● **PERSPECTIVE**

Age-at-injury effects of microglial activation following traumatic brain injury: implications for treatment strategies

Traumatic brain injury (TBI) remains one of the leading causes of disability and death in infants and children. Studies have demonstrated that the youngest age group (especially ≤ 4 years old) exhibit worse functional outcome following moderate to severe TBI compared to older children or adults (Anderson et al., 2005; Emami et al., 2017). These data suggest that age-at-injury may be an important determinant of outcome, as damage to the developing brain at a young age likely disrupts normal brain development which will influence cognitive and psychosocial skills. The negative consequences of early injury manifests not only during early childhood, but throughout their life as these individuals have difficulty in developing new cognitive or social skills. The acute and long-term cognitive deficits such as impairments of learning and memory, attention, and executive function are often associated with development of brain atrophy. Psychosocial problems such as depression, anxiety, and sleep disturbances become more apparent as these children become older. Other than the supportive care in the acute and chronic post-traumatic period being the usual standard, therapies targeted at reversing or attenuating the behavioral deficits do not exist for the brain-injured patient. These clinical observations demonstrate the need for clinically relevant pre-clinical animal models to better understand the age-at-injury responses to TBI.

In a clinically-relevant model of pediatric TBI, we demonstrated an age-at-injury response where closed head injury to the 11-day-old rat resulted in greater cognitive deficits and brain atrophy at 4 weeks post-injury compared to the 17-day-old rat (Raghupathi and Huh, 2007). Following lateral fluid percussion brain trauma, the youngest animals (17-day-old rats) demonstrated worse hypotension and mortality compared to older animals (28-day-old and adult rats) (Prins et al., 1996). While the mechanisms underlying age-specific pathologic alterations following TBI are not completely understood, one intriguing possibility is that cerebral inflammation may play an important role in the sequelae of secondary injury. TBI leads to activation of resident microglia and release of pro- and anti-inflammatory cytokines and chemokines. Following TBI in children, cytokines and chemokines such as interleukins-6 and -10 were increased in the cerebrospinal fluid with more prominent increases observed in the youngest age group (≤ 4 years of age) (Bell et al., 1997), suggesting that neuroinflammation may put these patients at higher risk for worse outcome.

As the primary mediators of the resident immune response in the brain, microglia are thought to play an important role in neuroinflammation affecting both acute and chronic neurodegeneration that are observed following brain injury. The development of an unregulated, highly reactive microglial activation state (M1-like) may result in an excessive release of pro-inflammatory and cytotoxic mediators which may contribute to neuronal dysfunction and cell death. However, microglial activation also plays a beneficial role by removing excess neurons, dendritic spines and axons especially during development by phagocytosis ("pruning"). M2-like microglia release anti-inflammatory cytokines and neurotrophic factors to help prevent or minimize neuronal injury and restore tissue integrity and function in the injured brain.

While the understanding of the functional roles of microglia in adult models of TBI has developed dramatically in recent years (Loane and Kumar, 2016), very little is known about the role of microglia in pediatric models of TBI. Recently, we and others have demonstrated microglial reactivity following experimental TBI to the immature brain that corresponded to areas containing degenerating neurons in the grey matter tracts and degenerating axons in the white matter tracts that was associated with tissue loss, spatial learning and memory deficits, and working memory deficits (Zhang et al., 2015; Chhor et al., 2016; Hanlon et al., 2016; Hanlon et al., 2017). These data suggest that microglial activity may be involved in the ongoing pathogenesis following TBI in the immature brain and may potentially serve as a therapeutic target.

Minocycline is a tetracycline derivative antibiotic with anti-inflammatory properties, effectively crosses the blood-brain barrier after systemic administration and has demonstrated neuroprotection in many models of neurodegenerative diseases and brain injury. Early treatment with minocycline in most models of adult TBI demonstrated neuroprotection with a reduction of microglial activation and proliferation which was associated with a decrease in pro-inflammatory cytokine response, cerebral edema, lesion volume and attenuation of locomotor and spatial learning and memory deficits. In contrast, we recently reported that short-term early minocycline administration (within the 1st week) initiated immediately following closed head injury in the 11-day-old rat reduced microglial proliferation and activation and was accompanied by an increase, in the extent of neurodegeneration (Hanlon et al., 2017). This observation suggests that microglia may either participate in clearance of degenerating cells or may play a more active role in neuronal survival following injury to the immature brain. Moreover, there was no attenuation of spatial learning and memory deficits by minocycline (Hanlon et al., 2017). To test whether extended dosing of minocycline may be necessary to reduce the ongoing pathologic alterations, a separate group of animals received minocycline into the second week post-injury. Immediately after completion of this extended duration, microglial activation, axonal degeneration and neurodegeneration were exacerbated in multiple brain regions and this effect was sustained in the cortex and hippocampus up to the third week post-injury. Furthermore, whereas spatial learning deficits were unaffected by extended dosing of minocycline treatment, retention of the learned task was worsened in the extended dosing of minocycline-treated, brain-injured group (Hanlon et al., 2017). It is possible that decreasing the microglial response for a prolonged period may have lessened the neuroprotective effects of microglia such as secretion of neurotrophic factors and clearance of unwanted cellular debris and thus contributed to reactive delayed exacerbation of the microglial response which may have contributed to further damage.

A recent study demonstrated that within the first week following closed head contusive TBI in a 7-day-old mouse, an early increase in microglial number was associated with a predominantly reparatory/regenerative or anti-inflammatory microglial-associated phenotype in the injured cortex (Chhor et al., 2016). Acute post-traumatic administration of minocycline decreased the number of microglia and the absence of long-term neuroprotection suggested that minocycline may have been interfering with the reparative properties of activated microglia (Chhor et al., 2016). This is different from most adult models of contusive TBI, where there is predominantly a cytotoxic/pro-inflammatory microglial-associated profile (Loane and Kumar, 2016), suggesting an age-at-injury inflammatory response. This is not surprising since recent experimental studies demonstrated robust age-dependent differences in microglia-associated gene expression patterns in normal neonate and adult brains (Bennett et al., 2016).

While repetitive brain trauma in adults on the battlefield or sports have received much deserved attention and research due to concern for long-term neurologic complications such as chronic traumatic encephalopathy, relatively little research has been done on the "signature" disease of repetitive brain trauma associated with very poor outcome in the infant population, abusive head trauma (AHT). Survivors of this devastating trauma often develop profound cognitive and behavioral deficits into adulthood. Victims of AHT have demonstrated increased levels of microglial/macrophage-associated neurochemicals in the cerebrospinal fluid (Berger et al., 2004). In a clinically relevant model of AHT in 11-day-old rats, we demonstrated that 3 impacts (24 hours apart) resulted in increased microglial reactivity associated with traumatic axonal injury, neuronal degeneration, cortical and white matter atrophy, and long-term spatial learning and memory deficits. While acute short-term post-traumatic administration of minocycline decreased phagocytic microglial reactivity in the white matter tracts (corpus callosum) of brain-injured animals, this effect was lost after cessation of minocycline treatment. Unfortunately, minocycline treatment failed to provide any overt neuroprotection as it failed to attenuate traumatic axonal injury, axonal neurodegeneration, tissue atrophy, or spatial learning deficits. Interestingly, minocycline-treated animals demonstrated exacerbated injury-induced spatial memory deficits (Hanlon et al., 2016). It is possible that the reduction in the number of phagocytic microglia in the corpus callosum as a result of minocycline treatment may have prevented the removal of unwanted cellular debris such as myelin fragments or apoptotic oligodendrocytes in the white matter tracts and/or negatively influenced microglial-associated pro- and anti-inflammatory cytokine release, thereby preventing proper white matter repair and contributing to worsening long-term cognitive deficits.

While more experimental studies must be done to better understand the role of age-at-injury related microglial responses, it is likely that the developmental status of the brain is important for microglial function and will be an important consideration for studies of neuroinflammation following TBI. A better understanding of the role of M1-like (pro-inflammatory) and M2-like (anti-inflammatory) microglia polarization state in the immature brain following trauma is needed. Broad-spectrum anti-inflammatory therapies have not been successful in clinical human TBI trials (Loane and Kumar, 2016). Further research is needed to discover critical mechanisms that control phenotype switching in microglia in order to enhance their beneficial and suppress their detrimental activation states following pediatric TBI. It is much more feasible that recovery after TBI requires both the M1-like and M2-like functional responses, and that this may be different during the acute and chronic phases of injury. Furthermore, there may be regional-specific effects (*e.g.*, gray matter *vs.* white matter) of microglial polarization. Caution is advised against initiating a poorly timed M1- to M2-like phenotypic shift especially in the immature brain because microglia is known to play an active role in sculpting neuronal circuits, synapse and axonal remodeling, and pruning of unwanted or excess cells. Conversely, a prolonged repair phase or anti-inflammatory phase after a rapid pro-inflammatory response that is driven by M2-like activity can promote aberrant repair, such as fibrosis (Loane and Kumar, 2016). Further research on the severity of pediatric TBI and its effect on microglial activity is also warranted. Additional studies on different types of pediatric TBI such as contusive *vs.* diffuse *vs.* repetitive and its effect on microglial polarization is also warranted. Repetitive brain trauma may be an area to investigate whether early microglia reactivity undergoes "priming" that potentiates chronic microglial activity associated with chronic neuroinflammation.

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References

Anderson V, Catroppa C, Morse S, Haritou F, Rosenfeld J (2005) Functional plasticity or vulnerability after early brain injury? Pediatrics 116:1374-1382.

Bell MJ, Kochanek PM, Doughty LA, Carcillo JA, Adelson PD, Clark RS, Wisniewski SR, Whalen MJ, DeKosky ST (1997) Interleukin-6 and interleukin-10 in cerebrospinal fluid after severe traumatic brain injury in children. J Neurotrauma 14:451-457.

Bennett ML, Bennett FC, Liddelow SA, Ajami B, Zamanian JL, Fernhoff NB, Mulinyawe SB, Bohlen CJ, Adil A, Tucker A, Weissman IL, Chang EF, Li G, Grant GA, Hayden Gephart MG, Barres BA (2016) New tools for studying microglia in the mouse and human CNS. Proc Natl Acad Sci U S A 113:E1738-1746.

Berger RP, Heyes MP, Wisniewski SR, Adelson PD, Thomas N, Kochanek PM (2004) Assessment of the macrophage marker quinolinic acid in cerebrospinal fluid after pediatric traumatic brain injury: insight into the timing and severity of injury in child abuse. J Neurotrauma 21:1123-1130.

Chhor V, Moretti R, Le Charpentier T, Sigaut S, Lebon S, Schwendimann L, Ore MV, Zuiani C, Milan V, Josserand J, Vontell R, Pansiot J, Degos V, Ikonomidou C, Titomanlio L, Hagberg H, Gressens P, Fleiss B (2016) Role of microglia in a mouse model of paediatric traumatic brain injury. Brain Behav Immun doi:10.1016/j.bbi.2016.11.001.

Emami P, Czorlich P, Fritzsche FS, Westphal M, Rueger JM, Lefering R, Hoffmann M (2017) Impact of Glasgow Coma Scale score and pupil parameters on mortality rate and outcome in pediatric and adult severe traumatic brain injury: a retrospective, multicenter cohort study. J Neurosurg 126:760-767.

Hanlon LA, Huh JW, Raghupathi R (2016) Minocycline transiently reduces microglia/macrophage activation but exacerbates cognitive deficits following repetitive traumatic brain injury in the neonatal rat. J Neuropathol Exp Neurol 75:214-226.

Hanlon LA, Raghupathi R, Huh JW (2017) Differential effects of minocycline on microglial activation and neurodegeneration following closed head injury in the neonate rat. Exp Neurol 290:1-14.

Loane DJ, Kumar A (2016) Microglia in the TBI brain: The good, the bad, and the dysregulated. Exp Neurol 275 Pt 3:316-327.

Prins ML, Lee SM, Cheng CL, Becker DP, Hovda DA (1996) Fluid percussion brain injury in the developing and adult rat: a comparative study of mortality, morphology, intracranial pressure and mean arterial blood pressure. Brain Res Dev Brain Res 95:272-282.

Raghupathi R, Huh JW (2007) Diffuse brain injury in the immature rat: evidence for an age-at-injury effect on cognitive function and histopathologic damage. J Neurotrauma 24:1596-1608.

Zhang Z, Saraswati M, Koehler RC, Robertson C, Kannan S (2015) A new rabbit model of pediatric traumatic brain injury. J Neurotrauma 32:1369-1379.