Immune Activation Promotes Depression 1 Month After Diffuse Brain Injury: A Role for Primed Microglia

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Background: Traumatic brain injury (TBI) is associated with a higher incidence of depression. The majority of individuals who suffer a TBI are juveniles and young adults, and thus, the risk of a lifetime of depressive complications is a significant concern. The etiology of increased TBI-associated depression is unclear but may be inflammatory-related with increased brain sensitivity to secondary inflammatory challenges (e.g., stressors, infection, and injury).

Methods: Adult male BALB/c mice received a sham ($n = 52$) or midline fluid percussion injury (TBI; $n = 57$). Neuroinflammation, motor coordination (rotarod), and depressive behaviors (social withdrawal, immobility in the tail suspension test, and anhedonia) were assessed 4 hours, 24 hours, 72 hours, 7 days, or 30 days later. Moreover, 30 days after surgery, sham and TBI mice received a peripheral injection of saline or lipopolysaccharide (LPS) and microglia activation and behavior were determined.

Results: Diffuse TBI caused inflammation, peripheral cell recruitment, and microglia activation immediately after injury coinciding with motor coordination deficits. These transient events resolved within 7 days. Nonetheless, 30 days post-TBI a population of deramified and major histocompatibility complex II^+ (primed) microglia were detected. After a peripheral LPS challenge, the inflammatory cytokine response in primed microglia of TBI mice was exaggerated compared with microglia of controls. Furthermore, this LPS-induced microglia reactivity 30 days after TBI was associated with the onset of depressive-like behavior.

Conclusions: These results implicate a primed and immune-reactive microglial population as a possible triggering mechanism for the development of depressive complications after TBI.

Key Words: Cytokines, depression, fluid percussion injury, lipopolysaccharide, major histocompatibility complex II, microglia

Traumatic brain injury (TBI) elicits immediate neuroinflammatory events that contribute to acute cognitive, motor, and behavioral disturbances (1–4). Despite resolution of these acute complications, depression can develop and persist years after TBI (5–7). Indeed, individuals who suffer a TBI are 5 to 10 times more likely to develop symptoms of depression compared with the general population (8). Depressive symptoms are diagnosed in 30% to 40% of individuals within the first year of TBI (5,9) and in 60% of individuals within 8 years of TBI (10), and 50 years after TBI patients continue to report higher rates of depression (11). Moreover, most (69%; Centers for Disease Control and Prevention, 2002–2006) brain injuries occur in juveniles (34.7%, 0–14 years) and young adults (34.3%, 15–34 years) implicating an increased risk for a lifetime of depressive complications that negatively affect quality of life and life span (11,12). Furthermore, the limited number studies on antidepressant therapies (e.g., amitriptyline, sertraline) after TBI show reduced efficacy in TBI patients (13,14). We propose that TBI-associated depression is inflammatory-based and associated with increased brain sensitivity to acute immune challenges.

In support of this premise, clinical and experimental data indicate a cause-and-effect relationship between inflammation and depression (15,16). Patients with higher inflammatory cytokine levels in circulation and within the central nervous system (CNS) report a higher incidence of treatment-resistant depression (15). These patients have elevated levels of the inflammatory cytokine interleukin (IL)-6 in circulation, and antidepressant therapies fail to reduce tumor necrosis factor (TNF)-α (17). Critically, TBI patients have increased levels of IL-6, IL-1β, and TNF-α in cerebrospinal fluid (18,19) and serum (20) immediately after injury. Moreover, markers of neuroinflammation (e.g., CD68, CR3/43) persist in the brain parenchyma up to 16 years after TBI (21). Although several studies report increased neuroinflammation after TBI and others report increased depression after TBI, the extent to which prolonged brain inflammation contributes to neurobehavioral complications after TBI is unclear.

One potential consequence of heightened and prolonged brain inflammation after TBI is increased sensitivity to secondary challenges including subsequent injuries, stressors, and infections (22). In models of aging, stress, early-life infection, sterile CNS injury, and preclinical neurologic disease increased sensitivity to inflammatory challenges corresponds with the development of a primed and more inflammatory microglia phenotype (e.g., increased major histocompatibility complex II [MHCII], IL-1β, CD68, complement receptor [CR]3) (23–27). Microglia are the innate immune cells of the CNS and responsible for interpreting and propagating inflammatory signals that affect neuronal function (28,29). Thus, enhanced microglial activation and

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amplified inflammatory cytokine production can impair normal neurologic function. In support of this idea, primed and MHCII$^+$ microglia in the aged brain become hyperreactive to a systemic injection of lipopolysaccharide (LPS) and produce exaggerated levels of IL-1β (23) corresponding to impaired cognitive performance (22,30,31), protracted sickness behavior (32,33), and depressive-like behavior (34). Relevant to the axonal and neuronal damage done during TBI, models of optic nerve crush also demonstrate microglia priming (CD68$^+$) and exaggerated IL-1β, TNF-α, and IL-6 expression after LPS challenge 28 days postinjury (dpi) (27). Although not discussed in the context of microglial priming, increased MHCII (OX6) expression has also been detected in the brain 16 dpi in a rat model of cerebral contusion (35). Thus, a primed microglia phenotype after TBI may set the stage for exaggerated responses to acute challenges, precipitating the development of chronic neuropsychiatric disorders.

On the basis of these data, we hypothesize that a diffuse TBI induces microglial priming and that an acute immune challenge weeks to months after injury results in a hyperinflammatory microglia response triggering the development of depressive-like behavior. To test this hypothesis, the midline fluid percussion injury (FPI) model of TBI was used in mice. Midline FPI causes mild neuronal pathology including diffuse axonal injury (36) and transient neurologic deficits (37) that recapitulate complications after mild to moderate concussive head injuries in humans (38). Here, we show that TBI caused immediate, but transient, neuroinflammation and behavioral impairments. Nonetheless, evidence of microglial priming was detected in the brain 30 dpi. Furthermore, activation of the peripheral immune system 30 days after TBI caused exaggerated microglial expression of IL-1β and TNF-α corresponding with induction of depressive-like behavior. Collectively, these data support the premise that a diffuse TBI sensitizes the brain to secondary inflammatory challenges that may precipitate depression.

Methods and Materials

Mice and LPS Injections

Adult (3-month-old) male BALB/c mice were obtained from a breeding colony at the Ohio State University. Mice were individually housed and maintained at 25°C under a 12-hour light-dark cycle with ad libitum access to food and water. For injections, mice were intraperitoneally injected 30 days postinjury (dpi) with saline or LPS (.33 mg/kg; serotype 0127:B8, Sigma-Aldrich, St. Louis, Missouri) 1 to 2 hours before the start of the dark phase (between 5:00 and 7:00 PM) (32,39). All procedures were in accordance with the National Institutes 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.

Midline Fluid Percussion Injury

Mice received a midline and diffuse TBI using a FPI apparatus (Custom Design and Fabrication, Richmond, Virginia) as previously described (40) and detailed in Materials and Methods in Supplement 1. This diffuse injury is well characterized and occurs in the absence of contusion, tissue cavitation, or gross neuronal loss and causes diffuse axonal injury in the neocortex, hippocampus, and dorsolateral thalamus (41–43). Immediately after sham injury or TBI, the injury hub was removed, dural integrity was confirmed, and mice were evaluated for injury severity using the self-righting test (2). On the basis of previous studies in FPI (44), self-righting inclusion criteria was modified for BALB/c mice as follows: sham ≤ 60 seconds; 60 seconds < mild ≤ 200 seconds; 200 seconds < moderate ≤ 540 seconds; severe > 540 seconds. Only mice with a moderate TBI were used.

Motor Function and Depressive-Like Behavior

Motor coordination was assessed using rotarod (Rotamex, Columbus, Ohio) as previously described (45) and detailed in Materials and Methods in Supplement 1.

Activity was determined using an activity box paradigm (Open Field and Fusion software; AccuScan Instruments, Columbus, Ohio). Mice were placed into independent 8 × 8 inch chambers and automated software packaging was configured to determine the total distance traveled and total movement time for 10 minutes. A subset of mice was tested for 30 minutes, and values were recorded in 10-minute increments.

Sickness and depressive-like behavior were determined through unmotivated (locomotor) (32) and motivated (social exploratory behavior, tail suspension test (TST), sucrose preference) (24,32,34,46–48) behavioral tests as described in Materials and Methods in Supplement 1. For the TST, the same mice were tested at both 7 and 30 dpi, with another subset of mice tested only at 30 dpi. At 30 dpi, immobility between the two subsets was not significantly different and data were collapsed.

Isolation of Enriched Brain CD11b$^+$ Cells

Enriched CD11b$^+$ cells (microglia/peripheral myeloid cells) were isolated from whole brain homogenates as previously described (32). In brief, brains were homogenized and resuspended in a discontinuous, isotonic Percoll gradient. Microglia were collected from the interphase of the 70% and 50% Percoll layers. We have previously characterized these cells as approximately 90% CD11b$^+$/CD45$^+$ "enriched CD11b$^+$" cells (23,24).

RNA Isolation and Real-Time Polymerase Chain Reaction

RNA was isolated from individual brain regions or enriched brain CD11b$^+$ cells using the Tri-Reagent protocol (Sigma-Aldrich), or the PrepEase kit (Affymetrix-USB, Santa Clara, California), respectively. RNA concentration was determined and RNA was reverse transcribed to complementary DNA. Real-time polymerase chain reaction was performed using the Applied Biosystems (Foster City, California) Taqman Gene Expression assay using an ABI PRISM 7300-sequence detection system as previously described (49). Data were analyzed using the comparative threshold cycle method, and results are expressed as fold difference from controls.

Flow Cytometry

Enriched brain CD11b$^+$ cells were assayed for surface antigens by flow cytometry as described (23,39). In brief, cells were incubated with rat anti-mouse antibodies (eBioscience, San Diego, California; CD11b-APC, CD45-PerCP-Cy5.5, and CD14-PE). Surface expression was determined using a Becton-Dickinson (Franklin Lakes, New Jersey) FACSCaliber four-color Cytometer. Twenty thousand events were recorded, and microglia (CD11b$^+$/CD45^{low}) and peripheral myeloid cells (PMCs) (CD11b$^+$/CD45^{high}) were identified by CD11b/CD45 expression (50). Gating was determined based on appropriate negative isotype controls. Data were analyzed using FlowJo software (Tree Star, Inc., Ashland, Oregon).

Immunohistochemistry

Fluorescent staining for glial fibrillary acidic protein (GFAP) and ionized binding association protein (Iba)-1 was performed as described in Materials and Methods in Supplement 1. Threshold staining was determined using National Institutes of Health ImageJ, and quantification was assessed for each image using digital image