Review Article

Current understanding of neuroinflammation after traumatic brain injury and cell-based therapeutic opportunities

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ABSTRACT

Traumatic brain injury (TBI) remains a major cause of death and disability worldwide. Increasing evidence indicates that TBI is an important risk factor for neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, and chronic traumatic encephalopathy. Despite improved supportive and rehabilitative care of TBI patients, unfortunately, all late phase clinical trials in TBI have yet to yield a safe and effective neuroprotective treatment. The disappointing clinical trials may be attributed to variability in treatment approaches and heterogeneity of the population of TBI patients as well as a race against time to prevent or reduce inexorable cell death. TBI is not just an acute event but a chronic disease. Among many mechanisms involved in secondary injury after TBI, emerging preclinical studies indicate that posttraumatic prolonged and progressive neuroinflammation is associated with neurodegeneration which may be treatable long after the initiating brain injury. This review provides an overview of recent understanding of neuroinflammation in TBI and preclinical cell-based therapies that target neuroinflammation and promote functional recovery after TBI.

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Introduction

Traumatic brain injury (TBI) is a leading cause of morbidity and disability worldwide with a substantial socioeconomic burden.[1] Approximately 1.7 million people experience TBI in the United States every year and up to 75% of these injuries are classified as mild TBI (mTBI).[2] The average annual number of TBI cases in China is 3—4 million.[3] It has been estimated that TBI affects over 10 million people annually leading to mortality and hospitalization worldwide.[1,4] TBI, according to the World Health Organization (WHO), will become the major cause of death and disability by the year 2020.[1] TBI has been associated with long-term cognitive deficits relating to trauma-induced neurodegeneration. These long-term deficits include impaired memory and attention, changes in executive function, emotional instability, and sensorimotor deficits.[5] Besides the pre-existing health conditions (including age, sex,

diseases, alcohol/drug abuse, and genetic factors), heterogeneity of injury location, mechanisms, severity, and polytrauma contribute to differences in the course and outcome of TBI.[6,7] TBI exacerbates pre-existing disorders and is an important risk factor for neurological diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), epilepsy, stroke, and chronic traumatic encephalopathy (CTE).[8,9] CTE is a neurodegeneration characterized by the abnormal accumulation of hyperphosphorylated tau protein measured in the postmortem brains of American football players, professional boxers and bull riders with histories of repetitive concussive injuries.[10–12] Despite improved supportive and rehabilitative care of TBI patients, unfortunately, over 30 clinical trials in TBI have yet to yield a safe and effective neuroprotective treatment.[13–19] Recent clinical trials for erythropoietin[20,21] and progesterone[22–24] fall into this category of failure, which is in contrast to the robust preclinical data.[25–27] Further study of the cellular and molecular posttraumatic processes is warranted for better understanding of TBI pathophysiology and for developing therapeutic targets for treatment of TBI.

Animal models of TBI are essential for studying the biomechanical, cellular, molecular and behavioral aspects of human TBI as

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well as for developing and characterizing novel therapeutic interventions that cannot be directly addressed in the clinical setting.[6,28–30] Although larger animals with gyrencephalic brains are closer in size and physiology to humans and have been increasingly used,[31–33] lissencephalic rodents are most frequently used in TBI research due to modest cost, small size, easy genetic manipulation, and availability of standardized functional outcome measurements among other reasons.[28] It is impossible to mimic all aspects of TBI in a single animal model and therefore, a variety of TBI models have been developed in animals with various ages, injury type, severity levels and comorbidities/polytrauma to study different aspects of TBI pathology observed in humans.[6,28,29,34,35] Among them, five animal models of TBI are widely used: fluid percussion injury (FPI),[36,37] cortical impact injury (CCI),[38,39] weight drop/impact acceleration injury,[40,41] gunshot penetrating injury,[42,43] and blast injury.[44,45] Repeated head impacts are likely associated with the development of the neurodegenerative disorders including CTE.[46] Over the last decades a number of rodent models of repeated mTBI have been developed with adaptations mainly based on these well-established TBI models to allow for better modeling of the mechanical forces associated with concussion.[47–50] Although animal models of mTBI using CCI and FPI in rodents have successfully reproduced some of the cognitive deficits frequently exhibited by patients with mTBI, modeling post-concussion symptoms is challenging.[49] Recent use of closed head and blast injury animal models more closely mimics clinical mTBI,[49,51–54] which will advance understanding of mTBI pathophysiology and accelerate clinical translation to benefit people affected by mTBI.

In TBI, primary injury occurs at the time of trauma and is the direct result of the external mechanical forces producing deformation of the brain tissue (contusion, damage to blood vessels, and axonal shearing) and disruption of normal brain function. Secondary injury is extensive, and lasting damage is sustained through a complex cascade of events including ischemic and hypoxic damage, cerebral edema, raised intracranial pressure, hydrocephalus, and infection,[6,55] which are induced by a multifactorial set of events including glutamate excitotoxicity, perturbation of cellular calcium homeostasis, membrane depolarization, mitochondrial dysfunction, inflammation, increased free radical generation and lipid peroxidation, apoptosis, and diffuse axonal injury.[17,56] Once TBI occurs, the immediate neurologic damage caused by the primary traumatic forces may not be treatable, while the secondary neurologic damage produced by a cascade of secondary events after the primary injury evolves minutes to years, and the extended temporal profile of injury may provide opportunities for therapeutic interventions.[57]

Neuroinflammation

Neuroinflammation in response to TBI involves the activation of resident glia (microglia and astrocytes), release of inflammatory mediators within the brain, and recruitment of peripheral immune cells (leukocytes).[58] TBI is a multi-system pathology with the complex interactions between the brain, the periphery and the immune systems.[59] Although all brain cell types (neurons, astrocytes, microglia, oligodendroglia, and endothelial cells) can produce proinflammatory cytokines, microglia are the major resident immune cells of the brain which are thought to arise from macrophage/monocytes from the bone marrow early during embryogenesis.[60] Peripheral macrophages can also infiltrate the brain and transform into microglia in response to TBI.[61] Microglia play a critical role in neuroinflammation as the first line of defense whenever injury occurs.[62] Microglia in the injured brain produce anti-inflammatory mediators, scavenger cellular debris and

orchestrate neurorestorative processes to promote neurological recovery after TBI.[62] However, microglia can produce excessive proinflammatory mediators that exacerbate brain damage, hinder brain repair and neurological functional recovery.[62] For example, levels of the proinflammatory cytokines interleukin 1 beta (IL-1β), IL-6, IL-17, tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), and as well as the chemokines macrophage chemotactic protein-1 (MCP-1), macrophage inflammatory protein 2 (MIP-2), chemokine (C-C motif) ligand 5 (CCL5) are significantly increased while levels of the anti-inflammatory cytokines IL-4, IL-10, IL-13, and transforming growth factor-β1 (TGF-β1) are reduced in the injured brain of TBI rats.[62–67] Anti-inflammatory cytokines have the ability to counteract and downregulate inflammatory and cytotoxic reactions.[62,64–69] Proinflammatory cytokines are produced mainly by microglia, with some also generated by astrocytes, neurons and endothelial cells, which in turn activate glial cells, inducing further cytokine production and astrogliosis.[68,70] Therefore, neuroinflammation can be detrimental and/or beneficial after TBI.[71,72] These dual roles of microglia may be accounted for by their polarization state and functional responses after injury. Recent reports suggest that microglia/macrophages not only display a polarized M1 (a classical pro-inflammatory state) or M2 (an alternative anti-inflammatory state) phenotype, but also a mixed phenotype over time after TBI.[73] Specifically, microglia/macrophages express M1- and M2-type phenotypic markers in mice early after moderate TBI induced by controlled cortical impact (CCI), but that the transient upregulation of the M2-type phenotype is followed by a predominant M1-type or transient mixed (Mtran) phenotype that expresses high levels of reactive oxygen species-producing nicotinamide adenine dinucleotide phosphate oxidase (NOX2) in reactive microglia at the site of injury, and this is accompanied by ongoing cortical and hippocampal neurodegeneration.[74] Inhibition of NOX2 activity in microglia/macrophages after TBI alters M1/M2-type balance towards an M2-type phenotype, which is associated with reduced oxidative damage in neurons post injury.[74] M1 response rises rapidly in young rats after a unilateral CCI, and overpowers the initial M2 response.[75] An early treatment that can modulate inflammation, reduce secondary injury cascades, and improve recovery should be introduced after TBI.

TBI compromises the integrity of the blood—brain barrier (BBB), a physical barrier separating the brain parenchyma from blood.[76–78] BBB damage is an early event that may persist for many months or years after TBI in animals[79] or humans.[80] Injury-induced BBB damage allows infiltration of peripheral immune cells into the brain to exacerbate neuroinflammatory response.[81] Increased permeability of the BBB after injury allows extravasation of plasma proteins into the brain.[71,82,83] One of the plasma proteins is fibrinogen, a plasma adhesion protein, and its deposition in extravascular space induces neuroinflammation and astrocyte scar formation by activating transforming growth factor-beta (TGF-β) signal pathway,[84–86] providing evidence that deposition of fibrinogen that leaks into the brain acts through TGF-β as a molecular link between vascular permeability and scar formation.[85,86] Although primary injury occurs in milliseconds during TBI, a prolonged state of inflammation after brain injury may linger for years and predispose TBI patients to develop other neurological disorders, such as AD.[79,87–89] Prolonged microglial activation is strongly associated with worsening pathology and outcomes.[87] Chronic alterations in neurons with reduced dendritic spine density lasting >1 year after TBI are in parallel with a long-lasting inflammatory response throughout the entire brain.[90] Persistent neuroinflammation in TBI patients may contribute to progressive and long-lasting impairments in their physical, cognitive, behavioral, and social performance.[72,91,92] Neuroinflammation-induced secondary injury after TBI has been linked to chronic

neurodegenerative diseases; however, anti-inflammatory agents have failed to improve TBI outcomes in clinical trials.[65,66,72,93,94] Inflammatory mediators not only induce secondary injury and neurodegeneration but also promote repair and regeneration following TBI. Early inflammation can set the stage for proper neural regeneration and functional recovery. Inhibition of these beneficial responses will likely enhance neural damage and impede the repair response to TBI. This may explain why immune-dampening drugs (e.g., methylprednisolone, and progesterone) have not achieved a clinical benefit in human TBI patients when administered shortly after injury.[95] Nevertheless, neuro-inflammation can become maladaptive over time, especially when macrophages and microglia remain in an inflammatory state in the brain for months or years and acquire abnormal functions. A chronic manipulation of inflammatory response, but not an acute manipulation alone, is more likely to result in long-lasting therapeutic effects.

Chronic traumatic brain inflammation manifested by extensive microglial and astroglial activation may be the most important cause of post-traumatic neurodegeneration including CTE.[96–104] Neuroinflammation is associated with greater p-Tau pathology in CTE.[104] The potential key role of increased neuroinflammation in CTE development and progression suggests that inflammatory molecules may serve as important diagnostic or predictive bio-markers as well as promising therapeutic targets in CTE.[104] The brains of animals with repeated mTBI induced by lateral FPI show substantial loss of neurons and increases in microglia in the hippocampus of brain.[105] These changes last for several weeks after the injury, and are associated with decreased memory.[105] Recently, a cadaver study highlights the presence of activated microglia (CD68-positive cells) close to tau deposits in brain with CTE pathology.[104,106–108] Repetitive mTBI produces acute and prolonged cognitive and anxiety-like disturbances associated with inflammatory changes (astrocytosis and microglial activation) in brain regions involved in spatial memory and anxiety in mice after closed head injury.[109,110] Acute (initiated 30 min post injury) or delayed (initiated 9 months post injury) treatment with anatabine that has putative anti-inflammatory actions improves long-term spatial memory and reduces pathological sequelae at late time-points in mice after repetitive mTBI.[111] These studies show that neuro-inflammation may be an important manipulatable aspect of secondary injury in animal[75,96,97,112] and human TBI.[58,113,114] However, the use of general anti-inflammatory drugs has not worked effectively for TBI.[66] The key to developing future anti-inflammatory treatments for TBI is to minimize the detrimental effects of neuroinflammation while promoting their beneficial effects, thereby creating optimal conditions for regeneration and repair after injury.[66] Developing precision effective treatments is challenging but they need to be designed based on severity and type of brain injury, age and sex of the patient, existing health and polytrauma conditions, and the inflammatory profiles over time following TBI.

Despite increasing appreciation of the critical role of neuroinflammation in brain injury and neurodegeneration in rodents, little is known about acute microglial reactivity in larger animals with diffuse axonal injury. Acute microglial activation converges on proximal axonal swellings undergoing diffuse axonal injury in pigs following mTBI, an interaction not previously recognized in the literature.[115] Using a porcine model of closed-head rotational velocity/acceleration-induced TBI that closely mimics the biomechanical etiology of inertial TBI in humans, Wofford et al. observed rapid microglial reactivity within 15min of both mild and severe TBI.[116] Strikingly, microglial activation was constrained to regions proximal to individual injured neurons, and microglial reactivity around injured neurons was exacerbated following repetitive TBI, suggesting further amplification of acute neuroinflammatory

responses. Their findings suggest that neuronal trauma rapidly activates microglia in a highly localized manner, which may rapidly influence neuronal stability and/or pathophysiology after diffuse TBI.[116] The combination of porcine TBI and shock results in an immediate activation of coagulation and complement systems with subsequent endothelial shedding, protein C activation, and inflammation.[117] Treatment with artificial colloid and valproic acid a histone deacetylase inhibitor improves hemodynamic parameters, reduces swelling and the size of brain lesion, and attenuates the inflammatory response in the combined porcine hemorrhagic shock and TBI model.[118–120]

Sex difference in neuroinflammation

TBI is a heterogeneous disease associated with different outcomes that vary by individual and by sex.[121] Based on the demographic data that TBI primarily occurs in young male civilian and military.[122,123] Sex differences in mortality and functional outcome exist after TBI.[124] However, controversy exists regarding the role of sex in TBI outcome and response to TBI treatments.[125–132] Male and female nervous systems respond differently to TBI and preclinical research relates this difference to neuroprotection from female sex hormones.[133] Microglia have sexually dimorphic roles in development and maintenance of the normal brain,[134] and have different responses and roles in TBI between males and females.[124] Microglia from female mice have a lower inflammatory status than male mice after middle cerebral artery occlusion.[135] Microglia play a central role in the secondary proinflammatory and pathologic responses after TBI, leading to disruption of the BBB and increased cerebral edema.[124] However, the vast majority of preclinical TBI studies of the inflammatory response to TBI have been conducted in male animals. Females respond differently to brain injury because of hormonal changes.[125,133] The specific mechanisms underlying these sex differences remain elusive. The temporal and spatial differences in the male/female microglia and macrophage response show complete divergence in the subacute phase post injury with male mice displaying faster and more pronounced microglia/macrophage activation and astrogliosis following moderate-to-severe TBI compared with females.[121] A divergent cytokine mRNA profile in these cells after injury is associated with early neuronal cell death and larger lesion volume in the first few days post injury in male mice compared with females.[121] Deletion of one allele of chemokine fractalkine (CX3CL1) mediates neural/microglial interactions via its sole receptor CX3CR1 receptor, limits infiltration of peripheral immune cells and largely prevents the chronic degeneration of the injured brain and provides improved functional recovery in female, but not in male, mice.[90,136] A recent study suggests that comorbidity of TBI and posttraumatic stress disorder (PTSD) in a military population sample are associated with inflammation, emphasizing the necessity for intervention in order to mitigate the risks associated with inflammation.[137] Women show higher incidence of trauma-related mental health disorders including PTSD than their male counterparts.[138] However, the data in the literature demonstrate that male animals are significantly more vulnerable to acute and chronic stress, whereas females are far more resilient.[139] This is in stark contradiction to epidemiological data regarding the prevalence of PTSD in humans.[138] TBI leads to a more aggressive neuroinflammatory profile in male compared with female mice during the acute and subacute phases postinjury.[121] The sex specific feature of the secondary inflammatory response may be connected to different prostaglandin regulation with higher level in males after TBI.[140] It is apparent that sex differences likely exist, but the contrary nature and magnitude of such differences existing in the literature does not allow for drawing definitive conclusions.[124] In order to develop personalized and effective treatments for TBI, it is

important to understand how sex affects the course of neuro-inflammation following brain injury. Pharmacological interventions that target the inflammatory cascade should consider sex differences as a significant variable factor following TBI.

Role of age in neuroinflammation

TBI incidence peaks between the ages 15 and 24 and after 75 years of age.[141] Age-at-injury is a key factor for acute and long-term functional recovery and advanced age is associated with poor outcome following TBI, and age has been shown to be a primary determinant of survival following isolated TBI.[141] The mechanisms behind the poor outcome of aged versus young individuals may be a result of a more severe secondary brain damage, increased neuroinflammation, or reduced plasticity of old individuals to compensate neurological deficits.[141–143] A recent study indicates that old animals are prone to increased functional deficits and strong ipsilateral cerebral inflammation without major differences in morphological brain damage compared to young animals.[141] Infiltration of peripheral monocytes significantly contributes to the etiology of trauma-induced inflammatory sequelae in the aged brain.[142] A recent study in the aged brain demonstrates that there is an increased accumulation of peripherally derived chemokine receptor 2 (CCR2+) macrophages after TBI compared to young animals, and excessive recruitment of this population of cells is associated with an augmented inflammatory response in the aged TBI animals.[142] Aging and injury are inflammatory priming events, and either one can exacerbate the other.[144] Genetic deletion of CCR2 reduces macrophage numbers by 80% early after TBI, and improves functional recovery and neuronal survival in young mice.[145] CCR2 antagonism significantly reduces macrophage accumulation and cognitive dysfunction 1 month in young mice after TBI.[146] Therapeutic treatments for targeting the CCR2 (+) subset of monocytes/macrophages may provide a new avenue of clinical intervention following TBI. Brain injury-induced production of cytokines and chemokines is age-dependent, and differentially regulated by microglial Kv1.3 channels and P2Y12 receptors.[147] TBI causes larger lesions associated with a shift in M1/M2 balance resulting in a pro-inflammatory profile in hippocampal microglia of aged mice compared to young adult mice.[148] While cell therapy appears effective for TBI, reduced efficacy is observed in aged rats.[149] Thus, further investigations on the effects of age-at-injury and after-injury on neuroinflammation after TBI and immunomodulatory therapy for TBI are warranted.

Role of extracellular vesicles (EVs) in neuroinflammation

Local (brain) and systemic (peripheral) inflammatory responses initiated early after TBI play a key role in the secondary injury processes resulting in neuronal death and long term neurological dysfunction.[71,82,150] However, the mechanisms responsible for the rapid expansion of neuroinflammation and its long-term progression have yet to be elucidated. The emerging field of EVs provides a potentially promising vehicle for intercellular communication.[151] EVs are divided into three main categories depending on their biogenesis or release mechanisms[152–155]: 1) exosomes (30—150 nm in diameter) small EVs released from endosomes; 2) microvesicles/microparticles (MPs) (100—1000 nm) budded directly from the plasma membrane; and 3) apoptotic bodies (1000—5000 nm) bulged from cells during the execution phase of the apoptotic process. Exosomes and MPs are secreted by almost all cell types including neurons, microglia, astrocytes, endothelial cells, and oligodendrocytes in the central nervous system (CNS).[156,157] Exosomes and MPs carry a cargo of protein, lipids, metabolites, and nucleic acid from the donor cells, and the release of EVs can facilitate intercellular communication by contact with or by internalization of contents, either by fusion with the plasma membrane or by endocytosis into recipient cells.[158] EVs present a novel form of intercellular communication by autocrine and paracrine actions, controlling multiple cell processes in development, proliferation, migration, and pathology.[158] EVs may induce extensive neuroinflammation and peripheral immune responses responsible for secondary damage post TBI.[151,153,159,160] For example, MPs mediate intercellular communication, resulting in secondary injures such as systemic coagulopathy and inflammation after TBI.[161,162] In this regard, MPs serve as functional mediators for TBI-induced injuries and their progression. MPs loaded with pro-inflammatory molecules initially released by microglia following trauma can activate additional microglia that may contribute to progressive neuroinflammatory response in the injured brain, as well as stimulate systemic immune responses.[163] Injecting MPs isolated from lipopolysaccharide-stimulated microglia into the brains of uninjured animals creates progressive inflammation at both the injection site and eventually in more distant sites.[163] Due to their ability to independently initiate inflammatory responses, MPs derived from activated microglia may provide a potential therapeutic target for other neurological disorders in which neuroinflammation may be a contributing factor. Selectively targeting EVs from macrophage/monocyte populations is likely to be of value in reducing the impact of the systemic inflammatory response on the outcome of TBI.[151] This study highlights the need for further work to elucidate both the differences in the molecular profile of EVs produced in response to injury, and to determine the exact routes of their communication, from biogenesis to uptake. Circulating exosomes not only represent a central mediator of the pro-inflammatory microenvironment linked with secondary brain injury, but their presence in the peripheral circulation may serve as a surrogate for biopsies, enabling real-time diagnosis and monitoring of neurodegenerative progression.[153] Circulating exosomes are a novel platform for diagnosis and monitoring of TBI.[153] Exosome-mediated siRNA delivery is also a strong candidate to block inflammasome activation following CNS injury.[164]

Classical and neurogenic inflammation

Classical inflammation is a well-characterized secondary response to many acute disorders of the CNS.[165] A robust classical inflammatory response develops acutely after TBI and is characterized by the activation of resident cells (microglia and macrophages), migration and recruitment of peripheral leukocytes, and the release of inflammatory mediators.[165] Agents with known anti-inflammatory properties such as corticosterone methylprednisolone in the CRASH trial (Corticosteroid Randomization After Significant Head injury),[166] progesterone in the SyNAPSE trial (Efficacy and Safety Study of Intravenous Progesterone in Patients With Severe Traumatic Brain Injury) and ProTECT III trial (The Progesterone for Traumatic Brain Injury, Experimental Clinical Treatment),[22,167] and erythropoietin in the EPO-TBI (Erythropoietin in Traumatic Brain Injury) study[20] have all shown no benefit to date. However, the recent post-hoc analyses indicate that erythropoietin treatment may reduce mortality at 6 months after TBI.[168,169] In addition, anti-inflammatory agents often have narrow therapeutic windows identified in pre-clinical studies. For example, minocycline appears most efficacious when delivered within the first hour post injury,[170] with interleukin-1 antagonists administered within hours following injury.[171] This may be due to the dual effects of the immune response following TBI, with some aspects of the inflammatory response necessary to promote repair.[172] In addition, this may reflect that classical inflammation may be partially responsible for TBI pathophysiology.

The role of neurogenic inflammation in the pathophysiology of neurological diseases has gained increasing attention, with a particular focus on its effects on modulation of the BBB.[165] Neurogenic inflammation is inflammation arising from the local release by afferent neurons of inflammatory mediators such as substance P, neurokinin A (NKA), calcitonin gene-related peptide (CGRP), and endothelin-3 (ET-3).[165] Once released, these neuropeptides induce the release of histamine from adjacent mast cells.[173] In turn, histamine evokes the more release of substance P and CGRP. The neuropeptide substance P has been shown to increase BBB permeability following acute injury to the brain and is associated with marked cerebral edema.[174,175] Substance P release has also been shown to modulate classical inflammation.[165] Blocking the neurogenic inflammation pathway may provide a novel alternative treatment for CNS injury.[174] Following TBI, activation of a classical inflammatory response only represents part of the neuroinflammatory response. Therefore, modulation of neuroinflammation following TBI may require addressing both the classical and neurogenic inflammation by preventing their deleterious effects of the response and facilitating their promotion of brain repair.

Role of brain lymphatic system in neuroinflammation

In peripheral organs, lymphatic drainage contributes substantially to tissue fluid homeostasis and immune surveillance by clearance of excessive interstitial fluid (ISF), macromolecules, and immune cells from the interstitium back into blood circulation.[176] The brain had long been thought to lack a classical lymphatic drainage system until 2015 when two studies of mice provided evidence that the brain has its own lymphatic system in the dura.[177,178] A network of true lymphatic vessels is present within the mouse dura mater and runs alongside blood vessels, notably the superior sagittal and transverse sinuses.[177,178] In 2017, Absinta et al. found the existence of meningeal lymphatic vessels in human and nonhuman primates (common marmoset monkeys) which can be visualized by employing noninvasive high-resolution clinical magnetic resonance imaging (MRI) scan with injection of the gadolinium-based dye gadobutrol.[179] These findings suggest that the lymphatic system is a common feature of mammalian brains, and that the peripheral immune system may interact directly with the brain. The discovery of the CNS lymphatic system sheds new light on the etiology of neuroinflammatory and neurodegenerative diseases associated with immune system dysfunction,[178] which may be involved in the causation of neurological diseases including AD.[180] It is imperative to investigate whether TBI impairs the brain lymphatic system and how increased waste products accumulated in the injured brain lead to neuroinflammation, brain damage and functional deficits or cause enhanced risk for developing neurodegenerative diseases, which will pave the way for developing new treatments for TBI.

Role of the glymphatic system in neuroinflammation

Recent work has led to the discovery of the glymphatic system (the added "g" referring to glial cells) in the brain.[181–185] Within the glymphatic system, cerebrospinal fluid (CSF) enters the brain via periarterial spaces, passes into the interstitium via perivascular astrocytic aquaporin4 (AQP4), and then drives the perivenous ISF drainage ISF and its solutes.[181] In addition to waste elimination, the glymphatic system also facilitates brain-wide distribution of glucose, lipids, amino acids, growth factors, and neuro-modulators.[181] Glymphatic system disruption serves as a mediator of brain trauma and CTE.[186] Assessment of glymphatic function using MRI with intrathecal contrast agent as a CSF tracer has been applied to rodents,[183,184,187–189] and hydrocephalus patients.[190] This

system is impaired in the acute phase following mTBI, in part due to re-localization of APQ4 channels away from astrocytic end feet, resulting in reduced potential for waste removal.[186] Long-term consequences of chronic dysfunction within this system in the context of repetitive mTBI and insomnia have not been established, but potentially provide one link connecting repetitive mTBI with later neurodegeneration. For example, extracellular tau can be efficiently cleared in normal mice from the brain along the glymphatic system.[191] After TBI, glymphatic pathway function is reduced by approximately 60%, and this impairment persists for at least 1 month post injury.[191] Genetic knock-out of the gene encoding the astroglial water channel AQP4, which is importantly involved in paravascular interstitial solute clearance, exacerbates glymphatic pathway dysfunction after TBI and promotes the development of neurofibrillary pathology and neurodegeneration in the post-traumatic brain.[191] The glymphatic system works to remove toxins and waste products from the brain while distributing the glucose, lipids, and amino acids which the brain needs to function properly.[181] These findings suggest that chronic impairment of glymphatic pathway function after TBI may be a key factor that renders the post-traumatic brain vulnerable to tau aggregation and the onset of neurodegeneration. Functional impairment in the glymphatic system has been confirmed in other neurological diseases including animal models of diabetes,[187] vascular dementia,[192,193] subarachnoid hemorrhage,[194] prolonged wakefulness,[195,196] aging,[197] AD,[198] and cortical spreading depression the neural correlate of migraine aura.[199] Dysfunction of this system leads to accumulation of waste products, neuroinflammation and cognitive problems.[200,201] Sleep disruption, which includes a loss of sleep and poor quality fragmented sleep, frequently follows TBI.[202,203] Sleep drives clearance of neurotoxic waste products that accumulate in the awake brain.[195,204] TBI-induced sleep disruption may impair glymphatic functioning and increase the risk for developing CTE-related pathology and subsequent clinical symptoms following repetitive brain trauma.[186] Dysfunction within this glial vascular network, which is a feature of the aging and injured brain, is a potentially critical link between brain injury, neuroinflammation and the development of chronic neurodegeneration.[201] A recent study indicates that voluntary wheel running in aged mice increases glymphatic clearance efficiency, improves astrocytic AQP4 expression and polarization, attenuates the accumulation of amyloid plaques and neuroinflammation, and ultimately protects mice against synaptic dysfunction and a decline in spatial cognition.[205] Our own work shows that decreased AQP4 and glymphatic dysfunction may play an important role in axonal/white matter damage and cognitive deficits in a multiple microinfarction (MM) model in retired breeder rats.[192] Widespread reactive gliosis, including mis-localization of the astrocytic water channel AQP4 persist long after injury in this MM model.[206] Suppressed clearance of ISF in the hippocampus and hypothalamus contributes to cognitive deficits in rats with Type-2 diabetes mellitus.[187] Whole brain MRI provides a sensitive, non-invasive tool to quantitatively evaluate CSF and ISF exchange in Type-2 diabetes mellitus and possibly in other neurological disorders, with potential clinical application.[187,192] Anesthetics appear to activate microglia, and increase uptake of drug-loaded nanoparticles directly to microglia after TBI.[207] Drugs that could restore glial and glymphatic function, enable efficient drainage of waste and fluid from the brain, may effectively reduce neuroinflammation and improve recovery after TBI.

Imaging monitoring of neuroinflammation after TBI

MRI can noninvasively monitor inflammatory response after TBI.[208,209] Positron emission tomography (PET) imaging can detect

the inflammation marker translocator protein (TSPO) in TBI patients in brain areas with no visually detectable MRI changes.[108,209,210] Further, the magnitude of the increase is significantly different among patients with predominant extra-axial hemorrhage, microhemorrhage, or contusion. A limitation of TSPO as a biomarker is that it is expressed in both activated microglia and astrocytes.[108,209,210] Astrocytic TSPO expression may reflect chronic rather than acute inflammation. Postmortem studies report the presence of activated microglia persisting years after injury,[211] indicating the utility of TSPO PET imaging in the clinical care of subjects with TBI. PET imaging may identify patients in whom post-traumatic neuroinflammation is a significant component of tissue response to injury that cannot be identified by MRI alone.[212] In clinical studies in humans, MRI has not been employed to directly assess neuroinflammation, but rather to indirectly provide a measure of BBB dysfunction and endothelial cell activation.[94,106] Multiple MRI signals, cerebral blood flow (CBF), and amide proton transfer-weighted (APTw) MRI in particular, are sensitive biomarkers for identification and assessment of neuroinflammation and drug efficacy in the TBI model.[208,213] The use of APTw MRI has the potential to introduce a novel molecular neuroimaging approach for the simultaneous detection of ischemia, hemorrhage, and neuroinflammation in TBI.[213] Recently, imaging vascular cell adhesion molecule-1 (VCAM-1) expression using micron-sized particles of iron oxide (MPIOs) coupled with MRI has shown to be a highly sensitive and specific method of detecting and locating inflammation in a rat model of neuroinflammation.[214] Localized macrophage population can be monitored using ultrasmall superparamagnetic iron oxide (USPIO) nanoparticle enhanced in vivo serial MRI in TBI mice.[215] Commonly, magnetic resonance spectroscopy (MRS) is used to measure metabolic activity in TBI, but it has also been used to detect neuroinflammation.[106] A recent review article provides an overview of state-of-the-art techniques for imaging human neuroinflammation which have potential to impact patient care in the foreseeable future.[216] Techniques such as TSPO PET imaging for microglia activation/monocyte infiltration, iron oxide particle enhanced MRI for phagocyte labeling, genetically engineered T-lymphocytes for PET, as well as myelin and neuronal death have been translated to and tested in humans.[106,217] In addition, preclinical methods such as myeloperoxidase (MPO), matrix metalloproteinases (MMP), and adhesion molecule imaging have improved our understanding of the pathophysiology of many neuroinflammatory diseases.[217]

Biomarkers of neuroinflammation

Various anti- and pro-inflammatory molecules, such as TNF-α, IL-1β, IL-6, IL-8 and IL-10, have been considered as biomarkers for TBI diagnosis and prognosis.[218] There are some limitations to the use of inflammatory cytokines as biomarkers for acute and chronic TBI. While many of biomarkers are sensitive to acute- and chronic-phase moderate-to-severe TBI, most of them lack specificity because inflammatory cytokines measured in blood are nonspecific to central inflammation (peripheral injuries can also alter these markers).[218] CSF levels of these markers may reveal central inflammation, but BBB dysfunction is a confounder for accurately measuring CSF concentration of inflammatory proteins. A CSF to plasma albumin quotient can be used to better quantify the incidence of BBB dysfunction.[219] In addition, dysfunction of lymphatic system and glymphatic system may be important confounders for low levels of blood biomarkers because of their compromised clearance efficiency after TBI, while treatment-induced variance in the brain's lymphatic and glymphatic clearance may be responsible for the gap between biomarker discovery and clinical translation.[220,221] Clinically relevant manipulation of glymphatic

activity, including sleep deprivation and cisternotomy, suppresses or eliminates TBI-induced increases in serum S100beta, glial fibrillary acidic protein (GFAP), and neuron specific enolase.[221] Routine management of TBI patients may limit the clinical utility of blood-based biomarkers because the management may alter the glymphatic activity.[221] The blood levels of molecular biomarkers of TBI may not truly represent their CSF levels due to glymphatic pathway dysfunction that reduces transport TBI biomarkers from CSF to blood. In order to provide a normalization index for blood biomarker levels and thus improve the diagnostic, prognostic, and therapeutic information provided by blood biomarker measurement, it would be necessary to detect both blood and CSF biomarkers in combination with MRI monitoring of glymphatic system function after TBI. Functional status of the glymphatic system can be noninvasively measured by MRI and near-infrared spectroscopy.[184,188,190,222—224] In addition, the glymphatic system is surprisingly delicate, and can be easily disrupted by brain injuries and affected by sleep disruption, aging and even different body positions.[189] Consequently, the level of impairment to the glymphatic system should be taken into account when measuring biomarkers after TBI and with treatment.

Neurorestorative approaches emerge as a potential treatment for TBI

Currently, most of neuroprotection is neurocentric.[17,225] Acute neuroprotective therapies attempt to block the molecular cascade of injury following TBI. Although neuroprotection is an important strategy for the treatment of TBI, to date, no effective neuroprotective agents have been identified from TBI clinical trials. Preclinical studies from us and others have revealed that TBI not only induces neuroinflammation but also promotes many neurorestorative processes including neurogenesis (generation of new neurons), axonal remodeling (axonal sprouting and pruning), synaptogenesis (formation of new synapses), oligodendrogenesis (generation of myelin-generating mature oligodendrocytes), and angiogenesis (formation of new blood vessels from pre-existing endothelial cells), which in concert may contribute to spontaneous functional recovery.[226—237] In addition, therapeutic treatments that reduce neuroinflammation and promote these neurorestorative processes have been demonstrated to improve functional recovery in animals after brain injury.[238]

Cell therapy for TBI

Although human embryonic stem cells (hESCs) or fetal tissues are suitable sources for cell-based therapies, their clinical application is limited by both ethical considerations and other practical challenges including cell viability, antigenic compatibility, and tumorigenicity.[239] Induced pluripotent stem cells (iPSCs) generated by reprogramming differentiated cells resemble embryonic stem cells and can potentially be applied to cell-based therapy for human diseases.[240] These iPSCs avoid the ethical issues and remove the major roadblock of immune rejection associated with the clinical use of hESCs, as well as potentially generate patient-specific cells for cell-replacement therapy. However, even with improvements in the virus-free and transgene-free reprogramming technologies, the safety (mainly the potential tumorigenicity) and therapeutic applications of iPSCs and iPSC-derived cells must be rigorously tested in appropriate animal models before advancing to any clinical trial.[241]

Cell-based therapy has been shown to reduce neuroinflammation and improve functional recovery after TBI.[63,242] Among therapeutically employed cells, multipotent mesenchymal stem cells (MSCs), a mixed cell population including stem and progenitor cells, are a promising source of cell-based therapy for TBI because they can be

easily isolated from many tissues and expanded in culture from patients without ethical and immune rejection problems.[243,244] MSC treatment shows promise for the treatment of various diseases including neural injuries.[243,245—254] MSCs have the ability to modulate inflammation-associated immune cells and cytokines in TBI-induced cerebral inflammatory responses.[255] Intravenous MSC transplantation after TBI is associated with a reduced density of microglia/macrophages and peripheral infiltrating leukocytes at the injury site, reduced levels of proinflammatory cytokines and increased anti-inflammatory cytokines; with the anti-inflammatory cascade possibly mediated by enhanced expression of the immunosuppression-related factors TNF-α stimulated gene/protein 6 (TSG-6), which may suppress activation of the nuclear factor-kappaB signaling pathway.[63] Systematically infused rat MSCs migrate into injured rat brains and survive after TBI.[256] Some of the implanted MSCs express cell markers for neurons and astrocytes.[247] The stromal-cell-derived factor-1 receptor, CXC-chemokine receptor-4, is expressed in MSCs both in vitro and in vivo.[257] Expression of the chemokine stromal-cell-derived factor-1 is significantly increased in the lesion boundary zone after brain injury.[257] The interaction of stromal-cell-derived factor-1 with CXC-chemokine receptor-4 may contribute to the trafficking of transplanted MSCs into the injured brain.[257,258] Direct implantation (6 h post injury) of MSCs enhances neuroprotection via activation of resident neural stem cell (NSC) nuclear factor−κB activity leading to an increase in interleukin-6 production and decrease in apoptosis.[259] The delayed administration (24 h or 1 week following injury) of MSCs also significantly improves functional outcome in rodents following TBI.[243,247,256,260—262] MSC-secreted factors may modulate the inflammation at the injury site and activate endogenous restorative responses in injured brain.[63,243,263,264] The significant therapeutic benefits of MSCs are not attributed to the few MSCs that differentiate into neural cells.[256] MSCs enhance recovery by paracrine effects including secretion of various growth factors, such as brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF) and bFGF (basic fibroblast growth factor), and increase the levels of these factors in the brain.[243,248] MSCs also induce intrinsic parenchymal cells to produce these growth factors.[248] MSCs appear to act as neurotrophic/growth factor generators and inducers to promote brain functional recovery via angiogenesis, neurogenesis, synaptogenesis and axonal remodeling.[243,265]

Exosomes as mediating cell-based therapy of TBI

Recent studies indicate that novel neurorestorative treatment with exosomes derived from MSCs improve functional recovery by inducing neuroplasticity and by reducing long-term neuroinflammation in TBI rats.[266—268] We have demonstrated that therapeutic effects of MSCs may be attributed to their robust generation of exosomes which alter the parenchymal cells, rather than a cell replacement effect.[266,267,269—276] MSCs robustly release exosomes, which contain a plethora of molecular constituents including proteins, lipids and RNAs from parental cells.[275,277—280] Contained among these constituents, are small non-coding RNA molecules, microRNAs (miRNAs), which play a key role in mediating biological function due to their prominent role in gene regulation.[269,273,281,282] MSCs transfer their therapeutic factors via exosomes, especially miRNAs, to recipient cells, and therein alter gene expression and thereby promote therapeutic response.[269,273,281,282] Our recent data demonstrate that MSC-derived exosomes administered intravenously post injury improve functional recovery and promote neuroplasticity in rats after TBI[267] and stroke.[274] MSC-generated exosomes effectively improve functional recovery, at least in part, by reducing neuroinflammation (GFAP + astrocytes and CD68 + microglia/

macrophages) in the injured brain and by promoting endogenous angiogenesis and neurogenesis in rats after TBI.[267] Our data show that using exosome-based therapy for TBI does not compromise efficacy associated with using complex cell-based therapeutic agents such as MSCs.[267] Developing a cell-free exosome-based therapy for TBI may open up a variety of means to deliver targeted regulatory genes (e.g., microRNAs) to enhance multifaceted aspects of neuroplasticity and to amplify neurological recovery, potentially for a variety of neural injuries and neurodegenerative diseases. We have developed "designer" exosomes, enriched in miRNAs that amplify therapeutic benefit in stroke rats.[270,275,276] In addition, we have generated exosomes from 3D cultured MSCs, which also augments benefit by reducing neuroinflammation and enhancing neuroplasticity.[266] Accumulating preclinical evidence has revealed that the peripheral inflammation is an important pathological culprit and possibly is a therapeutic target for cell therapies as a treatment for TBI.[242] MSC-derived exosomes may also modulate peripheral inflammation via the spleen after TBI.[242] Exosome/miRNA therapy by regulating neuroinflammation and multiple restorative therapeutic pathways can exceed the benefits of cell-based therapies.[160,267,283] In contrast to MSCs, nano-sized exosomes carry genetic materials and proteins and easily pass through the BBB, without vascular obstructive effect and risks of tumor formation.[284]

microRNAs in exosomes as possible mediators of anti-inflammation and neuroplasticity

miRNAs, small non-coding regulatory RNAs (usually 18 to 25 nucleotides), regulate gene expression at the post-transcriptional level by binding to complementary sequences on target message RNA (mRNA) transcripts, and cause mRNA degradation or translational repression and gene silencing.[272] Exosomes can transfer miRNAs to the brain and subsequently promote neuroplasticity and functional recovery after brain injury. For example, functional miRNAs transferred from MSCs to neural cells via exosomes promote neurite remodeling and functional recovery of stroke rats.[276] As a control of exosomes, treatment with liposome mimic consisting of the lipid components of the exosome (no proteins and genetic materials) provides no therapeutic benefit compared with naive exosome treatment after TBI,[266] indicating that the therapeutic effects of exosomes derive from the exosome content, including proteins and genetic materials, such as miRNAs. Our data indicate that genetically engineered exosomes harvested from miR-133b-overexpressing MSCs improve neural plasticity and functional recovery after stroke with a contribution from a stimulated secondary release of neurite-promoting exosomes from astrocytes.[271] A recent study indicates that the miR-124-3p level in microglial exosomes is increased apparently from the acute phase to the chronic phase of TBI in mice.[285] Transfected miR-124-3p in microglia inhibits neuroinflammation by targeting the mammalian target of rapamycin (mTOR) signaling pathway, and improves the neurologic outcome in TBI mice. Therefore, miRNA-manipulated microglial exosomes may provide a novel therapy for TBI and other neurologic diseases.[272,273,285]

Induced pluripotent stem cells (iPSCs) for treatment of TBI

The adult mammalian brain possesses regenerative potential through neurogenesis in the neurogenic regions including the subgranular zone of the hippocampal dentate gyrus and the subventricular zone of the lateral ventricles.[286—293] TBI induces neurogenesis which may contribute to cognitive recovery after injury.[98,232,294—296] Treatments that increase neurogenesis and reduce neuroinflammation promote functional recovery after

TBI.[97,266,267] However, neurogenesis is limited in the adult brain and no neurogenesis exists in non-neurogenic regions in the cortex. Reprogramming reactive glial cells in vivo has potential to generate neurons in non-neurogenic regions in the cortex. Retroviral mediated expression of four well-known transcription factors (Oct4, Sox2, Klf4, and c-Myc) is able to cooperatively reprograms reactive glial cells into iPSCs in the adult neocortex following TBI.[297] These iPSCs further differentiate into a large number of neural stem cells, which further differentiate into neurons and glia in situ, and fill up the tissue cavity induced by TBI.[297] The induced neurons show a typical neuronal morphology with axon and dendrites, and exhibit action potential. This innovative technology to transform reactive glia into a large number of functional neurons in their natural environment of neocortex without embryo involvement and without the need to grow cells outside the body and then graft them back to the brain offers hope for personalized regenerative cell therapies for repairing damaged brain.[297,298] Instead of employing four transcript factors, when infected with retrovirus encoding a single transcription factor NeuroD1, reactive glial cells (both astrocytes and NG2 cells) can be reprogrammed into functional glutamatergic and gamma-aminobutyric neurons that integrate into the host's neural circuitry in the adult mouse cortex after brain stab injury and in an AD model.[299,300] Once activated, many reactive glial cells will stay in the injury sites and secrete neuroinhibitory factors to prevent neuronal growth, eventually forming glial scar inside the brain. This in vivo regeneration of functional neurons from reactive glial cells may reduce neuroinflammation and provide a potential therapeutic approach to restore lost neuronal function in injured or diseased brain. NeuroD1 instructs neuronal conversion from astrocytes in non-reactive astrocytes in the brain and in a spinal cord injury model.[301,302] Chemical cocktails of small molecules can be used to directly convert fibroblasts into functional astrocytes without transgenes.[303–307] Another study reports that a combination of small molecules directly reprograms mouse fibroblasts into neural stem cells without genetic manipulation.[306] It is important to use virus-free or small molecule strategy to achieve the reprogramming in vivo for regenerative medicine.

Conclusion

Finally, we try to summarize with a diagram (Fig. 1). Briefly, neuroinflammatory responses after TBI have dual roles: chronically, they mainly contribute to worsening outcomes of the progressive TBI pathology, and acutely, they may promote functional recovery.[58] The prolonged local and systemic inflammation as a pathological culprit of TBI, provides an opportunity for therapeutic interventions for treatment of TBI. Sex differences and age effects are understudied in TBI research and therefore investigations of how sex and age alters time-course of inflammation in the brain after TBI are warranted. Microglia play a critical role in neuroinflammation after TBI. Emerging evidence indicates that EVs are a vehicle for intercellular communication and may be responsible for the rapid expansion of neuroinflammation and its long-term progression. Impaired lymphatic and glymphatic systems contribute to the accumulation of soluble proteins, and neuroinflammation after TBI, and may impact the relationship of biomarkers in the blood and CSF. AQP4, the major water channel primarily expressed in astrocytes, plays a critical role in the functioning of glymphatic system. To date, no specific therapeutic agents have been developed to inhibit or enhance AQP4. Thus, developing treatments that modulate AQP4 expression or function and improve the efficiency of AQP4-dependent bulk flow and clearance of soluble proteins including soluble Aβ a potential contributing factor involved in AD after TBI, should be considered. Direct regulation of neuroinflammation represents an exciting target for future study of TBI. Shifting the balance of neuroinflammation after injury toward cellular repair is a goal for future TBI therapies. Cell-based therapies offer promise in promoting neuroprotection and neuroregeneration by many means, including modulating neuroinflammation as well as peripheral inflammation. Nanosized exosomes can pass the BBB to directly target brain cells,[269] and abrogate the local and systemic inflammatory response after TBI.[242,266,267,283,285] MSC-generated exosomes effectively improve functional recovery, at least in part, by reducing inflammation and by promoting endogenous angiogenesis and neurogenesis in rats after TBI. Exosomes contribute to a therapeutic benefit in TBI rats, likely by shifting microglia polarization to reduce
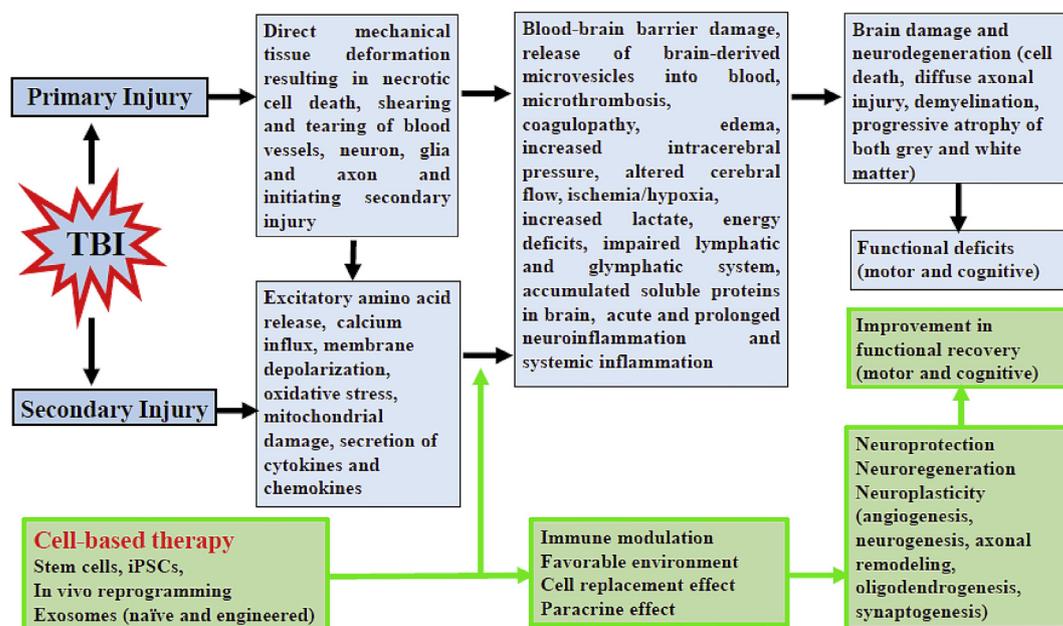

Fig. 1. TBI pathophysiology and cell-based therapy.

neuroinflammation.[283] Thus, cell-generated exosomes may provide a novel cell-free therapy for TBI and possibly for other neurological diseases. In contrast to transplantation of exogenous neural stem/progenitor cells, MSC-derived exosomes have several advantages including no ethical issue of embryonic and fetal cells, less invasiveness, low or no immunogenicity, and low or no tumorigenicity. They are also easily generated, can be readily scaled for clinical use and are very stable at room temperature, thereby readily facilitating clinical translation. Exosomes are promising therapeutic agents because their complex cargo of proteins and genetic materials contain diverse biochemical potential to participate in multiple biochemical and cellular processes. This multifactorial molecular targeting system provides an important attribute in the treatment of complex diseases such as TBI, with multiple secondary injury mechanisms including neuroinflammation. Cell-free exosome-based therapy by delivering targeted regulatory genes (miRNAs) to reduce neuroinflammation, enhance multifaceted aspects of neuroplasticity, and to amplify neurological recovery for efficacious restorative treatment of a variety of neural injuries and neurodegenerative diseases. In addition to the role of miRNAs, further investigation of exosome-associated proteins is warranted to fully appreciate the mechanisms of trophic activities underlying exosome-induced therapeutic effects in TBI. Although exosomes provide promising beneficial effects in the rodent TBI model, further studies are required for clinical translation. In addition to imperative safety studies, ongoing studies should be designed to fully elucidate the mechanisms (central and peripheral effects) underlying exosome mediation of improved functional recovery after TBI, to maximize, scaling consistency and reproducibility of exosome production, to identify the optimal sources and characteristics of exosome parent cells, e.g., age and sex of parent cells, and to identify the optimal dose and therapeutic time window and potential routes of administration. Next generation exosome studies would include modification of exosome content, e.g., mRNA, miRNA, lipids, and proteins for specific disease and injury treatment, develop exosomes as a drug delivery system that can target specific cell populations, and to refine 3D culture methods such as scaffolds, or tissue-engineered models, cell spheroids, and micro-carrier cultures for advanced exosome production. These efforts would facilitate applications of exosome therapeutics to other diseases. Further investigations on whether there are cell specific differential responses to exosome therapy, which factor (s) of exosomal contents plays a key role in the treatment of TBI, and what efficacy exosomes can achieve in large animal models of TBI are warranted. Thinking beyond the present, there are also additional novel restorative therapeutics on the horizon for the treatment of TBI, particularly in vivo reprogramming of parenchymal cells.

Fund

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