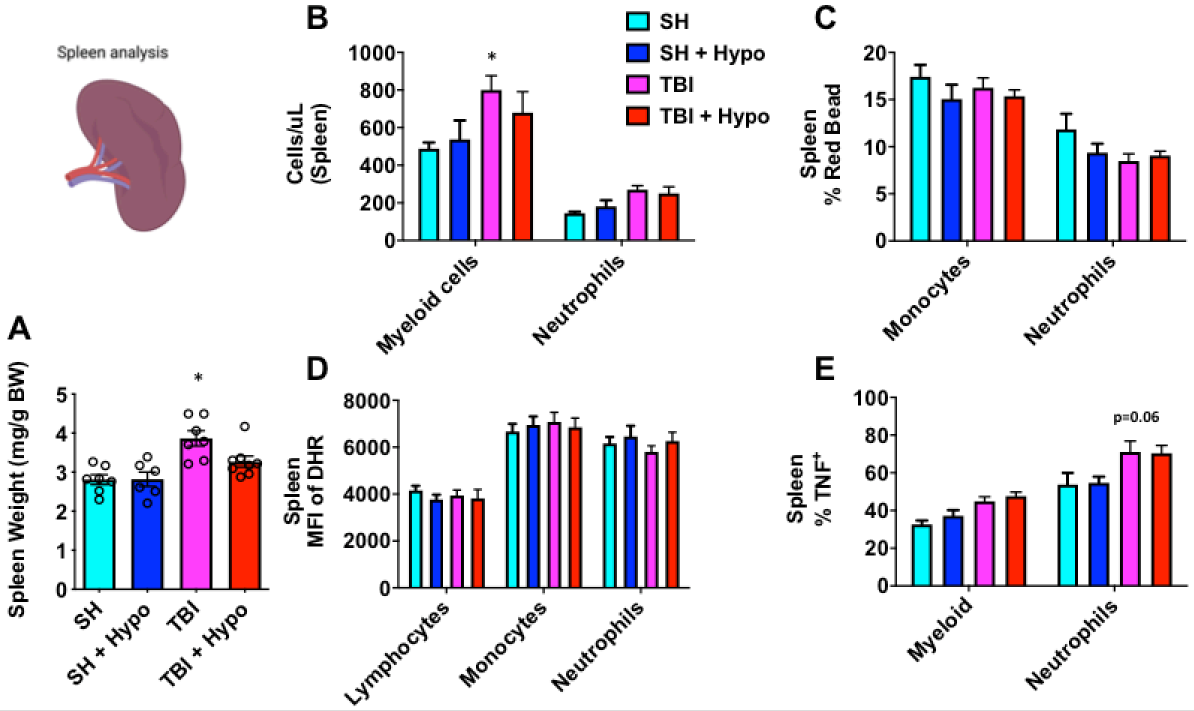

Figure 3. Hypobaria exposure did not significantly alter TBI-induced immune dysfunction in spleen at 3 days post-injury

*Analysis of gross changes in spleen as measured by spleen weight (expressed as mg/g body weight; A). Analysis of lymphocytes and myeloid cells (monocytes and neutrophils) in spleen at 3 days post-injury (B). Ex vivo functional assessments of phagocytosis (red latex bead engulfment; C) and oxidative burst (DHR123; D) performed using flow cytometry in spleen at 3 days post-injury. Ex vivo assessments of TNF cytokine expression in myeloid cells in spleen (E). SH = sham; SH + Hypo = sham + hypobaria treatment; TBI = controlled cortical impact; TBI + Hypo = controlled cortical impact + hypobaria treatment. N = 6-8/group. Statistical analysis: Two way ANOVA (injury x treatment (hypo)). (A) Post-hoc analysis: Spleen mass: *p<0.05 versus Sham only group. (B) Post-hoc analysis: Myeloid cells: *p<0.05 versus Sham only group. (E) Post-hoc analysis: Neutrophils: p=0.06 versus Sham only group. For all experiments, N=7-8/group. Error bars show mean SEM.*

5.2 Hypobaria exposure increases macrophage accumulation in the injured brain and increases microglial ROS production and phagocytosis activity

We next analyzed brain tissue by flow cytometry to assess resident microglial function and infiltration of inflammatory brain macrophages from the periphery. These cell populations in brain were discriminated by levels of CD45 ($CD45^{high}$ = infiltrating brain macrophage; $CD45^{int}$ = resident microglia). Analysis of absolute cell numbers in brain revealed that there was an increase in numbers of resident microglia ($CD11b+CD45^{int}$) in the TBI + Hypo group compared to all other groups (Sham, Sham + Hypo, TBI only; **Figure 4A**), but the increased proliferation of these microglia did not reach statistical significance. Assessment of infiltrating macrophages ($CD11b+CD45^{high}$) demonstrated a robust and significant increase in numbers of brain macrophages in TBI group compared to levels in Sham ($p<0.001$ versus sham; **Figure 4A**). There was a further increase in numbers of accumulating brain macrophages with hypobaria exposures following TBI, but the increased accumulation of macrophages in TBI + Hypo mice did not reach statistical significance when compared with levels in TBI only mice (**Figure 4A**).

We then investigated functional markers in microglia using DHR123 and Red latex bead assays for ROS and phagocytosis, respectively. There was a significant increase in ROS levels (as assessed by DHR123 MFI) in microglia from TBI only group compared to levels in Sham mice ($p<0.01$ versus sham; **Figure 4B**). Notably, there was a further increase microglial ROS levels with hypobaria exposure following TBI, such that there was a significantly increased ROS production in microglia from TBI + Hypo mice when compared with levels in TBI only mice ($p<0.05$ versus TBI only; **Figure 4B**). When we assessed phagocytic activity using the Red latex bead assay there was a significant increase in phagocytic function in microglia from TBI only group compared to levels in Sham mice ($p<0.001$ versus sham; **Figure 4C**). Notably, there was a further increase microglial phagocytic function with hypobaria exposure following TBI, such that there was a significantly increased latex bead uptake in microglia from TBI + Hypo mice when compared with levels in TBI only mice ($p<0.05$ versus TBI only; **Figure 4C**). Combined, these functional assays in microglia indicate that hypobaria exposure increased ROS production and phagocytosis activity following TBI.

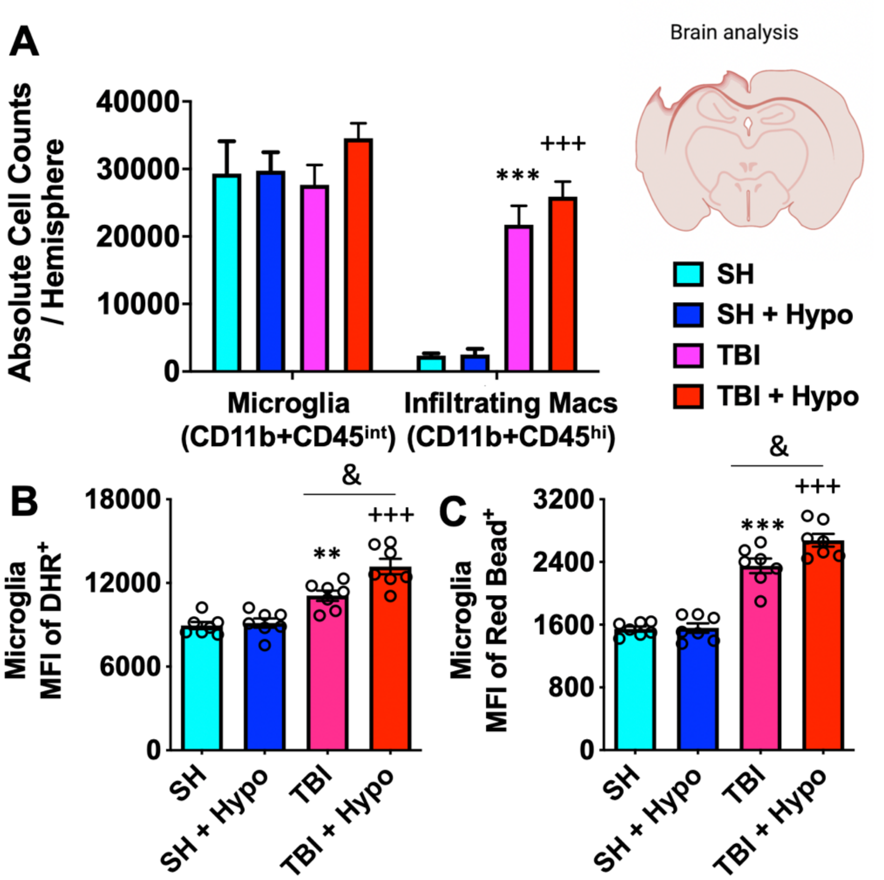

Figure 4. Hypobaria exposure increased infiltrating macrophage accumulation in brain and increased microglial ROS levels and phagocytic activity

Analysis of resident microglia (Cd11b+CD45^{int}*) and infiltrating brain macrophages (Cd11b+CD45*$^{h}{}_{igh}$*) in brain at 3 days post-injury (A). Ex vivo functional assessments of oxidative burst (DHR123; B) and phagocytosis (red latex bead engulfment; C) in resident microglia performed using flow cytometry at 3 days post-injury. SH = sham; SH + Hypo = sham + hypobaria treatment; TBI = controlled cortical impact; TBI + Hypo = controlled cortical impact + hypobaria treatment. N = 6-8/group. Statistical analysis: Two way ANOVA (injury x treatment (hypo)). (A) Post-hoc analysis: Infiltrating brain macrophages: ***p<0.001 versus Sham only group; +++p<0.001 versus Sham+Hypo. (B) Post-hoc analysis: Microglia DHR123: **p<0.01 versus Sham only group; +++p<0.001 versus Sham+Hypo group; &p<0.05 versus TBI only group. (C) Post-hoc analysis: Microglia Red Bead phagocytosis: ***p<0.001 versus Sham only group; +++p<0.001 versus Sham+Hypo group; &p<0.05 versus TBI only group. Error bars show mean SEM.*

We also measured inflammatory mRNA markers in the cortex from sham and TBI mice that had been exposed to hypobaria to determine tissue level neuroinflammatory responses. As expected, there was a very robust induction of pro-inflammatory mRNA expression in the cortex following TBI, such that there was a significant increase in levels of TNF, NOX2, CD68, CCL2, and CCL5 mRNA in the TBI only group when compared to the sham group (p<0.001 versus sham; **Figure 5A-E**). Hypobaria exposure did not significantly increase the TBI-induced levels of each pro-inflammatory mRNA marker at 3 days post-injury, indicating that it did not further exacerbate the neuroinflammatory response.

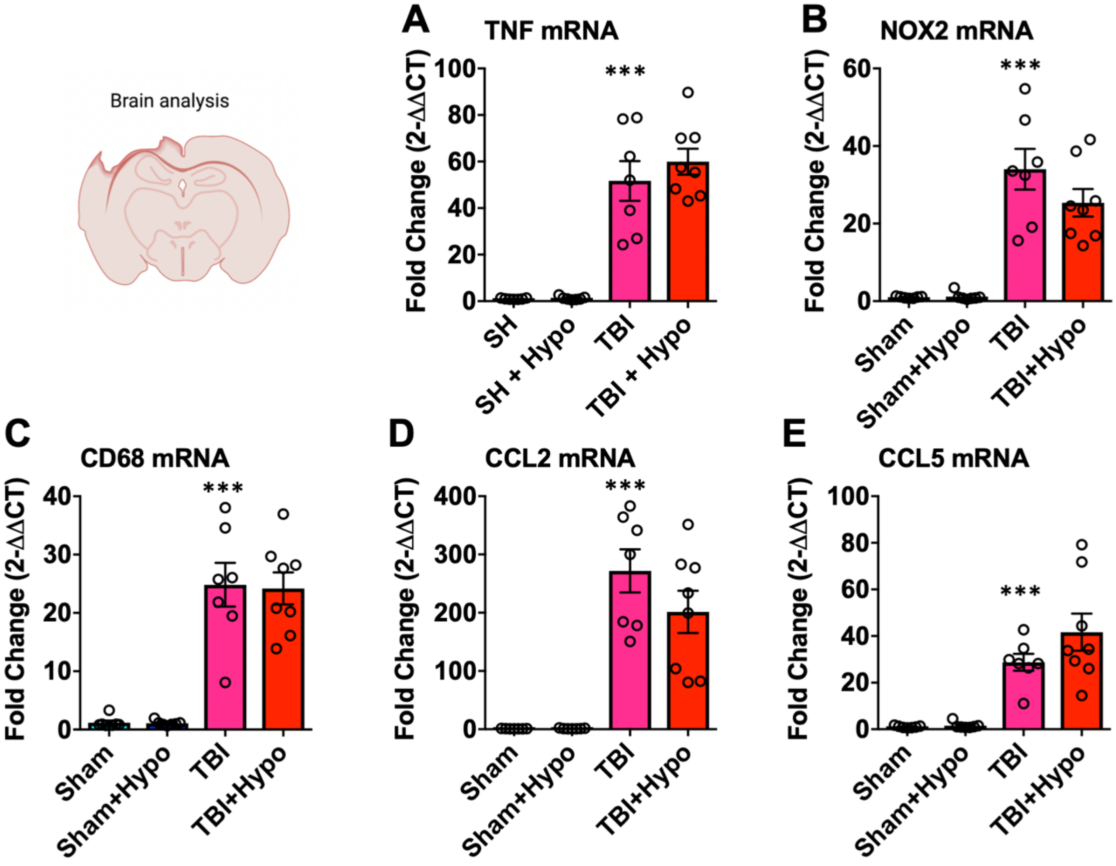

Figure 5. Hypobaria exposure does not exacerbate inflammatory mRNA expression levels in the injured cortex

*Analysis of several inflammatory mRNA in the cortex from sham and TBI mice at 3 days post-injury by real-time qPCR. TNF (A), NOX2 (B), CD68 (C), CCL2 (D) and CCL5 (E). SH = sham; SH + Hypo = sham + hypobaria treatment; TBI = controlled cortical impact; TBI + Hypo = controlled cortical impact + hypobaria treatment. N = 6-8/group. Statistical analysis: Two way ANOVA (injury x treatment (hypo)). (A-E) Post-hoc analysis: ***p<0.001 versus Sham only group. Error bars show mean SEM.*

5.3 Hypobaria exposure does not worsen long-term neurological impairments after TBI

To determine the long-term functional consequences of short duration hypobaria exposure after TBI we followed sham and TBI mice that were/were not exposed to hypobaria treatment (568 mmHg for 6 hours) for up to 28 days post-injury. A battery of neurological tests for motor and cognitive function were performed as described in the experimental protocol.

Adult male C57Bl/6J mice were trained on the beam walk task prior to TBI and had to obtain a minimum competence in the fine motor coordination test (<10 foot faults) prior to surgery. All groups of mice achieved this baseline training competence. As expected, TBI induced significant fine motor coordination deficits such that TBI mice had a high number of foot faults (>45) after injury when compared to sham mice (p<0.001versus sham group; **Figure 6**). These motor function deficits persisted up to 28 days post-injury with modest levels of recovery (40 foot faults). Hypobaria exposure did not exacerbate the fine motor coordination deficits after TBI. In addition, hypobaria exposure of sham mice did not alter their performance in the fine motor coordination task.

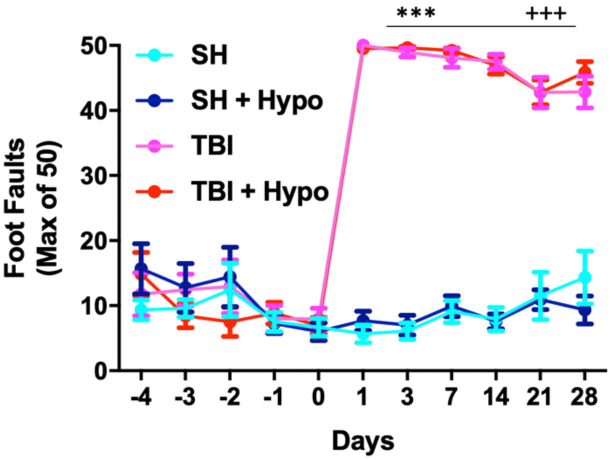

Figure 6. Hypobaria exposure does not worsen fine motor coordination in the beam walk test through 28 days post-injury

*Analysis of fine motor coordination prior to and following TBI +/- Hypobaria exposure using a beam walk coordination task. Repeated measures Two way ANOVA (injury x treatment (hypo)). Interaction: F (30, 460) = 39.02, p<0.0001. Injury factor: F (10, 460) = 99.81, p<0.0001. Treatment factor: F (3, 46) = 88.82, p<0.0001. Post-hoc analysis by Tukey's multiple comparisons test: ***p<0.001 versus Sham group, and +++p<0.001 versus Sham+Hypo. N = 12/group. Abbreviations: SH = sham; SH + Hypo = sham + hypobaria treatment; TBI = controlled cortical impact; TBI + Hypo = controlled cortical impact + hypobaria treatment. Error bars show mean SEM.*

To assess the effect of hypobaria exposure on hippocampal dependent spatial learning and memory the mice were assessed using a Morris Water Maze test from days 21-25 post-injury. As expected, TBI induced significant deficits in spatial learning and memory in this task. When compared to sham levels, TBI mice took longer to find the hidden submerged platform on each of the training days (1-4) in the latency analysis (**Figure 7A**). There was no difference in latency times between the TBI only and TBI + Hypo groups. The Sham + Hypo mice took more time to find the platform in the acquisition trials when compared to Sham only mice, but this difference was not statistically significant. A probe trial was performed on the 5th day of the test (at 25 days post-injury), and the predicted impairments in retention memory was observed in TBI mice, such that they spent more time looking for the missing platform in the target quadrant when compared to Sham mice (**Figure**

7B). Hypobaria exposure did not significantly increase latency times in the probe trial, indicating that it did not further worsen spatial learning and memory in TBI mice.

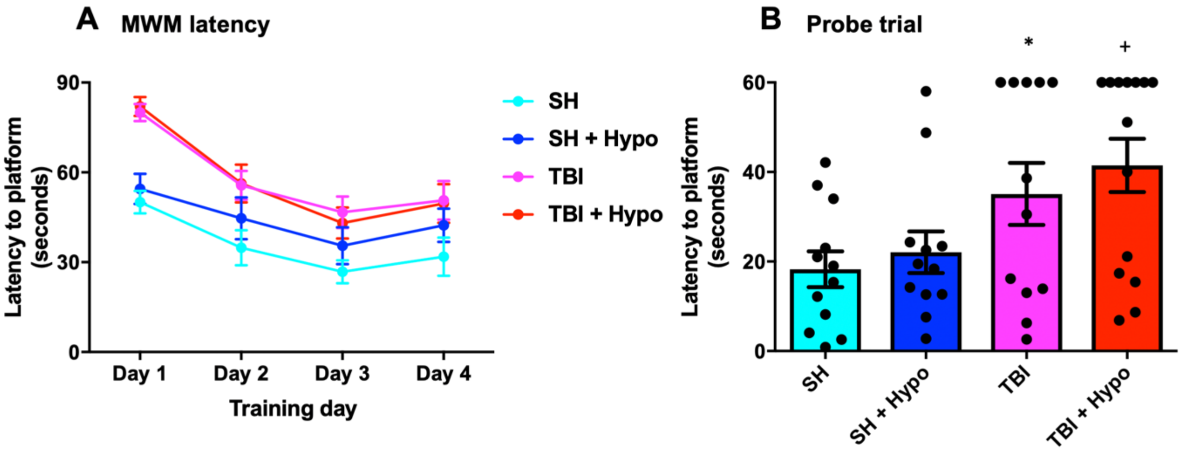

Figure 7. Hypobaria exposure did not worsen cognitive function impairments after TBI in the Morris Water Maze test

*Analysis of cognitive function following TBI with/without hypobaria using a Morris Water Maze task. (A) Latency to find the submerged platform on training days 1-4. Repeated measures Two way ANOVA (injury x treatment (hypo)). Interaction: F (9, 138) = 0.8664, p=0.5569. Injury factor: F (3, 138) = 25.94, p<0.0001. Treatment factor: F (3, 46) = 9.992, p<0.0001. (B) Probe trial on day 5. Two way ANOVA (injury x treatment (hypo)). Injury factor: F (3, 46) = 3.944 p=0.0138. Treatment factor: F (3, 46) = 9.992, p<0.0001. Post-hoc analysis by Tukey's multiple comparisons test: *p<0.05 versus Sham group, and +p<0.05 versus Sham+Hypo. N = 12/group. Abbreviations: SH = sham; SH + Hypo = sham + hypobaria treatment; TBI = controlled cortical impact; TBI + Hypo = controlled cortical impact + hypobaria treatment. N = 12/group. Error bars show mean SEM.*

Finally, to assess the effect of hypobaria exposure on declarative memory the mice were assessed using a novel object recognition test from days 26-27 post-injury. As expected, TBI induced significant deficits in declarative memory in this task. When compared to sham levels, TBI mice spent less time with the novel object in the arena indicating deficits in memory storage and declarative memory function (**Figure 8**). Notably, there was no difference in time spent with novel object between the TBI only and TBI + Hypo groups, indicating that hypobaria exposure did not further increase memory deficits in this task.

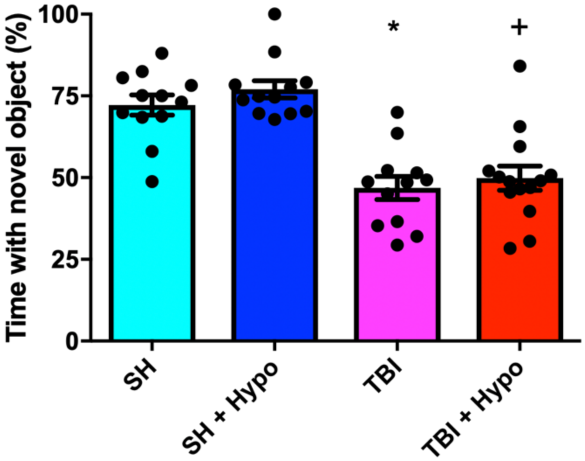

Figure 8. Hypobaria exposure did not worsen cognitive function impairments after TBI in the novel object recognition test

*Analysis of declarative memory following TBI with/without hypobaria treatment using a novel object recognition task. Time spent with novel object (%). Two way ANOVA (injury x treatment (hypo)). Injury factor: F (3, 46) = 21.16 p<0.0001. Post-hoc analysis by Tukey's multiple comparisons test: *p<0.05 versus Sham group, and +p<0.05 versus Sham+Hypo. N = 12/group. Abbreviations: SH = sham; SH + Hypo = sham + hypobaria treatment; TBI = controlled cortical impact; TBI + Hypo = controlled cortical impact + hypobaria treatment. N = 12/group. Error bars show mean SEM.*

6.0 DISCUSSION

Our advanced analysis using flow cytometry and functional assays has enabled up to characterize the impact of TBI on systemic and central immune responses and to better understand the pathogenesis of TBI-induced immune dysfunction in secondary organs such as spleen. We showed that:

- TBI + hypobaria (576 mmHg for 6 hours; 6-12 hours post-injury) had minor effects of systemic immune function in blood at 3 days post-injury
- Hypobaria exposure did not significantly alter TBI-induced immune dysfunction in spleen at 3 days post-injury
- Hypobaria exposure increased infiltrating macrophage accumulation in brain and increased microglial ROS levels and phagocytic activity
- Hypobaria exposure does not exacerbate inflammatory mRNA expression levels in the injured cortex
- Hypobaria exposure does not worsen fine motor coordination in the beam walk test through 28 days post-injury
- Hypobaria exposure did not worsen cognitive function impairments after TBI in the Morris Water Maze test or in the novel object recognition test

7.0 CONCLUSIONS

Here we demonstrated that TBI results in activation of systemic immune components such as neutrophils and monocytes leading to dysregulation of functional response (production of cytokines, ROS, phagocytosis) in blood and spleen. Our data show that hypobaria had modest effects on TBI-induced systemic and central activation of inflammatory responses except for a further increase microglial ROS levels and microglial phagocytic function with hypobaria exposure following TBI when compared with levels in TBI only mice. Importantly, we observed no effect of hypobaric to increase TBI-induced motor and cognitive dysfunctions. Overall, the current findings differ from our previous work using *rat lateral fluid percussion model of TBI* in which hypobaria amplified posttraumatic secondary injury and neurological dysfunctions. We conclude that hypobaria effects on TBI outcomes is species and brain trauma model specific.

8.0 REFERENCES

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LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

%	Percent
ft	Feet
FPI	Fluid Percussion Injury
USAF	United States Air Force
DoD	Department of Defense
ADSM	Injured Active Duty Service Members
AE	Aeromedical Evacuation
CCI	Controlled Cortical Impact
TBI	Traumatic Brain Injury
mmHg	Millimeters of Mercury
mm	Millimeter
ROS	Reactive Oxygen Species
TNF	Tumor Necrosis Factor