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Review Article

Neuroimmune responses in the developing brain following traumatic brain injury

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ABSTRACT

Traumatic brain injury (TBI) is one of the leading causes of both acute and long-term morbidity in the pediatric population, leading to a substantial, long-term socioeconomic burden. Despite the increase in the amount of preclinical and clinical research, treatment options for TBI rely heavily on supportive care with very limited targeted interventions that improve the acute and chronic sequelae of TBI. Other than injury prevention, not much can be done to limit the primary injury, which consists of tissue damage and cellular destruction. Secondary injury is the result of the ongoing complex inflammatory pathways that further exacerbate tissue damage, resulting in the devastating chronic outcomes of TBI. On the other hand, some level of inflammation is essential for neuronal regeneration and tissue repair. In this review article we discuss the various stages of the neuroimmune response in the immature, pediatric brain in the context of normal maturation and development of the immune system. The developing brain has unique features that distinguish it from the adult brain, and the immune system plays an integral role in CNS development. Those features could potentially make the developing brain more susceptible to worse outcomes, both acutely and in the long-term. The neuroinflammatory reaction which is triggered by TBI can be described as a highly intricate interaction between the cells of the innate and the adaptive immune systems. The innate immune system is triggered by non-specific danger signals that are released from damaged cells and tissues, which in turn leads to neutrophil infiltration, activation of microglia and astrocytes, complement release, as well as histamine release by mast cells. The adaptive immune response is subsequently activated leading to the more chronic effects of neuroinflammation. We will also discuss current attempts at modulating the TBI-induced neuroinflammatory response. A better understanding of the role of the immune system in normal brain development and how immune function changes with age is crucial for designing therapies to appropriately target the immune responses following TBI in order to enhance repair and plasticity.

1. Introduction

There is growing interest in traumatic brain injury (TBI) due to an increase in public recognition of its impact on each individual as well as society. This is more so in pediatrics where the long-term consequences and the societal burden are amplified. The CDC is reporting an increase in the number of pediatric TBI-related emergency room visits. There was an alarming increase in the incidence of TBI related visits from 1374 per 100,000 from the years 2007–2008 to 2194 per 100,000 between the years 2009–2010 (Centers for Disease Control and Prevention, 2016) in the 0–4 years age group. Additionally, the 5–14 and 15–24 age groups also showed an increase in the incidence of TBI during those same years (Centers for Disease Control and Prevention, 2016). Between the years 2009–2010, the death rates in the age groups 0–4, 5–14, 15–24 were 4.3, 1.9, and 15.6 per 100,000 respectively (Centers for Disease Control and Prevention, 2016). The majority of pediatric TBI emergency room visits were classified as mild (70–90%). Although only 14% of children with mild injuries had long-term consequences, almost 60% of the moderate to severe TBI pediatric patients had chronic disability requiring specialized services (Kuehn, 2018). The long-term morbidity in the pediatric population is often difficult to quantify and is typically challenging to diagnose (Wechsler et al., 2005). This is because the recovery phase after injury usually varies based on the age of the patient at the time of injury. Typically, the acquisition of new knowledge and motor skills are easily affected and are more vulnerable to injury as compared to previously acquired skills

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(Smith et al., 2015). This is often not detected until much later during development when higher level executive and cognitive functions develop. There are ongoing studies to better measure and treat these disabilities that result from pediatric TBI (Maddux et al., 2018). Short-term recovery after mild forms of TBI could be measurable (Zuckerman et al., 2018) and manageable. However, recovery after more severe forms of TBI can be unpredictable. The younger population is not only at a higher risk of suffering from acute morbidity such as post traumatic epilepsy (PTE) (Webster et al., 2017), but are subject to a greater risk of long-term disability (Anderson et al., 2009; Giza and Prins, 2006; Anderson et al., 2012). There is a growing body of evidence that addresses the permanent changes to the brain after TBI. A study by Ewing-Cobbs et al. used diffuse tensor imaging to conclude that in pediatric patients, TBI leads to the arrest of normal brain development as well as neuronal and axonal damage, which correlates well with neurobehavioral outcomes (Ewing-Cobbs et al., 2008).

Long-term morbidities described in pediatric TBI patients range from arrested development and lack of age-appropriate responsiveness in infants and children with severe TBI, to learning disabilities secondary to deficits in verbal working memory, visuo-spatial memory as well as attention deficit disorders which are typically detected in school age children (Masel and DeWitt, 2010; McKinlay et al., 2002). A study analyzed data from the National Survey of Children's Health and concluded that pediatric patients with a history of TBI are more likely to have behavioral/cognitive conditions compared to a control population that has not suffered from TBI (Haarbauer-Krupa et al., 2018). These social and behavioral impairments include psychosocial issues such as depression, anxiety and sleep disorders in older patients, while disinhibited speech, aggression and irritability are more common in younger patients (Anderson et al., 2009; Mayer et al., 2017; Keenan and Bratton, 2006). In addition, there is evidence that patients younger than age 2 have worse outcomes than older children when assessed for cognitive deficits (Anderson et al., 2009). In children, younger age (less than 5 years) was found to be an independent risk factor for post-traumatic seizures and was associated with adverse outcomes (Rumalla et al., 2018). Similar to adults, pediatric patients with severe TBI had a greater risk of developing post-traumatic epilepsy (DeGrauw et al., 2018).

This age-dependent susceptibility to TBI within the pediatric population may be dependent on the stage of brain development at the time of the injury. The immune system plays a crucial role in the normal development of the brain, therefore any injury that triggers an inflammatory response leading to immune dysregulation, may result not only in a variable age-dependent response but may also have implications for plasticity and repair. The old notion of CNS immune privilege is debunked since modern data has demonstrated an active involvement of the immune system with the CNS (Carson et al., 2006). There are several potential entry sites for immune cells to migrate from the blood into the CNS such as the choroid plexus, the subarachnoid space, and the parenchymal perivascular space. Additionally, cells of the immune system can exit out of the CNS through both blood vessels and the lymphatic system in a normal brain (Ransohoff et al., 2003). TBI results in an acute immune response that involves both the adaptive and the innate immune systems, eventually leading to chronic changes in certain immune cells of the brain that has been demonstrated in animal models and in patients (Witcher et al., 2018; Faden et al., 2016; Zhang et al., 2019; Loane and Kumar, 2016).

2. Role of the immune system in brain development

The central nervous system has always been labelled as an immune privileged site, until recent discoveries that have pointed towards the crucial role that the immune system plays in brain function as well as development (Tanabe and Yamashita, 2018). This is supported by the recent discovery of a CNS lymphatic system that connects to the deep cervical lymph nodes and may have implications for a localized role of the immune system in neuroinflammatory conditions (Louveau et al., 2015; Aspelund et al., 2015). The term “glymphatic” (glial-associated lymphatic system) was coined to describe a system that facilitates the influx and efflux of cerebrospinal fluid as well as interstitial fluid to perfuse the CNS. This system could potentially explain the different possible routes that the immune cells and the antigens could take in order to access the CNS (Louveau et al., 2017). Disorders in sleep pattern are commonly seen after TBI and may contribute to impaired metabolite clearance through these lymphatics (Viola-Saltzman and Watson, 2012; Xie et al., 2013). In addition, the CNS lymphatic system's function in waste removal declines with age and may contribute to pathologic findings with the aging brain (Kress et al., 2014). The disruption of the glymphatic pathway has also been implicated in chronic neurodegeneration after TBI (Iliff et al., 2014).

During development, neurons have been shown to express classical and non-classical MHC1 class I proteins in vivo, indicating that the immune system plays a role in normal neuronal development, activity-dependent plasticity and behavior (Boulanger and Shatz, 2004). Using mice that are genetically deficient for either MHC class I or the class I MHC receptor component CD3ζ, the crucial role of these molecules in activity dependent plasticity and remodeling of connections in the developing and mature brain have been elucidated (Huh et al., 2000). The immune cells that are normally present in the brain are comprised of the resident myeloid cells (microglia) and the infiltrating cells that are present in the parenchyma, meninges and choroid plexus.

The resident myeloid cells populate the early embryonic brain and are responsible for the clearance of debris and apoptotic neurons, in addition to the regulation of neuronal excitability and synaptic pruning. Microglial cells contribute towards the maintenance of the brain microenvironment via their normal physiologic functions such as surveillance, phagocytosis, neuro-modulation, synaptic pruning, proliferation and communication with the BBB (Gomez-Nicola and Perry, 2015). Microglia also support the homeostatic development of neurons, providing a supportive role which includes synaptic ‘pruning’(Schafer et al., 2012; Tremblay et al., 2011). The microglial cells modulate the synaptic development through the complement cascade (Stevens et al., 2007), and the complement proteins are used to tag unwanted debris in synapses in order to be subsequently cleared by phagocytosis (Stephan et al., 2012). Opsonization also leads to synaptic pruning which is necessary for axonal and synaptic maturation (Stevens et al., 2007). The crucial role of microglia in brain development has been further demonstrated in experiments where ablation of abnormal microglia followed by engraftment of ‘normal’ wild type bone marrow-derived myeloid cells resulted in normal brain development (Derecki et al., 2012).

Leukocytes are normally present in the meninges and the choroid plexus but their proximity to the brain indicates that in the presence of injury they may have a major role in modulating the inflammatory response of ependymal and endothelial cells. Leukocytes also secrete cytokines in the CSF thereby altering the microenvironment and subsequently activating resident immune cells such as microglia (Korin et al., 2017). T cells have been shown to play an important role in neurogenesis and normal cognition, in addition to spatial learning and maintenance of plasticity (Ziv et al., 2006; Wolf et al., 2009; Ron-Harel and Cardon, 2011). These studies indicate that regulated immune activity is necessary for plasticity and normal development. An imbalance of this regulation following injury can disrupt parenchymal homeostasis leading to impaired repair mechanisms (Schwartz et al., 2013) which can in turn affect normal development.

The maintenance of a local homeostatic environment, which is isolated due to the blood brain barrier (BBB) is an especially important role of the immune system (Obermeier et al., 2013). The astrocytes in the brain are key modulators of BBB homeostasis (Gee and Keller, 2005). The maintenance of the BBB and the selective isolation of the brain microenvironment is essential for normal brain development (Moretti et al., 2015). Indeed, dysfunction of the BBB has been linked to

disorders such as epilepsy, Alzheimer's disease, and Parkinson's disease (Daneman, 2012).

3. Immune response to TBI

The neuroinflammatory reaction that ensues as a result of TBI can be described as a highly intricate interaction between the cells of the innate and the adaptive immune systems. Immediately after brain injury, the highly regulated blood brain barrier which is composed of and coordinated by complex interactions between neurons, glial cells, and endothelial cells (Banerjee and Bhat, 2007; Persidsky et al., 2006), is broken down due to the mechanical insult as well as the resulting edema from TBI. This leaky BBB will in turn allow the activated immune cells to gather at the site of the injury as they gain entry through the damaged interface (Loane and Kumar, 2016). Soon after the insult, the resident microglial population becomes activated, and cells of the innate immune system such as neutrophils, infiltrate the lesion. Following that, monocyte-derived macrophages and cells of the adaptive immune system such as lymphocytes migrate to the site of injury.

3.1. Innate immune response

3.1.1. Initial signals

3.1.1.1. DAMPs and PAMPs. The initial traumatic insult to the brain leads to tissue disruption as well as cellular destruction. The mechanical forces that result in the breakdown of tissues and cells lead to the release of intracellular molecules referred to as damage and pathogen associated molecular patterns (DAMPs and PAMPs) (Bianchi, 2007; Corps et al., 2015). Previously referred to as alarmins, these DAMPs are endogenous molecules that are released through non-programmed cell death (Bianchi, 2007). The release of DAMPs in turn activates the innate immune system which results in the initiation of the inflammatory response (Corps et al., 2015) (see Fig. 1). This initial innate neuroinflammatory response leads to the activation of the adaptive immune system in an attempt to facilitate CNS tissue repair. (Ransohoff and Brown, 2012; Hauwel et al., 2005). On the other hand, an overactive inflammatory reaction could lead to the devastating sequela of traumatic brain injury.

Traumatic brain injury (TBI) causes tissue damage and neuronal injury which results in the release of damage associated molecular patterns (DAMPs) into the microenvironment. These DAMPs in turn activate the surrounding innate immune cells and recruited peripheral immune cells to the site of injury leading to the initiation of the secondary phase of injury. This is further exacerbated by the release of more chemokines and cytokines that contribute to additional neuronal injury. After the acute inflammatory phase, the activated microglia could return to a resting state with full recovery. However, with enough inflammatory damage, the microglia are unable to return to a resting state and become chronically activated. The astrocytes participate in a similar manner resulting in the formation of a chronic scar. This chronic inflammatory state may potentially disrupt brain homeostasis and normal CNS development leading to cognitive and behavioral dysfunction.

DAMPs encompass several molecules such as ATP, S-100 proteins, uric acid and the chromatin protein, high mobility group box 1 (HMGB1). Of particular interest is HMGB1, which is a nuclear protein that normally interacts with nucleosomes, transcription factors and histones. It also binds to distorted DNA and facilitates the bending of linear DNA (Agresti and Bianchi, 2003). When HMGB1 is actively released into the extracellular space by macrophages and activated monocytes or passively released by necrotic cells, it binds to the RAGE (the receptor for advanced glycation end products) thus inducing inflammation (Scaffidi et al., 2002). Interestingly, intracellular HMGB1 plays an important role in early brain development and has a spatially different expression pattern throughout murine brain development. HMGB1 expression significantly decreases in adult murine brains (Guazzi et al., 2003).

The amount of HMGB1 release is a measure of cellular damage and injury since it has been shown that CSF levels of HMGB1 can be correlated with clinical outcomes after pediatric traumatic brain injury (Au et al., 2012). That same study also found that the mean and the peak HMGB1 levels in the CSF of TBI patients is increased compared to control patients (Au et al., 2012). Another study in adult human TBI patients showed that HMGB1 plasma levels correlated with 1-year mortality and dismal outcomes (Wang et al., 2012). The release of HMGB1 in damaged tissue leads to the recruitment of neutrophils through Mac-1 and is most significant at 1 day after TBI (Gao et al., 2012; Orlova et al., 2007). The clearance of HMGB1 from the brain microenvironment is accomplished by phagocytic microglia between days 2 and 6 after TBI (Gao et al., 2012). The detrimental effects of HMGB1 is partly due to the activation of inflammatory pathways via toll like receptor 4 (TLR4), in addition to the downstream increase in expression of the astrocyte water channel aquaporin-4 (AQP4) (Laird et al., 2014). Therefore, HMGB1 is a marker of cellular injury as well as a potential target for therapy (Parker et al., 2017).

Similar to many other pathophysiologic states, TBI results in a state of oxidative stress, which exacerbates the secondary neuroinflammatory phase of brain injury. The immune cells of the brain have

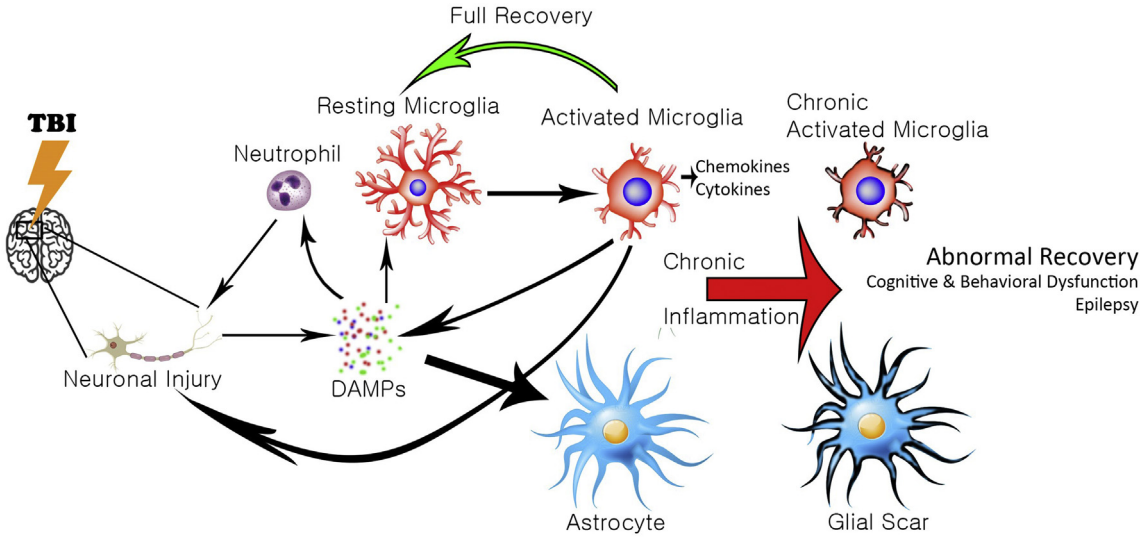

Fig. 1. Potential neuroimmune mechanisms leading to chronic inflammation and neurodevelopmental impairment in pediatric TBI.

antioxidative enzymes that counteract the oxidative stress and the damage resulting from free radicals (Abdul-Muneer et al., 2019). There is growing amount of evidence suggesting that the immature brain is prone to more secondary damage as a result of the reduced amount of antioxidant activity as well as the diminished antioxidant reserve (Bayir et al., 2006). For example, the antioxidative enzyme glutathione peroxidase has decreased activity in pediatric-aged mice (postnatal day 21) compared to adult mice (Fan et al., 2003).

3.1.1.2. Complement system. Another under-recognized component of innate immunity is the complement system, which plays a role in brain homeostasis (Alawieh et al., 2015). The ontogeny of most complement proteins and complement regulatory proteins demonstrate lower levels in the neonates when compared to adults (De Paula et al., 2003; Davis et al., 1979; Norman et al., 1975; Moalic et al., 1988). In addition to its role in the normal physiologic and developmental functions of the brain that involve its role in neurogenesis, migration and synaptic pruning, there is evidence suggesting that the complement system plays a role in neuroprotection (Brennan et al., 2012; Pisalyaput and Tenner, 2007; Rupprecht et al., 2007; Hammad et al., 2018). The complement protein C1q has been shown to be protective against neurodegeneration and improves neuronal viability in culture (Pisalyaput and Tenner, 2007). Another study using a murine model of pneumococcal meningitis has demonstrated that the complement system has protective properties in the setting of infectious neuroinflammation (Rupprecht et al., 2007).

In the context of TBI-induced neuroinflammation, the complement system has been found to have both detrimental as well as neuroprotective effects on inflammation (Hammad et al., 2018). The destruction of the BBB secondary to trauma allows for the entry of peripheral proteins, including complement, into the brain. The activation of the endogenous CNS complement system as well as the influx of innate and adaptive immune cells into the injured brain further exacerbate the secondary neuroinflammatory reaction (Brennan et al., 2012). Elevated levels of several complement factors have been detected after TBI in human samples of brain tissue as well as CSF, compared to controls (Bellander et al., 2001; Kossmann et al., 1997). Several animal models have explored the role of complement in the pathogenesis of TBI-induced neuroinflammation by either using complement knockouts or complement inhibitors (Hammad et al., 2018). Pharmacologic inhibition of both the classical and alternative pathways of complement by using the soluble human recombinant complement receptor 1 (sCR1) prior to inducing trauma (open head weight drop) in a rat model demonstrated a significant decrease in the amount of neutrophil infiltration and accumulation at the site of injury when compared to untreated controls (Kaczorowski et al., 1995). Similarly use of the Complement receptor related protein y (Crry), which inhibits the classical and alternative complement pathways also demonstrated improved outcomes in TBI. The overexpression of Crry in astrocytes in a transgenic murine closed head injury model improved BBB function as well as neurologic recovery (Rancan et al., 2003). Subsequent studies by that group demonstrated that pharmacologic inhibition of Crry by using recombinant Crry-Ig showed improved neurologic outcomes and increased upregulation of neuroprotective genes compared to controls (Leinhase et al., 2006).The membrane attack complex (MAC), which forms as a result of the binding of several complement factors is also involved in cell death following TBI. The MAC complex functions by inserting into cell membranes ultimately leading to cell lysis. Mice that are lacking in CD59, which is a MAC regulatory protein have been shown to have increased attachment of MAC to the cell membrane. When these $CD59^{-/-}$ mice were subjected to a focal closed head injury model they showed a decrease in neuronal cell death (Bellander et al., 2001).

3.1.2. Cellular signals

3.1.2.1. Neutrophils. It is well established that neutrophils play a primary role in the innate immune system's ability to mount an inflammatory response. A traumatic insult results in the recruitment of neutrophils to the site of the injury within minutes (Nourshargh and Alon, 2014) and within hours to the brain (Engelhardt et al., 1994; Liu et al., 2018). Neuroinflammation results in the recruitment of neutrophils to the site of the injury within minutes (Nourshargh and Alon, 2014) and within hours to the brain (Engelhardt et al., 1994; Liu et al., 2018). A study using a rat TBI model demonstrated neutrophil migration to the brain within 2–12 h after injury (Soares et al., 1995). Chemotaxis is the process by which neutrophils gain access to the various sites of active inflammation in the body (Liu et al., 2018). Proinflammatory cytokines and complement are detected through various cell surface receptors on neutrophils, allowing them to migrate to areas of inflammation (Liu et al., 2018). To facilitate this migration, neutrophils express selectins that allows them to bind to endothelial cells (Liu et al., 2018). Neutrophils also phagocytose organisms and particles that have undergone antibody opsonization, thus facilitating clearance of cellular debris. Several studies have confirmed that neutrophils infiltrate the brain after TBI, where they peak at 24–48 h post injury and resolve by day 7(Jassam et al., 2017). Other experimental models of neuroinflammation have demonstrated that these neutrophils will subsequently become activated, leading to further neurotoxicity (Allen et al., 2012), which is mediated by the release of various agents including pro-inflammatory cytokines such as TNF-α, reactive oxygen species, and matrix metalloproteinases (MMP) (Dinkel et al., 2004; Nguyen et al., 2007). This timeline of neutrophil infiltration has been found to correlate well with certain clinical variables such as brain edema and intracranial hypertension (Clark et al., 1994). The properties of the immature neutrophils in the developing brain have been extensively studied in order to determine whether they differ from the adult neutrophils in their ability to mount an immune response. Certain studies that focused on immature neonatal neutrophils have determined that those cells have impaired expression of integrins and selectins as well as defective phagocytosis (Levy, 2007). Another study demonstrated that these immature neutrophils have reduced expression of complement receptor 3 (CR3) (Reddy et al., 1998) which serves as both an adhesion molecule and a membrane receptor that allows neutrophils to have bactericidal properties in addition to its role in diapedesis (Ross and Vetvicka, 1993; Springer, 1994). In addition, immature neutrophils have decreased expression of the adhesion molecule L-selectin as well as reduced up-regulation of CD11b, which also plays a role in adhesion (Kim et al., 2003). Other studies demonstrated that neutrophil adherence in human samples is markedly impaired during the neonatal period and does not achieve adult levels until 15 years of age (Eisenfeld et al., 1990) The impaired function of these neutrophils was found to be directly correlated with cell maturation and development rather than global neutrophil dysfunction in the early developmental stages (Makoni et al., 2016; Lawrence et al., 2017). Neutrophil elastase is released by activated neutrophils and is a proteolytic enzyme with damaging properties to the surrounding tissues such as the initiation of apoptosis and the degradation of the extracellular matrix (Zhou et al., 2012; Kawabata et al., 2002). Another characteristic of immature human neutrophils is the diminished expression of TLR4 as well as their poor responsiveness to LPS stimulation, which leads to an impaired innate immune response compared to adults (Förster-Waldl et al., 2005).

Both neutrophil elastase knockouts as well as the use of a neutrophil elastase inhibitor resulted in the attenuation of the acute neuroinflammatory response in a murine controlled cortical impact (CCI) model of TBI (Semple et al., 2015). When compared to the adult brain, TBI in the juvenile murine brain at P21 resulted in a greater infiltration of GR-1 granulocytes and CD45 + leukocytes that remained elevated up to 2 weeks after injury. This is in contrast to the adult brains where the time course was limited to 3 days after injury, indicating that neutrophils may play a significant role in TBI in the immature brain (Claus et al., 2010).

3.1.2.2. Microglia. Microglia are cells of the innate immune system that share similar characteristics with macrophages. They originate from the embryonic yolk sac and comprise about 10% of the cells of the adult brain (Ginhoux et al., 2010). More importantly, they play an important role in both the development of the brain and the initiation of the CNS immune response (Jassam et al., 2017; Anderson and Vetter, 2018; Lenz and Nelson, 2018). The microglial progenitor cells migrate to the brain early on in the prenatal period, and populate the CNS at approximately embryonic day 8(Ginhoux et al., 2010; Andjelkovic et al., 1998; Monier et al., 2006). Microglia are derived from a different cell line than tissue macrophages (Sheng et al., 2015), and peripheral cells do not contribute to the CNS microglial population during normal development (Ginhoux et al., 2010). One other characteristic feature of microglia is that they are exquisitely sensitive to minute metabolic changes in their microenvironment (Nimmerjahn et al., 2005). Microglia become primed and function as mediators of the immune response when it is exposed to an "activating" microenvironment (Aihara et al., 1995; Simon et al., 2018; Norden et al., 2015). It has been shown that microglial activation is relatively rapid, whereby the cells autonomously converge to the site of injury in an ATP dependent mechanism (Koshinaga et al., 2000; Davalos et al., 2005). Activated microglia aggregate at the site of the injured tissue and subsequently isolate the injured site within an hour of the insult, forming a stable structure resembling a "honeycomb"(Davalos et al., 2005; Roth et al., 2014).

Defining the role that the microglial population plays in normal CNS development is key in order to understand its contribution to the pathophysiologic response to TBI in the immature brain. Microglia play a key role in clearing the cellular debris that accumulates during both inflammation and normal development (Schafer et al., 2012). Neuronal synapses make frequent contact with the microglia, and these interactions are thought to play an important role in the normal development and maturation of the brain (Panzanelli et al., 2011). Matcovitch-Natan et al. described transcription factor dependent changes in microglial gene expression profiles throughout brain development thus illustrating the close association between microglial and brain development (Matcovitch-Natan et al., 2016). Under normal developmental conditions, microglia participate in the promotion of neuronal apoptosis via signals that are dependent on the immunoreceptor DAP12 as well as the integrin CD11b (Wakselman et al., 2008). When microglial development is disrupted by neuroinflammatory processes, several homeostatic properties associated with the microglial population are gravely affected, possibly leading to devastating long-term neurocognitive deficits (Matcovitch-Natan et al., 2016). Studies by us and other groups have shown that the pro-inflammatory activation of microglia in the intrauterine or neonatal period leads to the development of neurodevelopmental conditions such as cerebral palsy and autism spectrum disorders (Kannan et al., 2012; Niño et al., 2018; Bilbo et al., 2018; Zhang et al., 2018). The timing of the insult related to microglial function during development appears to dictate the extent and type of injury. In addition, specifically targeting microglia to modulate its function mitigates the effects thereby promoting normal development (Kannan et al., 2012; Niño et al., 2018; Nance et al., 2017; Lei et al., 2017).

Microglia are thought to interact with the developing synapses via the classical complement system (Stephan et al., 2012; Panzanelli et al., 2011). The complement proteins C1q and C3 are both required for the elimination of synapses (Stevens et al., 2007). Microglia have C3 receptors that bind to C3b opsonized synapses thus eliminating these synapses through phagocytosis (Stevens et al., 2007). The process of phagocytosis is inhibited by CD47, which is a transmembrane protein that is upregulated during the process of synapse pruning in order to protect from excessive microglial phagocytosis (Lehrman et al., 2018). The role of microglia in synaptic pruning is especially important during brain development, whereby microglial phagocytosis of the synapses allows the synaptic structures to continually re-wire and to develop synaptic connections (Schafer et al., 2012). Therefore, any event that effectively changes the microglial number or function during development can lead to dysfunction of activity-triggered synaptic plasticity which is vital to learning and memory (Salter and Stevens, 2017). Abnormal phagocytosis by the microglial population has been linked to neurodevelopmental disorders such as Rett syndrome where a mutation in methyl-CpG binding protein 2 (MECP2) is linked to excessive phagocytosis at the synapses (Schafer et al., 2016). It is possible that the dysregulation of microglial activity that is seen after traumatic brain injury may impair the activity-related synaptic plasticity and could result in the cognitive deficits seen in children following TBI.

The microglial population is characterized as being long-lived in addition to having a low turnover rate (Norden et al., 2015). These features are unique and beneficial in the role that the microglia play as neuronal macrophages, however it also exposes them to the build-up of oxidative molecules, increasing their exposure to inflammatory states over time (Norden et al., 2015). Unlike other hematopoietic lineage cells, microglia renew at a rate of about 25% per year in the human brain thereby giving them an average age of ~4 years (Réu et al., 2017). The young microglia respond to extracellular ATP by becoming motile whereas the adult microglia are shown to have a slower, more dampened response (Damani et al., 2011). There is evidence to suggest that microglia undergo both phenotypic and physiologic changes after TBI and become more susceptible to an exaggerated immune response (Norden et al., 2015; Robinson et al., 2017). Microglia also undergo a burst of mitotic activity as a result of neuorinflammation, followed by programmed cell death soon afterwards to reduce the cell numbers back down to baseline (Streit, 2006). The mechanism behind microglial activation and "deactivation" is not fully understood (Schwartz et al., 2006), moreover, the microglial cells that are "chronically activated" can be sustained up to 17 years after traumatic brain injury (Ramlackhansingh et al., 2011). The aged microglia have altered immunologic activity (Sierra et al., 2007) that can be attributed in part to the buildup of undigested cellular waste (i.e. lipofuscin) (Gray and Woulfe, 2005).

Microglial activation has been typically classified as M1 (pro-inflamamtory) or M2 (anti-inflammatory). This classification however has been recently challenged and considered to be somewhat limiting since the microglia often exist in a spectrum between these two states. In addition, the ensuing chronic changes often result in dysfunction or microglial dysregulation (Loane and Kumar, 2016; Salter and Stevens, 2017; Jassam et al., 2017; Holtman et al., 2015). In the immature brain this has more significant consequences since normal developmental processes that are dependent on physiological microglial function may be disrupted and can result either in an arrest in maturation and an inability to achieve developmental milestones or a severe delay in achieving them. Targeting appropriate pathways specifically in the dysregulated or 'activated' microglia that is tailored to the developmental maturation and timing of injury can potentially change the microglial function and phenotype to one that promotes repair and normal development. Our group has previously shown that targeting activated microglia with dendrimer nanoparticles that deliver small molecule agents to modulate microglial function at a single time point after injury to the immature brain, has significant effects in improving motor and cognitive function in the animals both short-term and long-term (Kannan et al., 2012; Niño et al., 2018; Burd et al., 2012). Although these applications were in cerebral palsy and in necrotizing enterocolitis-induced brain injury, similar strategies may have implications for targeting microglia in pediatric TBI. This indicates that 'normalizing' microglial function appropriately after the injury results in sustained and long-lasting effects allowing normal brain development to occur. We have previously shown that these neutral, hydroxyl-terminated dendrimer nanoparticles localize in activated microglia in various models of brain injury irrespective of the species (Kannan et al., 2012; Zhang et al., 2015a; Dai et al., 2010; Nance et al., 2015, 2016; Mishra et al., 2014; Guo et al., 2016; Kambhampati et al., 2015). We

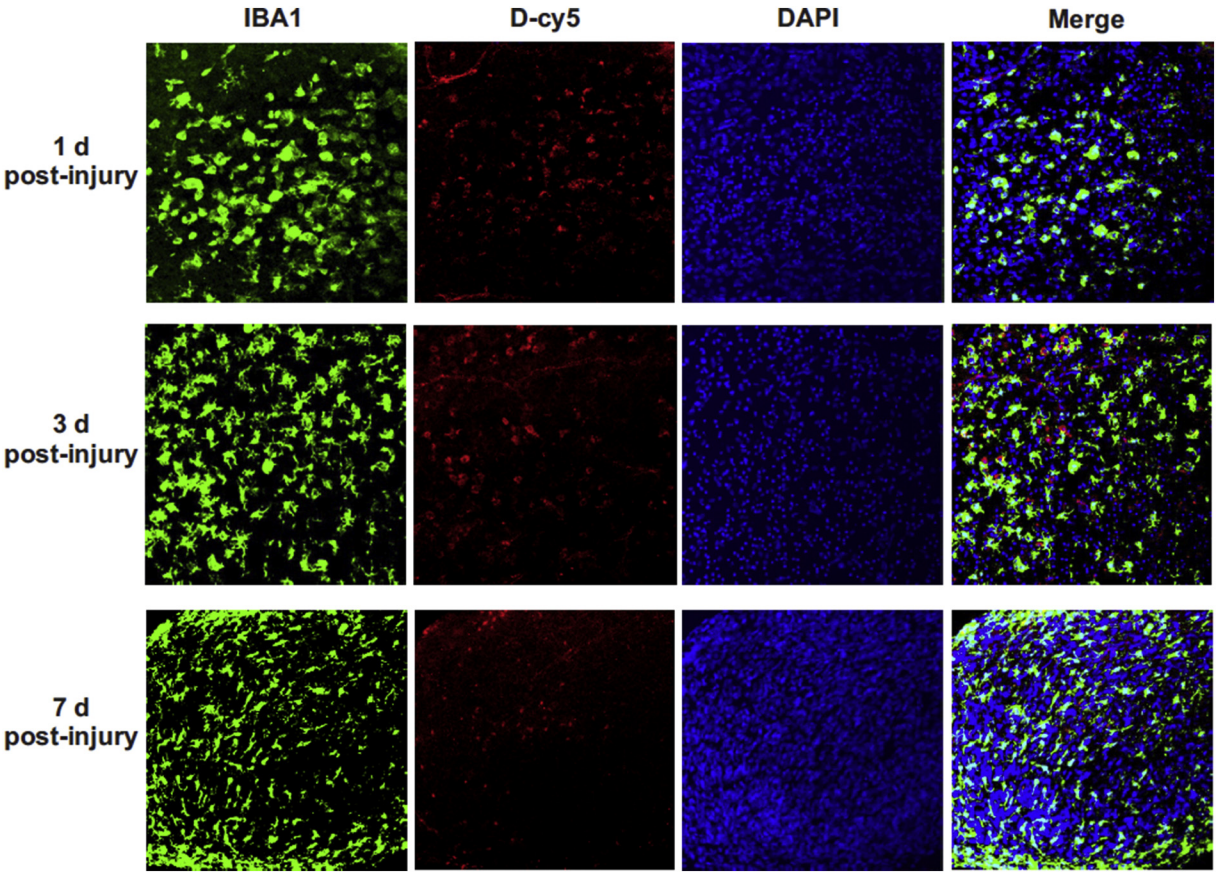

Fig. 2. Dendrimer (D) distribution in the ipsilateral hemisphere following CCI in the immature rabbit brain (P5-P7). D-cy5 (55 mg/kg) was injected (i.p.) at 1, 3 and 7 days post-injury and the rabbit kits were sacrificed 24 h after the D-cy5 injection. After PBS perfusion, the brain were harvested and co-stained with IBA1 (microglial maker). D-Cy5 localized in activated microglia in the ipsilateral hemisphere around the site of injury and in the white matter tracts remote from the injury. Peak localization of D-cy5 was at 3 day post-injury. Localization was seen even at 7 days after injury indicating ongoing microglial activation.

demonstrate here that they also localize in 'activated' microglial cells till at least 7 days post-injury in our previously described rabbit model of pediatric traumatic brain injury induced by controlled cortical impact (Zhang et al., 2019; Zhang et al., 2015b) (see Fig. 2). This data indicates that dendrimer nanoparticles can potentially be used to deliver novel therapeutics to modulate microglial function following traumatic brain injury.

3.1.2.3. Astrocytes. Astrocytes are the most abundant cells in the CNS and are organized in a contiguous, and non-overlapping anatomic structure. Astrocytes also play a key role in facilitating the communication between both neurons and other astrocytes (Parpura et al., 1994; Nedergaard et al., 2003). Discoveries in the last decade or so have shed light on the important role they play in neuronal function, regeneration, as well as the immune response in the brain (Nave, 2010; Attwell et al., 2010; Song et al., 2002; Sriram, 2011). The organized structure of astrocytes serves as a 'guide' for the migration of axons (Yang et al., 2005) as well as synaptic development (Ullian et al., 2001), which demonstrates the important role they play in CNS maturation and potentially in recovery after TBI. In addition, astrocytes are capable of promoting synaptogenesis by secreting thrombospondins which in turn stimulate cell-cell and cell-matrix interactions (Christopherson et al., 2005). Thrombospondin concentrations are highest in the young brain and decline in levels in adulthood indicating that the immature astrocytes are those that are most involved with synaptogenesis (Christopherson et al., 2005). During development, astrocytes are involved in the maintenance and tightening of the BBB (Wang and Bordey, 2008). The secretion of complement proteins by astrocytes initiates synaptic pruning and microglial phagocytosis (Stevens et al., 2007). Astrocytes also help regulate electrolyte levels thus maintaining the functionality of the synapses (Barres, 2008), and are important regulators of cerebral and neuronal blood flow (Mulligan and MacVicar, 2004; Blanchard et al., 2016). Astrocytes have been shown to be more diverse and electrophysiologically active in the young developing brain in contrast to the less diverse and less electrophysiologic phenotype that becomes dominant with age (Zhou et al., 2006). Therefore, traumatic injury to the young brain can potentially lead to significant effects in neuronal-glial interactions due to injury-related changes to the astrocytes that have functional and phenotypic differences in the developing brain when compared to the adult brain.

The ability of astrocytes to support myelination exhibits phenotypic variation in response to injury which implies that therapeutically targeting remyelination could potentially improve the recovery process after TBI (Nash et al., 2011). It is possible that the extent of injury following TBI could dictate the extent and type of astrocyte activation and hence the related inflammatory response. In addition to playing a central role in CNS homeostasis, astrocytes are capable of responding to various types of brain pathology, including TBI via a process referred to as reactive gliosis. TBI-induced mechanical forces create astrocyte reactivity and astrocyte-microglial interactions which in turn disrupt the role that the astrocytes play in development and neuronal maintenance thus initiating a cellular response that leads to both inflammation as well as tissue repair (Burda et al., 2016). Astrocytes have mechanosensitive ion channels that open as a result of the traumatic injury leading to an increase in intracellular Ca^{2+} (Liang et al., 2010). This Ca^{2+} wave leads to the release of ATP, which acts as an extracellular messenger that ultimately plays a role in microglial migration and activation (Davalos et al., 2005; Stout et al., 2002). Astrocytes also

undergo a sharp upregulation of cytoskeletal proteins indicating that these proteins are essential for the appropriate initiation and maintenance of reactive astrogliosis (Burda et al., 2016). Reactive gliosis and astrocytic responses have been shown to be dependent on the type of injury with protective responses seen in ischemia and detrimental responses seen in inflammation (Zamanian et al., 2012). Similarly, in TBI, the astrocytic response varies based on the severity and the extent of the injury resulting in a range of responses that varies from neuroprotection to immune activation and polarization into a proinflammatory phenotype that eventually leads to neurodegeneration (Burda et al., 2016). Astrocytes express pattern recognition receptors such as TLRs (Toll-Like Receptors) and RAGEs (Receptor for advanced glycation end-products) which make them responsive to the inflammatory stimuli that are released from the necrotic neurons into the micro-environment such as DAMPs and PAMPs (Gorina et al., 2011; Villarreal et al., 2014; Serramía et al., 2015). Astrocytes have also been shown to upregulate the expression of TLR4 and TREM-2 in response to an ischemic insult which in turn influences their conversion into a more pro-inflammatory phenotype (Rosciszewski et al., 2018). Using a global Trem2−/− model, Saber et al. demonstrated that Trem2 deficiency acutely decreased macrophage activation throughout the brain without changing astrogliosis but resulted in improved hippocampal atrophy and behavioral outcomes long term following TBI (Saber et al., 2017). The ability of astrocytes to support myelination exhibits phenotypic variation in response to injury which implies that therapeutically targeting remyelination could potentially improve the recovery process after TBI (Nash et al., 2011). It is possible that the extent of injury following TBI could dictate the extent and type of astrocyte activation and hence the related inflammatory response.

3.1.2.4. Mast cells. Mast cells belong to the innate immune system and are derived from the myeloid cell line. They function as amplifiers of the inflammatory response via the release of their granules that contain histamine as well as proteolytic enzymes. In addition, Mast cells contribute to inflammation by releasing prostaglandins as well as proinflammatory cytokines such as TNFα. The majority of mast cells are primarily located in the skin and mucosal surfaces, however they can be found in almost all other tissues including the dura of the CNS (Varatharaj et al., 2012). In fact, a study by Khalil et al. details the important role that CNS mast cells play in brain development, specifically their crucial role in thalamic angiogenesis (Khalil et al., 2007). Aging seems to have a negative effect on peripherally located mast cells, resulting in impaired immune function and diminished immune cell trafficking through the lymphatic system (Pal et al., 2017). Another study uses a rat model to correlate the diminished function of aging mesenteric mast cells with a defective cellular immune response (Chatterjee and Gashev, 2014). Mast cell activation and histamine release promote cell death following an ischemic insult (Wilcock et al., 2018). Certain CNS diseases that are associated with prematurity, such as hypoxic ischemic encephalopathy (HIE) have implicated mast cells in its pathophysiology. Pharmacologically stabilizing the mast cells in those studies seemed to impart neuroprotective effects (Biran et al., 2008; Jin et al., 2009). Moretti et al. investigated the role of mast cells in a closed head pediatric murine TBI model, and concluded that neither the genetic deletion nor the pharmacologic inhibition of mast cells attenuated the neuroinflammatory response (Moretti et al., 2016).

Mast cells are also thought to be involved in the chronic immune response to subdural hematomas (SDH) (Greeley and Karst, 2013). Subdural hematomas sometimes develop into chronic fluid collections (Hwang and Kim, 2000), referred to as hygromas, the mechanism of which is still unknown. The formation of hygromas may result in the chronic activation of mast cells which in turn leads to local inflammation and an increase in vascular permeability (Greeley and Karst, 2013; Dimitriadou et al., 1997; Theoharides et al., 2005). Hence, mast cells that are located at the borders of the fluid collection may contribute to the breakdown of the blood-brain barrier (Stokely and Orr, 2008). The pathophysiology of mast cells after TBI, specifically histamine release may explain some of the chronic symptoms associated with head injury such as post traumatic headache (Levy et al., 2016; Kamins and Charles, 2018).

3.2. Adaptive immune response

While the innate immune system is responsible for providing a rapid, non-specific inflammatory response when faced with infection or tissue damage, the adaptive immune system provides a more purposeful and specific antigen dependent response. More importantly, adaptive immunity is distinguished by its ability to form memory cells, which allows it to mount a more efficient immune response during subsequent encounters with the same antigen. The cells of the adaptive immune system are the (1) T lymphocytes, which are divided into the cytotoxic CD8 T cells, the helper CD4 T cells as well as the regulatory T cells (T regs), (2) B cells whose main function is antibody production but are also crucial in the memory function of the adaptive immune system, and (3) antigen presenting cells (APC), that process self or foreign antigens in order to activate the T cells (Warrington et al., 2011). Additionally, CD4 helper cells can be divided into the Th1 or Th2 helper cells which secrete proinflammatory and immunosuppressive cytokines, respectively. The function of T regs is to attenuate the immune response in order to prevent the damaging effects of autoimmunity and chronic inflammation. Another T cell subset includes the Th17 cells which are stimulated by IL17 and have been implicated in autoimmune inflammatory conditions. The interplay between the adaptive immune response and the CNS spans over several pathological and inflammatory states such as multiple sclerosis (MS), encephalitis as well as trauma. For example, Th1 and Th17 subtypes play an important role in the pathophysiology of experimental autoimmune encephalitis, MS, and other disease states that lead to neurodegeneration (Stromnes et al., 2008; Pierson et al., 2012). On the other hand, the Th2 phenotype has a neuroprotective role by supporting resting microglia and slowing down neurodegeneration (Gimsa et al., 2001).

The immature adaptive immune system has several features that differentiates it from the adult immune system. These differences may have several implications on the role of the adaptive immune response in the pathogenesis of TBI. For example, a newborn's immune system has an imbalance in the Th1:Th2 systems, whereby the pro-inflammatory Th1 lymphocytes are diminished, compared to an increase in the anti-inflammatory Th2 cells. This results in an increased susceptibility to both infections as well as allergic reactions (Zaghouani et al., 2009). In addition, the dendritic cells of newborns are immature and their numbers are decreased compared to adults, which leads to a decrease in IL-12 production and the subsequent imbalance of the Th1 and Th2 ratio (Zaghouani et al., 2009)

The role of the adaptive immune system in the pathophysiology of TBI remains unclear. Several studies have shown that T cells are a necessary component of CNS development since T cell-deficient mice (RAG knockouts) characteristically have cognitive and behavioral impairment (Filiano et al., 2015). In addition, T cell deficient mice have been shown to have poor outcomes in murine CNS injury models which potentially implies that T cells serve in a neuroprotective capacity (Wolf et al., 2009; Simon et al., 2018). Several studies have investigated the effect of immunosuppression in spinal cord injuries with promising results in terms of attenuating the neuroinflammatory response, promoting neuronal tissue regeneration, and improving functional outcomes (Lee et al., 2009; Norimatsu et al., 2012; Zhang et al., 2009). A study by Weckbach et al. contradicts earlier findings that T-cell deficient mice ($Rag1^{-/-}$) demonstrate improved neuroprotection. They were not able to find any evidence that these mice had better outcomes than wildtypes in terms of the extent of neurological impairment, neuroinflammation, cell death, as well as BBB dysfunction (Weckbach et al., 2012).

In an attempt to characterize the nature of the adaptive immune

Table 1
Physiologic and pathologic age-related immune response.

	Neonate/Infant	Adult
Neutrophils	Greater number of immature neutrophils with incomplete function (Makoni et al., 2016; Lawrence et al., 2017) Quantitative neutrophil deficiency during stress (Levy, 2007) Unsteady blood neutrophil concentration (Melvan et al., 2010) Reduced TLR4 expression (Förster-Waldl et al., 2005) Decreased L-selectin level (Kim et al., 2003) Neutrophil chemotaxis function is delayed for up to 10 days of life (Eisenfeld et al., 1990) More sensitive to neutrophil elastase due to lower anti-oxidant levels (Semple et al., 2015)	Steady blood neutrophil concentration (Melvan et al., 2010)
Microglia	No increase in TLR2 expression in murine stroke model (Lalancette-Hébert et al., 2017) Deletion of scavenger receptor CD36 increases injury in murine stroke model (Woo et al., 2012) Depletion of activated microglia increases inflammation (Faustino et al., 2011)	Acute increase in TLR2 expression in murine stroke model (Lalancette-Hébert et al., 2009) Deletion of scavenger receptor CD36 reduces injury in murine stroke model (Kuehn, 2018) Associated with tau pathology and Alzheimer's disease (Katsumoto et al., 2018) Increased inflammatory signaling (Frank et al., 2006) Decreased anti-inflammatory signaling (Sierra et al., 2007) Decreased proteolytic activity (Tremblay et al., 2012)
Astrocytes	More diverse and electrophysiologically active (Zhou et al., 2006) Helps with the maintenance of cerebrovascular resistance at 50% of adult (Longo and Goyal, 2013)	Become more reactive with age (Clarke et al., 2018) Increase in complement protein gene expression (Boisvert et al., 2018) Retain reactive oxidative species (ROS) (Ishii et al., 2017) Less diverse and electrophysiologically passive (Zhou et al., 2006)
Mast Cells	Stabilization of mast cells provides protection against hypoxic-ischemic injury in rats (Jin et al., 2009) Interact with astrocytes to induce angiogenesis in brain (Khalil et al., 2007)	Mast cell activation is associated with Alzheimer's disease (Kempuraj et al., 2017) Aging increases concentration of mast cells in perilymphatic tissue (Pal et al., 2017) Aging decreases mast cell-directed recruitment of immune cells towards mesenteric lymphatic tissue (Chatterjee and Gashev, 2014)
T Cells	Dendritic cells are immature and are lower in number compared to adults, resulting in decrease in IL-12 and the subsequent imbalance in the Th1/Th2 ratio (Zaghouani et al., 2009). The pro-inflammatory Th1 lymphocytes are diminished, compared to an increase in the anti-inflammatory Th2 cells (Zaghouani et al., 2009).	

response in pediatric patients with severe TBI, Amick et al. collected cerebrospinal fluid samples from ventriculostomy drains. Specifically, they looked at cytokine profiles to determine whether the T cells have a predominant pro-inflammatory Th1 or an anti-inflammatory Th2 phenotype. They concluded that there is a predominance of a Th2 response as seen by an increase in the anti-inflammatory cytokines IL-6 and IL-10. However, the pro-inflammatory cytokine, IL-12 was also increased, indicating that the adaptive immune response consists of a mixed Th1 and Th2 response (Amick et al., 2003). Another study by Braun et al. demonstrated that after TBI, T cells were skewed to take on a Th1 and Th17 phenotype, with a simultaneous decrease in the Treg population. They describe that stimulus driving this polarization is a result of infiltrating peripheral M1 macrophages (Braun et al., 2017). Future studies that are based on age specific evaluations of Th1, Th2 and Th17 responses following TBI would be crucial in better defining the adaptive immune response and its role in ongoing inflammation in pediatric TBI. For a summary of age-related differences in the innate and adaptive immune responses in the brain, please refer to Table 1.

4. Therapies targeting inflammation

The current management of pediatric patients suffering from TBI is supportive and is primarily focused on the reduction of secondary injury. The 2019 clinical guidelines for the acute management of pediatric TBI revolve around controlling intracranial pressure, optimizing ventilation strategies, maintaining normothermic core temperatures, and correcting coagulopathy in addition to other supportive maneuvers (Kochanek et al., 2019). This further illustrates the lack of targeted TBI specific interventions that can significantly restore brain function following trauma. Moreover, the pediatric population requires special consideration due to the multiple physiologic differences between the pediatric and the adult brain (Figaji, 2017). Clinicians that are involved in pediatric TBI care have recognized the lack of pediatric specific knowledge and have initiated the Approaches and Decisions in Acute Pediatric TBI trial (ADAPT) to help further elucidate pediatric TBI management (Bell et al., 2017). The goal of acute TBI management should consider not only the short-term outcomes but also the long-term consequences of acute TBI management. Due to the crucial role of the immune system in both the acute and the chronic injury, as well as in repair and long term plasticity, targeting the immune system in a nuanced manner would offer novel opportunities especially in the context of pediatric TBI (Huh and Raghupathi, 2019).

Steroid therapy was at one point considered to be a candidate for reducing the inflammatory response secondary to TBI and was used in an attempt to achieve improved neurologic outcomes. Corticosteroids were also thought to diminish edema and cerebrospinal fluid production as reported in feline and canine models (Pappius and McCann, 1969; Weiss and Nulsen, 2009), but direct evidence that steroids lead to clinical improvement in pediatric patients is lacking. Since the use of steroids has neither been associated with acute nor long-term improvement, its use is currently not part of the clinical guidelines for treating pediatric TBI patients (Kochanek et al., 2012).

Other anti-inflammatory drugs that were tested in the treatment of TBI include the nonsteroidal anti-inflammatory drugs (NSAIDs), which were previously shown to improve cognitive outcomes in mouse models of Alzheimer's disease (Morihara et al., 2005). However, a subsequent study by Browne et al. demonstrated that the chronic administration of ibuprofen in a rat TBI model had the opposite effect, which is worsening cognitive outcomes (Browne et al., 2006). It was initially thought that ibuprofen, which nonspecifically inhibits both the inflammatory and the anti-inflammatory COX pathways is the reason behind worse outcomes after TBI. However, the use of COX-2 specific inhibitors also failed to show improvement in TBI outcomes in a rat model (Hickey et al., 2007). Studies using NSAIDs demonstrated that the inflammatory pathways in TBI are at least partly required in order to achieve better outcomes. Another immunosuppressive drug under investigation for its

potential role as a neuroprotective agent in the setting of TBI is cyclosporine. A continuous infusion of cyclosporine over 5 days in a porcine CCI model was found to be promising, demonstrating improvement in the neuronal injury as well as the extent of the parenchymal injury. The proposed neuroprotective effects of cyclosporine were attributed to its effects on the desensitization of mitochondrial permeability transition pore activation in addition to the prevention of excess ROS formation (Karlsson et al., 2018).

Targeting microglial activation and proliferation using the antibiotic minocycline is a strategy that proved successful in adult TBI models by imparting neuroprotective effects as well as enhanced neurocognitive and motor deficits (Homsi et al., 2009; Homsi et al., 2010; Siopi et al., 2011; Siopi et al., 2012; Abdel Baki et al., 2010; Sangobowale et al., 2018). Short-term minocycline treatment was tested in a neonatal rodent model without being able to replicate the neuroprotective effects that were observed in the adult models. Decreased microglial proliferation and activation were observed, however neurodegeneration was increased and there was no effect on neurocognitive variables (Hanlon et al., 2016; Chhor et al., 2017), suggesting an age-related therapeutic effect.

The sex-linked hormone, progesterone has been shown to have beneficial effects on the immune system in a multiple sclerosis model by inhibiting microglial nitric oxide (NO) and TNF-α production (Drew and Chavis, 2000). Progesterone treatment in a repeated mild TBI rat model resulted in decreased neuroinflammation and oxidative stress as well as an improvement in cognitive and sensorimotor deficits (Webster et al., 2015). There are two phase 3 trials investigating the role of progesterone treatment: the PROTECT (Progesterone for the Treatment of Traumatic Brain Injury) III (Wright et al., 2014) and the SYNAPSE (the Study of a Neuroprotective Agent, Progesterone in Severe Traumatic Brain Injury) trials (Skolnick et al., 2014). The PROTECT III trial was terminated early at 882 patients after a futility analysis revealed no benefit from progesterone treatment (Wright et al., 2014). The SYNAPSE trial enrolled 1195 adult patients (ages 16–70) with severe TBI and also did not show any benefit from progesterone treatment (Skolnick et al., 2014). Unlike the adult studies, there are very few studies evaluating progesterone following pediatric TBI. Progesterone receptors are abundant in the developing brain and may play a critical role in maturation (Quadros et al., 2007). A concern about using progesterone in the developing brain is that progesterone is known to potentiate GABAa receptors that are normally inhibitory in the adult brain but excitatory in the developing brain, which can potentially lead to neurotoxicity in the young brain. Related to this, studies have shown an age-dependent worsening in hypoxic-ischemic injury with progesterone therapy with younger animals showing worsening of the injury when compared to older animals (Rice et al., 1981; Tsuji et al., 2012). In a pre-clinical model of pediatric TBI induced by CCI in immature rats, mitochondrial respiratory control ratio was found to be improved in male rats treated with progesterone but not in females (Robertson and Saraswati, 2015). Similarly another study in 4 week old mice undergoing CCI demonstrated improvement in motor function in males but worsening in females after progesterone treatment (Mannix et al., 2014).

Another treatment modality is the use of cellular therapy such as autologous bone marrow-derived mononuclear cell treatment. Studies in a rat model of TBI have shown that the intravenous administration of multipotent adult progenitor cells (MAPC) in multiple doses leads to the preservation of the blood-brain barrier in addition to decreasing the number of activated microglia and macrophages in the brain. This was associated with improved spatial learning in their CCI rat model. The authors concluded that the optimal timing of the treatment that leads to the most effective outcomes was at 2 and 24 h post injury (Bedi et al., 2018). This was translated into a human trial which determined the feasibility of autologous bone marrow-derived mononuclear cell injections in the pediatric population (Cox et al., 2011).

Other therapeutic modalities include immune modulation using receptor-specific targets such as the Toll-Like Receptor 4 (TLR4) signaling pathway (Jiang et al., 2018; Corrigan et al., 2017). There is mounting evidence suggesting that TLR4 signaling is involved in neuronal injury that is mediated by oxidative stress (Kenny and O'Neill, 2008). TLR4 inhibitors such as resatorvid (Matsunaga et al., 2011) have been shown to improve neurocognitive outcomes after TBI in a murine model (Zhang et al., 2014). Interestingly both TLR4 inhibition and stimulation have been shown to improve neurobehavioral outcomes (Jiang et al., 2018; Corrigan et al., 2017). This highlights the fact that we do not yet fully understand the scope of the immunologic interactions that are associated with TBI-induced neuroinflammation. A combination therapy of progesterone and vitamin D attenuated the TLR4-mediated neuroinflammatory reaction resulting in neuroprotection (Yousuf et al., 2015), further demonstrating the complexity and the overlap between the various pathways of TBI-induced inflammation.

Another molecule that has been investigated as a potential target that would attenuate the neuroinflammatory response is the high-mobility group box 1 (HMGB1) molecule, a Damage Associated Molecular Pattern (DAMP) that has been implicated in initiating the neuroinflammatory response. Postmortem sections of human brains from patients that died from a TBI were used to study the pattern of HMGB1 expression. The same group corroborated those findings in a rat TBI model, demonstrating that in the early stages after trauma, HMGB1 is translocated from the nucleus into the cytoplasm to be phagocytosed by the microglia at a later time point (Gao et al., 2012). Taking it a step further, Okuma et al. used a monoclonal antibody against HMGB1 (Anti-HMGB1 mAb) in both a rat and a mouse fluid percussion TBI model to successfully target and reduce HMGB1 translocation from the nucleus to the cytoplasm in neurons within the lesion, suppressing the reduction of HMGB1 from the site of injury (Okuma et al., 2012). More importantly, treatment with the anti-HMGB1 mAb lead to a reduction in brain edema, neuronal cell death as well as the improved maintenance of the BBB. Animals that were treated with anti-HMGB1 mAb also showed improvement in motor function as reflected in the rotarod and cylinder tests (Okuma et al., 2012). Similarly, ethyl pyruvate (EP), an ester of pyruvic acid, which has been shown in other models of tissue injury to attenuate sepsis by inhibiting HMGB1 release was evaluated in TBI. Treatment with EP in a rodent TBI model attenuated HMGB1 signaling, and significantly reduced HMGB1 and TLR4 protein levels which subsequently led to the reduction in brain edema and the improvement in neurocognitive outcomes in the beam walking balance test (Su et al., 2011).

5. Conclusion

It is well-known that both the adaptive and the innate immune systems play a significant role in normal brain development. Since the neuroinflammatory response after TBI plays an important role in the extent of injury and recovery after injury, we would anticipate that these may be different in the immature versus the adult brain. Certain pharmacologic therapies demonstrate an age-dependent response and this discrepancy in therapeutic responsiveness between the different age groups highlights the importance of tailoring the therapeutic strategies to take into account the developmental age of the brain and the immune system in order to optimize the inflammatory response and to facilitate repair.

Although there have been several successful pre-clinical trials using anti-inflammatory agents, none of those results have translated into positive clinical trial outcomes. This indicates that a multi-pronged approach that targets several pathways including the immune system may be necessary for attenuating injury and for improving plasticity and repair. Novel delivery tools such as dendrimer nanoparticles may be helpful in targeting the innate immune system by delivering therapeutic agents specifically to glial cells. Considering the crucial role that the immune system plays in normal brain development and maturation, designing therapies that would target the neuroinflammatory

process at the appropriate time points to facilitate long-term recovery and promote normal brain maturation would be necessary for treatment of pediatric TBI.

Additionally, an important point that was highlighted in this review is the paucity of literature that exists with regards to the age-related neuroimmune response to TBI. Future research is needed that specifically investigates the relationship between the age-related developmental differences and the ensuing neuroinflammatory response. The roles of both the adaptive and the innate immune responses would need to be evaluated to better understand their roles in repair following TBI in the young brain. Elucidating these relationships will facilitate the design of targeted immunomodulatory therapies. Additionally, variations in the dosage as well the duration of the therapeutic agents will have to be carefully considered when being tested in the developing brain since there is potential for great variability in responsiveness when compared to adult brains.

Pre-clinical pediatric age trials that target the immune response are crucial for the future of the field of pediatric TBI and need to be carefully designed bearing in mind the differences that exist between the mature and the developing brain.

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