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MORPHOLOGICAL AND GENETIC ACTIVATION OF MICROGLIA AFTER DIFFUSE TRAUMATIC BRAIN INJURY IN THE RAT

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Abstract—Traumatic brain injury (TBI) survivors experience long-term post-traumatic morbidities. In diffuse brain-injured rats, a chronic sensory sensitivity to whisker stimulation models the agitation of TBI survivors and provides anatomical landmarks across the whisker-barrel circuit to evaluate post-traumatic neuropathology. As a consequence of TBI, acute and chronic microglial activation can contribute to degenerative and reparative events underlying post-traumatic morbidity. Here we hypothesize that a temporal sequence of microglial activation states contributes to the circuit pathology responsible for post-traumatic morbidity, and test the hypothesis by examining microglial morphological activation and neuroinflammatory markers for activation states through gene expression and receptor-binding affinity. Adult male, Sprague–Dawley rats were subjected to a single moderate midline fluid percussion injury (FPI) or sham injury. Microglial activation was determined by immunohistochemistry, quantitative real-time PCR and receptor autoradiography in the primary somatosensory barrel field (S1BF) and ventral posterior medial nucleus (VPM) of the thalamus at 7 and 28 days following FPI. Morphological changes indicative of microglial activation, including swollen cell body with thicker, shrunken processes, were evident in S1BF and VPM at 7 and 28 days post-injury. Principally at 7 days post-injury in VPM, general inflammatory gene expression (major histocompatibility complex I, major histocompatibility complex II, translocator protein 18 kDa [TSPO]) is increased above sham level and TSPO gene expression confirmed by receptor autoradiography. Further, CD45, a marker of classical activation, and TGF-βI, an acquired deactivation marker, were elevated significantly above sham at 7 days post-injury. Daily administration of the anti-inflammatory ibuprofen (20 mg/kg, i.p.) significantly reduced the expression of these genes. Evidence for alternative activation (arginase 1) was not observed. Thus, these data demonstrate concomitant classical activation and acquired deactivation phenotypes of microglia in diffuse TBI in the absence of overt contusion or cavitation. Anti-inflammatory treatment may further alleviate the neuropathological burden of post-traumatic inflammation.

Key words: classical activation, alternate activation, anti-inflammatory, neuroplasticity.

INTRODUCTION

Diffuse traumatic brain injury (TBI) arises from abrupt acceleration and deceleration forces found in motor vehicle accidents and contact sports concussion. These external forces primarily impact gray–white matter interfaces, the axon hillock and the blood–brain barrier; which result in mechanically sheared axons, vasculature and membranes (Graham et al., 1995; Povlishock and Katz, 2005; Farkas and Povlishock, 2007). Immediate prolonged unconsciousness associated with diffuse TBI is not necessarily accompanied by an intracranial mass lesion and accounts for two-thirds of all TBI (Graham et al., 2002). The vast numbers of patients that survive a brain injury can develop varying degrees of post-traumatic morbidities, including cognitive, social, emotional and sensory deficits (McAllister, 1992; Millis et al., 2001; Povlishock and Katz, 2005; Yeates et al., 2008).

Post-TBI morbidities are brought on, if not exacerbated, by the secondary molecular, biochemical and cellular events that compound the neuronal, glial and vascular injuries across multiple brain areas (Povlishock and Katz, 2005; Farkas and Povlishock, 2007). In diffuse brain injury modeled by midline fluid percussion injury (FPI) these secondary cascades can occur in the absence of overt edema, hemorrhage or cavitation (Kelley et al., 2007). In the spared tissue of the thalamocortical relays that mediate whisker somatosensation, including somatosensory barrel field (S1BF) and ventral posterior medial nucleus (VPM) of thalamus, axonal transport is disrupted (Kelley et al., 2006), neurons atrophy (Lifshitz

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Abbreviations: FPI, fluid percussion injury; GAP43, growth-associated protein 43; Iba-1, ionized calcium binding adaptor molecule 1; MHC, major histocompatibility complex; qRT-PCR, quantitative real-time PCR; S1BF, somatosensory barrel field; TBI, traumatic brain injury; TSPO, translocator protein 18 kDa; VPM, ventral posterior medial nucleus.

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et al., 2007; Lifshitz and Lisembee, 2012), microglia classically activate (Kelley et al., 2007), glutamate neurotransmission becomes hypersensitive (Thomas et al., 2012) and neurons show hyperactivation with whisker stimulation (Hall and Lifshitz, 2010). These neuropathological changes along the whisker circuit correlate with the robust avoidance and apprehensive behavioral responses to whisker stimulation (increased sensory sensitivity) over 1 month in FPI-injured animals (McNamara et al., 2010; Learoyd and Lifshitz, 2012).

An intensely investigated feature of diffuse TBI is the prominent histological evidence for microglial activation (Lenzlinger et al., 2001; Morganti-Kossmann et al., 2007; Venkatesan et al., 2010). Currently, the range and extent of microglial activation states are being investigated in various neurological conditions, including spinal cord injury, Alzheimer's disease and TBI (Grossman et al., 2003; Colton et al., 2006; Bye et al., 2007; Donnelly and Popovich, 2008; Farfara et al., 2008; Popovich and Longbrake, 2008; Venkatesan et al., 2010). For simplicity, specific cellular functions have been associated with particular activation states (Gordon, 2003; Chen and Guilarte, 2008; Graeber, 2010), however it is more likely that functions overlap. Broadly, microglia can function to regulate both degenerative and reparative events in the injured and recovering brain. As such, microglia have been reported to exist in a state of dynamic equilibrium between a classical and alternative activated state following an insult, contributing to seemingly contradictory cellular processes (Popovich and Longbrake, 2008). Discrete signals in the pathological microenvironment induce action of resident microglia (Gordon, 2003; Mantovani et al., 2004; Colton et al., 2006; Popovich and Longbrake, 2008; Colton and Wilcock, 2010). Classically activated microglia participate in the host defense system as a part of innate and adaptive immunity, of which phagocytosis by macrophages is a primary role (Ransohoff and Cardona, 2010; Prinz et al., 2011). Microglial activation and their progression toward phagocytotic macrophages can lead to progressive and cumulative neuronal cell loss, as demonstrated by the toxicity associated with lipopolysaccharide (LPS) injection (Gao et al., 2002; Ling et al., 2006). Alternative activated microglia are purported to promote neuroplasticity and axonal regeneration, in addition to the monitoring and pruning of synapses (Gordon, 2003; Chen and Guilarte, 2008). For example, experimental spinal cord injury revealed microglia expressing genes linked to the alternative activated state in the presence of IL-4, which resulted in longer distance axon projections and promoted axon outgrowth overcoming chondroitin sulfate proteoglycan (CSPG) inhibition (Kigerl et al., 2009), however morphological features of alternative activated microglia have yet to be defined. Microglia with an acquired deactivation phenotype express TGF-βI and likely down-regulate the inflammatory response (Mantovani et al., 2004; Cullheim and Thams, 2007). In diffuse brain injury, the relative contributions of activated microglial phenotypes remain unknown.

Injury-induced behavioral deficits are likely due to interrupted neural network structure and function (Ghajar and Ivry, 2008). Microglial activation can modify neural networks in multiple ways: promoting neuronal cell death, influencing neural circuitry by neuroplastic reorganization or synaptic stripping (reviewed in Perry and O'Connor, 2010). In this study, for the first time, we survey the time course of microglial activation states during which late-onset sensory sensitivity develops and the consequence of inhibiting classical activation after experimental diffuse TBI.

EXPERIMENTAL PROCEDURES

Surgical preparation and fluid percussion brain injury

Adult male Sprague–Dawley rats (350–375 g) were subjected to midline FPI consistent with methods described previously (Lifshitz et al., 2007; Lifshitz, 2008; Hosseini and Lifshitz, 2009; McNamara et al., 2010). Briefly, rats were anesthetized with 5% isoflurane in 100% O_2 prior to the surgery and maintained at 2% isoflurane via nose cone. During surgery, animals' body temperature was maintained with a Deltaphase® isothermal heating pad (Braintree Scientific Inc., Braintree, MA). In a head holder assembly (Kopf Instrument, Tujunga, CA), a midline scalp incision exposed the skull. A 4.8-mm circular craniotomy was performed (centered on the sagittal suture midway between bregma and lambda) without disrupting the underlying dura or superior sagittal sinus. An injury hub was fabricated from the female portion of a Luer-Loc needle hub, which was cut, beveled, and scored to fit within the craniotomy. A skull screw was secured in a 1-mm hand-drilled hole into the right frontal bone. The injury hub was affixed over the craniotomy using cyanoacrylate gel and methyl-methacrylate (Hygenic Corp., Akron, OH) was applied around the injury hub and screw. The incision was sutured at the anterior and posterior edges and topical Lidocaine ointment was applied. Animals were returned to a warmed holding cage and monitored until ambulatory (approximately 60–90 min).

For injury induction, animals were re-anesthetized with 5% isoflurane 60–90 min after surgery to standardize anesthesia levels at the time of injury. The dura was inspected through the injury-hub assembly, which was then filled with physiological saline and attached to the male end of the fluid percussion device (Custom Design and Fabrication, Virginia Commonwealth University, Richmond, VA). As rat's reflexive responses returned, a moderate injury (1.9–2.0 atm) was administered by releasing the pendulum onto the fluid-filled cylinder. Animals were monitored for the presence of a forearm fencing response and the return of the righting reflex as indicators of injury severity (Hosseini and Lifshitz, 2009). Sham animals were connected to the FPI device, but the pendulum was not released. The injury-hub assembly was removed *en bloc*, integrity of the dura was observed, bleeding was controlled with Gelfoam (Pharmacia, Kalamazoo, MI) and the incision was stapled. Moderate brain-injured animals had righting reflex recovery times that averaged 6 min, and sham-injured animals recovered within 15 s. After recovery of the righting reflex, animals were placed in a warmed holding cage before being returned to the vivarium. In our hands, one of 25 brain-injured animals dies within 3 days from consequences of pulmonary edema. Surgical recovery was monitored post-operatively for 3 days, for which no overt differences (e.g. weight, coat, movement, grooming) were observed between animals. Staples were removed 7–10 days post-injury as needed. Experiments were conducted in accordance with NIH and institutional guidelines concerning the care and use of laboratory animals. Adequate measures were taken to minimize pain or discomfort. Animal numbers are reported in the figure legends for each study.