Brain, Behavior, and Immunity xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Full-length Article

Cognitive deficits develop 1 month after diffuse brain injury and are exaggerated by microglia-associated reactivity to peripheral immune challenge

Megan M. Muccigrosso [a], Joni Ford [a], Brooke Benner [a], Daniel Moussa [a], Christopher Burnsides [a], Ashley M. Fenn [a], Phillip G. Popovich [a,b,c], Jonathan Lifshitz [d], Fredrick Rohan Walker [e], Daniel S. Eiferman [f], Jonathan P. Godbout [a,b,c,*]

[a] Department of Neuroscience, The Ohio State University, 333 W. 10th Ave, Columbus, OH, United States
[b] Center for Brain and Spinal Cord Repair, The Ohio State University, 460 W. 12th Ave, Columbus, OH, United States
[c] Institute for Behavioral Medicine Research, The Ohio State University, 460 Medical Center Dr., Columbus, OH, United States
[d] Barrow Neurological Institute at Phoenix Children's Hospital, Department of Child Health, University of Arizona, College of Medicine-Phoenix, Phoenix, AZ, United States
[e] School of Biomedical Sciences and Pharmacy, University of Newcastle, Callaghan 2308, South Wales, Australia
[f] Department of Surgery, The Ohio State University, 395 W. 12th Avenue, Columbus, OH, United States

ARTICLE INFO

Article history:
Received 22 October 2015
Received in revised form 5 January 2016
Accepted 12 January 2016
Available online xxxx

Keywords:
Traumatic brain injury
Fluid percussion injury
Microglia
Astrocytes
Cognitive decline
Lipopolysaccharide
Neuroinflammation

ABSTRACT

Traumatic brain injury (TBI) elicits immediate neuroinflammatory events that contribute to acute cognitive, motor, and affective disturbance. Despite resolution of these acute complications, significant neuropsychiatric and cognitive issues can develop and progress after TBI. We and others have provided novel evidence that these complications are potentiated by repeated injuries, immune challenges and stressors. A key component to this may be increased sensitization or priming of glia after TBI. Therefore, our objectives were to determine the degree to which cognitive deterioration occurred after diffuse TBI (moderate midline fluid percussion injury) and ascertain if glial reactivity induced by an acute immune challenge potentiated cognitive decline 30 days post injury (dpi). In post-recovery assessments, hippocampal-dependent learning and memory recall were normal 7 dpi, but anterograde learning was impaired by 30 dpi. Examination of mRNA and morphological profiles of glia 30 dpi indicated a low but persistent level of inflammation with elevated expression of GFAP and IL-1β in astrocytes and MHCII and IL-1β in microglia. Moreover, an acute immune challenge 30 dpi robustly interrupted memory consolidation specifically in TBI mice. These deficits were associated with exaggerated microglia-mediated inflammation with amplified (IL-1β, CCL2, TNFα) and prolonged (TNFα) cytokine/chemokine expression, and a marked reactive morphological profile of microglia in the CA3 of the hippocampus. Collectively, these data indicate that microglia remain sensitized 30 dpi after moderate TBI and a secondary inflammatory challenge elicits robust microglial reactivity that augments cognitive decline.

Statement of Significance: Traumatic brain injury (TBI) is a major risk factor in development of neuropsychiatric problems long after injury, negatively affecting quality of life. Mounting evidence indicates that inflammatory processes worsen with time after a brain injury and are likely mediated by glia. Here, we show that primed microglia and astrocytes developed in mice 1 month following moderate diffuse TBI, coinciding with cognitive deficits that were not initially evident after injury. Additionally, TBI-induced glial priming may adversely affect the ability of glia to appropriately respond to immune challenges, which occur regularly across the lifespan. Indeed, we show that an acute immune challenge augmented microglial reactivity and cognitive deficits. This idea may provide new avenues of clinical assessments and treatments following TBI.

* Corresponding author at: 231 IBMR Bldg., 460 Medical Center Dr., The Ohio State University, Columbus, OH 43210, United States.
E-mail address: jonathan.godbout@osumc.edu (J.P. Godbout).

http://dx.doi.org/10.1016/j.bbi.2016.01.009
0889-1591/

1. Introduction

TBI is the leading cause of neurological disability in the United States and there is significant risk of neuropsychiatric illness after injury (Centers for Disease Control and Prevention, 2015). For instance, depressive complications and cognitive decline often occur in humans after TBI (Rosenthal et al., 1998; Hart et al., 2012). Indeed, 15–30% of TBI patients experience cognitive decline over time (Ruff et al., 1991; Himanen et al., 2006; Salmond et al., 2006; Till et al., 2008; Almeida et al., 2015) that may continue to progress after injury (Millis et al., 2001; Wang et al., 2012). For example, in a 5 year study, 27% of the TBI patients presented with reduced verbal fluency and verbal list learning (Till et al., 2008). The underlying cause of these neuropsychiatric complications after TBI is unclear but may be related to ongoing neuroinflammatory processes (Norden et al., 2014b).

Mounting evidence indicates that microglia-mediated inflammation persists long after TBI. For instance, clinical studies report elevated metabolic activity, white matter abnormalities and microglial activation persisting long after injury (Brooks et al., 2000; Ramlackhansingh et al., 2011). These findings are paralleled in rodent models of both penetrating and diffuse brain injury. For example, $CD68^+$ microglia were detected in the lesion site 1 year after controlled cortical impact injury (CCI) (Loane et al., 2014) and increased major histocompatibility complex (MHC)II and CD68 were also detected 14–30 days after diffuse brain injury in rats (Ziebell et al., 2012) and mice (Fenn et al., 2014). These studies provide evidence that microglia maintain a primed profile after TBI (Witcher et al., 2015). In the context of cognition, enhanced glial inflammation and concomitant cognitive decline are evident after TBI. For example, repeated closed head injury (CHI) in mice resulted in increased GFAP (astrocytes) and Iba-1 (microglia) labeling, and evidence of white matter damage that corresponded with deficits in hippocampal-dependent learning 12–18 months after injury (Mouzon et al., 2014). Clinical studies also link glial reactivity with cognitive impairment after injury. For example, PET scan shows amplified microglial activation in the thalamus that was associated with lower performance on tests of processing speed (Ramlackhansingh et al., 2011). Thus, inflammation is likely a contributing factor of cognitive decline after TBI.

Microglia and astrocytes have dynamic roles in coordinating responses between the immune system and the brain (Norden et al., 2014a, 2015). Therefore, persistent glial reactivity after TBI (Fenn et al., 2014; Hazra et al., 2014) may impair immune surveillance and cause maladaptive behavioral responses (Norden et al., 2014b). We and others have identified a population of "primed" microglia in the aged brain that become hyper-reactive following acute immune challenge (lipopolysaccharide (LPS) or *Escherichia coli*) (Chen et al., 2008; Barrientos et al., 2009b; Henry et al., 2009). These primed microglia have elevated MHCII expression and produce exaggerated levels of pro-inflammatory cytokines (Henry et al., 2009) that correspond with impaired cognitive performance (Chen et al., 2008; Barrientos et al., 2009b, 2010) and prolonged depressive-like behavior (Godbout et al., 2008). Similarly, 30 days after a diffuse brain injury in mice, an acute immune challenge (LPS) caused a hyper-reactive microglial inflammatory response that triggered the development of depressive-like behavior (Fenn et al., 2014). Thus, a primed profile of microglia and astrocytes after TBI may set the stage for exaggerated responses to acute challenges precipitating the development of neuropsychiatric complications.

The goals of this study were to determine the extent of cognitive decline after diffuse TBI and ascertain if immune challenge potentiates cognitive decline by augmenting glia-mediated inflammation. The midline fluid percussion injury (FPI) model was used because it causes mild neuronal pathology including diffuse axonal injury and transient neurological deficits that recapitulate complications after mild to moderate concussive head trauma (Morales et al., 2005; Lifshitz et al., 2007; Lifshitz, 2009). Here we extend our previous findings to show that mice recover from TBI and have normal learning and memory 7 dpi, but begin to develop cognitive deficits by 30 dpi. Moreover, microglia and astrocytes have heightened inflammatory gene expression 30 dpi and induction of an immune response 30 dpi further amplifies microglial activation and exacerbates deficits in memory recall.

2. Materials and methods

2.1. Mice and LPS injections

Adult (3 mo) male BALB/c mice were bred at The Ohio State University (OSU). Mice were individually housed and maintained at 25 °C under a 12 h light/12 h dark cycle with *ad libitum* access to food and water. For injections, mice were intraperitoneally (i.p.) injected 30 dpi with saline or LPS (0.33 mg/kg; serotype 0127:B8, Sigma) 1–2 h before the start of the dark phase (Godbout et al., 2005; Fenn et al., 2012). The LPS dosage was selected because it elicits a pro-inflammatory cytokine response in the brain resulting in a transient sickness response (24 h) in uninjured adult mice (Berg et al., 2004; Godbout et al., 2005). To control for sickness induction and subsequent recovery, food intake and body weight were determined over a 72 h time course (data not shown). All procedures were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and were approved by The Ohio State University Institutional Laboratory Animal Care and Use Committee.

2.2. Midline fluid percussion injury

Mice received a midline diffuse TBI using a fluid percussion injury (FPI) apparatus (Custom Design & Fabrication, Richmond, VA) as previously described (Fenn et al., 2014, 2015). This diffuse injury occurs in the absence of a contusion, does not induce tissue cavitation or gross neuronal loss, and causes diffuse axonal injury in the neocortex, hippocampus, and dorsolateral thalamus (Kelley et al., 2006, 2007; Bachstetter et al., 2013; Fenn et al., 2014). To prepare for FPI, mice were anesthetized with 5% isoflurane and were stabilized using head ear bars in a stereotaxic frame. Anesthesia was maintained with continuous inhalation of isoflurane (1.5–3%) through a nose-cone. Next, a midline craniectomy between bregma and lambda was performed with a 3 mm outer diameter trephine. A rigid Luer-loc needle hub was secured over the craniectomy and capped. After 4–6 h, injury was induced by filling the injury hub with saline and imposing a 10 ms pulse of saline (1.2 atmospheres; 670–720 mV) onto the dura through the hub (Witgen et al., 2006; Lifshitz et al., 2007; Fenn et al., 2014). All Sham controls received the same procedure without the fluid pulse. Immediately after injury, the injury hub was removed, dural integrity was confirmed, and mice were evaluated for injury severity using the self-righting test (Lifshitz et al., 2007). Mice with a confirmed dura breach were euthanized immediately after injury and excluded from the study. Self-righting inclusion criteria were based on our previous work with BALB/c mice (Fenn et al., 2014). Only mice with a moderate TBI were used: Sham $\leqslant$ 60 s; 60 s < mild $\leqslant$ 200 s; 200 s < moderate $\leqslant$ 540 s; severe > 540 s.

2.3. Recovery and nesting behavior

For all Sham and TBI mice, recovery after injury was monitored post-operatively (e.g. grooming, body weight, nesting behavior) for

Please cite this article in press as: Muccigrosso, M.M., et al. Cognitive deficits develop 1 month after diffuse brain injury and are exaggerated by microglia-associated reactivity to peripheral immune challenge. Brain Behav. Immun. (2016), http://dx.doi.org/10.1016/j.bbi.2016.01.009