Astrocytes, reactive astrogliosis, and glial scar formation in traumatic brain injury

María Belén Cieri, Alberto Javier Ramos*

https://doi.org/10.4103/NRR.NRR-D-23-02091

Date of submission: December 27, 2023

Date of decision: March 21, 2024

Date of acceptance: April 14, 2024

Date of web publication: May 17, 2024

From the Contents

Introduction	973
Search Strategy	974
Traumatic Brain Injury	974
Reactive Astrogliosis and Molecular Mechanisms in Traumatic Brain Injury	976
Therapeutic Approaches Targeting Astrocytes and Glial Scar Formation after Traumatic Brain Injury	978
Challenges and Future Directions	985
Conclusion	986

Abstract

Traumatic brain injury is a global health crisis, causing significant death and disability worldwide. Neuroinflammation that follows traumatic brain injury has serious consequences for neuronal survival and cognitive impairments, with astrocytes involved in this response. Following traumatic brain injury, astrocytes rapidly become reactive, and astrogliosis propagates from the injury core to distant brain regions. Homeostatic astroglial proteins are downregulated near the traumatic brain injury core, while pro-inflammatory astroglial genes are overexpressed. This altered gene expression is considered a pathological remodeling of astrocytes that produces serious consequences for neuronal survival and cognitive recovery. In addition, glial scar formed by reactive astrocytes is initially necessary to limit immune cell infiltration, but in the long term impedes axonal reconnection and functional recovery. Current therapeutic strategies for traumatic brain injury are focused on preventing acute complications. Statins, cannabinoids, progesterone, beta-blockers, and cerebrolysin demonstrate neuroprotective benefits but most of them have not been studied in the context of astrocytes. In this review, we discuss the cell signaling pathways activated in reactive astrocytes following traumatic brain injury and we discuss some of the potential new strategies aimed to modulate astroglial responses in traumatic brain injury, especially using cell-targeted strategies with miRNAs or lncRNA, viral vectors, and repurposed drugs.

Key Words: astrocyte; glial scar; innate immunity; neuroinflammation; stab injury; Toll-like receptors

Introduction

The importance of traumatic brain injury in human health

Traumatic brain injury (TBI) is recognized as a global public health crisis because it is a major cause of death and disability worldwide and it represents a very significant portion of life-years with disability, with effects devastating not only to patients but also to their families (Maas et al., 2022). Population-based studies showed that 50–60 million people worldwide are affected by a new TBI each year, with young people in low- and middle-income countries contributing the most to the global TBI morbidity rate (Karthigeyan et al., 2021). It is widely recognized that the incidence is underestimated due to the small proportion of cases that are admitted to hospitals in low-income countries (Lefevre-Dognin et al., 2021). TBI poses a substantial pressure on health systems costing the global economy around US$400 billion annually (Maas et al., 2022). TBI not only results in acute conditions, but TBI also increases long-term mortality and reduces life expectancy, associated with a higher incidence of anxiety, depression, post–traumatic stress disorder, seizures, and even neurodegenerative diseases that can emerge and/or persist for months or even years post-TBI (Masel and DeWitt, 2010; Muresanu et al., 2022). Although TBI is caused by an initial mechanical force, it is followed by a cascade of events that lead to secondary damage. It is widely known that neuroinflammation is one of the most relevant consequences involved in the secondary response to initial damage (Jassam et al., 2017). Concerning this, a recent study from a bibliometric approach has shown that from 2007 to 2021 there was a global increase in the number of articles on inflammation associated with TBI in academic journals (Li et al., 2023). Particularly, the astroglial response, together with microglia and other immune cells, is fundamental in the neuroinflammation triggered after the initial TBI that participates in the propagation of secondary damage (Fraunberger and Esser, 2019; Zheng et al., 2022).

Astrocytes are the main homeostatic cells in the central nervous system

Astrocytes are the main cells responsible for maintaining homeostasis in the central nervous system (CNS) since they play key roles in all aspects of neurodevelopment, metabolic support, injury, and repair processes (Knox et al., 2022; Li et al., 2022a). Astrocytes occupy the entire CNS in a contiguous manner. In the healthy CNS, each astrocyte occupies a unique territory, demarcated by numerous, essentially non-overlapping astroglial processes extending from the cell soma (Sofroniew and Vinters, 2010; Verkhratsky et al., 2019). Even so, astrocytes are interconnected forming a network or functional syncytium through gap junctions (Sofroniew and Vinters, 2010; Verisokin et al., 2021). In particular, the astroglial end-feet are in contact with the brain capillaries and these are essential for the blood–brain barrier (BBB) (Iadecola and Nedergaard, 2007; Mathiisen et al., 2010). This complex astroglial organization is related to the

Laboratorio de Neuropatología Molecular, IBCN UBA-CONICET, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina

***Correspondence to:** Alberto Javier Ramos, PhD, jramos@fmed.uba.ar.

https://orcid.org/0000-0003-4009-6337 (Alberto Javier Ramos)

Funding: *This work was supported by grants PICT 2019-0851; 2017-2203, UBACYT and PIP CONICET (to AJR).*

How to cite this article: *Cieri MB, Ramos AJ (2025) Astrocytes, reactive astrogliosis, and glial scar formation in traumatic brain injury. Neural Regen Res 20(4):973-989.*

functions they perform, including the metabolic support they provide to neurons, the regulation of the concentration of ions and neurotransmitters, and synaptic transmission, among others (reviewed in Verkhratsky and Nedergaard, 2018; Verkhratsky et al., 2019). Astrocytes are also an essential source of neurotrophic and neuroprotective factors that maintain CNS homeostasis and regulate blood–brain barrier permeability, oligodendrocyte maturation, and myelination (Hart and Karimi-Abdolrezaee, 2021). Astrocytes play a key role in supporting neuronal metabolism, this has been proposed by the astrocyte-to-neuron lactate shuttle model to sustain neuronal mitochondrial function necessary for high neuronal energy demands (Pellerin and Magistretti, 1994; Magistretti and Allaman, 2018). In agreement with this model, astrocytes respond to glutamate released at the synapse, which triggers increased glucose incorporation into astrocytes, which they use for their metabolism, mainly for glycolysis, and to produce lactate that is secreted to neurons, being lactate the preferred energy source during neuronal activity (Magistretti and Allaman, 2018; Beard et al., 2021). Particularly, at the level of synapses, astrocytes can take up excess glutamate, through two types of Na^+-dependent glutamate transporters called EAAT1/SLC1A6 and EAAT2/SLC1A2, known in rodents as glutamate-aspartate transporter and glutamate transporter 1, respectively (Verkhratsky and Rose, 2020; Beard et al., 2021). This is accompanied by a significant influx of Na^+, which activates the Na^+/K^+ ATPase (Pellerin and Magistretti, 1994). Therefore, astroglial adenosine 5′-triphosphate (ATP) consumption is increased, and this stimulates the activity of the glucose transporter GLUT1/SLC2A1, which allows astrocytes to uptake glucose from the astroglial endfeet that are in intimate contact with the vascular endothelium (Muraleedharan et al., 2020; Verkhratsky and Rose, 2020). Astroglial glycolysis then increases to produce pyruvate and lactate, and that lactate is released into neurons in a specific and controlled manner through specialized monocarboxylate transporters (Pellerin and Magistretti, 2012). At a functional level, it has been shown that lactate secretion from astrocytes to neurons is essential to mediate synaptic plasticity and memory consolidation (reviewed in Bonvento and Bolaños, 2021) and neuroprotective effects of lactate have even been reported after brain injury (Zhou et al., 2018). Furthermore, astrocytes can act as sensors and modulators of synaptic transmission and plasticity, by responding to different synaptically released neurotransmitters, which, upon activation, lead to intracellular calcium elevations. These calcium elevations stimulate the release of gliotransmitters, such as glutamate, D-serine, ATP, and adenosine, which act on neuronal receptors, regulate neuronal activity and synaptic transmission in several brain areas and impact animal behavior (reviewed in Noriega-Prieto and Araque, 2021). Another essential astroglial function is to maintain the homeostasis of neurotransmitters, such as glutamate, not only regulating neurotransmission but also preventing excitotoxicity. Because the glutamate that astrocytes reuptake is converted to glutamine (glutamine-glutamate cycle), through the astroglial enzyme called glutamine synthetase or also known as ammonium glutamate ligase (Sandhu et al., 2021). Likewise, the astroglial enzyme glutamine synthetase is essential for the elimination of ammonia from the CNS, since it uses the ammonia absorbed by the astroglial endfeet to convert glutamate into glutamine (Sandhu et al., 2021). Glutamine can then be used in astroglial metabolism or exported to both glutamatergic and GABAergic neurons (Hayashi, 2018). Furthermore, astrocytes are involved in water homeostasis in the CNS through the expression of aquaporin-4 (AQP4), the main bidirectional water channel that is located in a highly polarized manner in the astroglial endfeet (Vandebroek and Yasui, 2020). Meanwhile, deregulation in the expression or polarized localization of AQP4 affects the homeostasis of tissue fluids and facilitates the formation of edema (Stokum et al., 2015; Li et al., 2022a). Likewise, AQP4 has been observed in the context of TBI or stroke to be key to the reabsorption of extracellular edema fluid, resulting in a reduction in vasogenic edema (Michinaga and Koyama, 2021). It has even been proposed that perivascular AQP4 mislocalization after TBI affects the elimination of interstitial solutes, which promotes neurodegeneration and persistent neuroinflammation in the post-TBI brain, as a consequence of malfunctioning of the glymphatic system (Iliff et al., 2014).

The objective of this review is to discuss the role of astrocytes in the response to TBI and the possible therapeutic strategies to modulate astroglial-mediated neuroinflammation, with the final purpose of reducing the secondary damage after TBI.

Search Strategy

In this narrative review, PubMed database was used utilizing the appropriate terms (traumatic brain injury, reactive gliosis, neuroprotection, glial scar, miRNA). Searches were performed between November 2023 and March 2024 without limiting the time of publication.

Traumatic Brain Injury

Definition and classification of human TBI

TBI is defined as an insult to the brain caused by an external force exerted on the head that can result in temporary or permanent impairment of normal brain function (Jacquens et al., 2022). The clinical stratification of TBI in humans can be determined using the Glasgow Coma Scale (GCS) scores, which are based on neurological function and neuroimaging (Dinet et al., 2019). According to GCS scores, there are three stratifications severe (GCS 3–8), moderate (GCS 9–13), and mild (GCS 14–15) TBI, respectively (Datta et al., 2023). Furthermore, most patients with mild TBI recover within a few weeks, but approximately 10–15 percent of TBI patients do not recover even after 1 year and may continue to have cognitive impairments post-TBI even increasing the risk of neurodegenerative consequences such as Parkinson's and Alzheimer's diseases (Petersen et al., 2021).

Depending on the physical mechanism of injury, TBI can be classified as penetrating (open-head) or nonpenetrating (closed-head or blunt) injury, depending on whether or not the skull is ruptured, respectively (Naumenko et al., 2023). Penetrating injury is mainly defined as an open wound in the head caused by a foreign body, such as a gunshot wound or sharp object, which produces a focal alteration along the path taken by the object through the tissue. It is associated with perforation or often results in a skull fracture, laceration of the meninges, and structural damage to the nervous tissue (Freire et al., 2023). Penetrating brain injury constitutes the most severe form of TBI and the risk of infection increases with penetrating trauma (Petersen et al., 2021). Conversely, closed-head injury is the most common type of TBI which is characterized by tissue damage caused primarily by an external impact from a sudden and violent movement that does not lead to a skull fracture, nor does it produce structural disruption in the meninges (Petersen et al., 2021; Freire et al., 2023).

In experimental studies, several models of TBI have been used, and they try to reproduce or imitate clinical situations observed in human patients suffering from TBI. Different experimental models for TBI are summarized in **Table 1**.

Table 1 | **Summary of experimental models of TBI**

TBI models	Descriptions	Advantages	Disadvantages	References
Controlled cortical impact	Direct impact on the exposed dura mater directly with a rigid piston.	• Control the depth and velocity of the impulse, affecting the severity of brain damage. • It eliminates the risk of a rebound injury.	• It is not representative of the full spectrum of TBI severity in human patients. • It relies on a single impact, whereas many human TBI result from multiple impacts.	Deshetty and Periyasamy, 2023; Zhao et al., 2023
Fluid percussion injury	Fluid impulse on the exposed dura mater generated by a pendulum that strikes a piston at the end of a fluid-filled tube.	• Reproducible and standardized. • It reproduces human brain injuries. • It produces transient pressure, similar to that recorded in the human skull after sudden head trauma.	• Requires accurate placement of the craniotomy to ensure reproducibility. • The device needs calibration of pressure.	Petersen et al., 2021; Kundu and Singh, 2023
Weight drop	Impact on the dura mater or skull with freely falling weights, but the weight and height of the fall are controlled.	• Cost-effective and easy to use. • It allows for adjusting the magnitude of tissue damage. • It recreates mild and diffuse injuries.	• Limitations in its reproducibility and consistency. • It does not replicate the complex and diverse nature of human TBI.	Petersen et al., 2021; Deshetty and Periyasamy, 2023; Zhao et al., 2023
In vitro models of TBI	Mechanical damage to the cell culture with sharp objects (e.g., a needle and a pipette tip) or compression models that mimic brain injuries induced by weight loss in the skull. Those models can be applied to immortalized cell lines; 2D dissociated primary cultures and 3D hydrogel cultures or organotypic (organ-like) brain slices.	• They allow understanding the pathophysiology of TBI at the cellular and molecular level. • They can replicate the extracellular matrix or introduce biomaterial scaffolds into the lesion to evaluate their pro-regenerative properties.	• They do not reproduce the three-dimensional and multicellular environment of the brain, and can be difficult to scale and compare.	Ribeiro et al., 2021; Basit et al., 2023; Kundu and Singh, 2023; Zargari et al., 2023

TBI: Traumatic brain injury.

Pathophysiology of TBI

Traumatic brain injury pathophysiology involves two types of damage: primary and secondary, and the recently proposed tertiary damage (Jacquens et al., 2022). Primary traumatic injury to the CNS causes direct damage to the tissue, producing a limited area called the core, where all cell types suffer rapid death by necrosis, releasing various damage signals to the extracellular environment. Primary damage produced directly by the external force results in focal and diffuse lesions. Focal lesions are mainly neuronal lesions due to an external contact force, which are most frequently related to epidural hematomas and contusions. In contrast, the diffuse injury subtype also known as the diffuse axonal injury is often seen in cases of injury caused by rapid acceleration-deceleration or an external rotational force, which produce shearing and stretching effects and are primarily found in areas of least resistance of the axons such as in the corpus callosum.

Then, from a few minutes to days after the initial damage, the area surrounding the injured core called the traumatic penumbra suffers neuronal death, expanding the initial damage into a secondary injury, where neuroinflammation plays a key role (Burda et al., 2016; Jassam et al., 2017; Michinaga and Koyama, 2021). Importantly, secondary damage mechanisms are responsible for the persistence of symptoms and increased vulnerability to new brain trauma or other neurodegenerative disorders (Mira et al., 2021). Furthermore, a late tertiary injury has recently been proposed to be involved with neuroinflammation leading to neurodegeneration in the long-term prognosis after TBI (Jacquens et al., 2022) and astrocytes have shown an important role in this late damage (Cieri et al., 2023). In this context, longitudinal studies have found microglial activation and neuroinflammation in patients with TBI from 1–2 weeks to 3–4 months after injury (Ebert et al., 2019). Furthermore, activated microglial cells have been detected in patients with moderate to severe TBI, even up to 17 years after the injury (Ramlackhansingh et al., 2011). Together, these findings demonstrate persistent brain inflammation post-TBI (reviewed in Shao et al., 2022). Understanding the pathogenesis of TBI is essential to explain why a patient with a small TBI worsens and the original TBI may become a large injury in the following days after TBI. This evolution is due to cellular and molecular events that lead to neuroinflammation and propagate the initial damage, but that are triggered late after the initial injury, therefore they may be preventable or treatable taking advantage of the potential therapeutic window (Dinet et al., 2019).

Cellular and molecular responses to TBI

The acute neuroinflammation that follows TBI may and promote tissue regeneration, however, when neuroinflammation is chronic, it is detrimental to cells (Datta et al., 2023). At the cellular level, within minutes of the initial brain injury, an immune response develops under sterile conditions initiated by the release of damage-associated molecular patterns (DAMPs) from dying cells. Once released to the extracellular space, DAMP activates Toll-like receptors activating astrocytes and microglia, initiating the reactive gliosis that rapidly propagates from the injury core to distal tissues (**Figure 1**; Rosciszewski et al., 2019; Cieri et al., 2023; Naumenko et al., 2023). Professional immune cells from the periphery are recruited to the tissue through a permeabilized BBB thus generating an inflammatory storm (Jacquens et al., 2022). In particular, in post-mortem tissue from TBI patients, neutrophils can be observed in the first hours after injury (Alam et al., 2020). Present evidence shows that neutrophils can enter the brain within minutes because inflammatory mediators induce a state of hyperactivation that facilitates the release of metalloproteinases and proteases that alter the BBB (Alam et al., 2020). On the other hand, proliferation of microglia has been demonstrated at 3 days post-TBI (Cieri et al., 2023) and the infiltrate of monocytes/macrophages trends to decrease at 5 days post-injury (Frik et al., 2018). Pericytes are also important players in TBI and they have an important role in the vascular repair after TBI. In later stages post-TBI, pericytes become reactive and

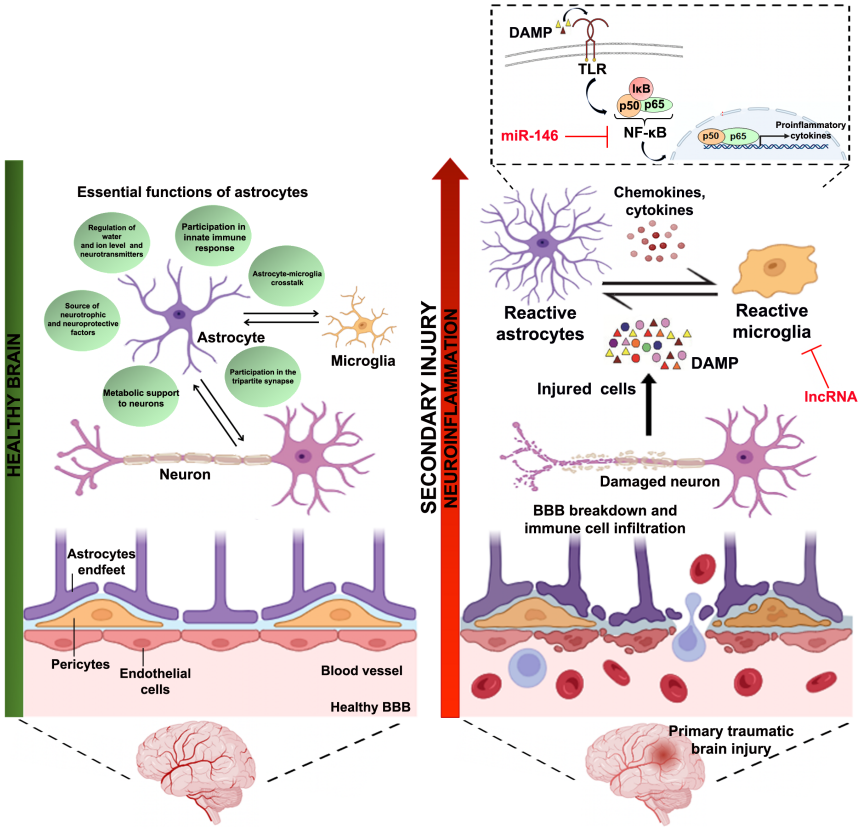


Figure 1 | Summarized cellular and molecular events after TBI.
The cascade of events following TBI involves the activation of microglia, astrocytes, pericytes, and endothelium. Released DAMPs activate PRR-like Toll-like receptors and others. Potential strategies aimed to block this pathway resulted effective in experimental models of TBI. Created with BioRender.com. BBB: Blood–brain barrier; DAMP: damage-associated molecular patterns; IκB: inhibitor of kappa B; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; TBI: traumatic brain injury; TLR: Toll-like receptor.

increase dramatically around the injury (pericytosis), where they secrete angiogenic growth factors that promote endothelial activity and vascular integrity (Salehi et al., 2017). At the site of TBI, oligodendrocytes are very sensitive to the damaging insult, however, NG2-glia or oligodendrocyte precursor cells increase their proliferation rate after TBI (peaking at 24 hours post-injury) and they can differentiate into mature oligodendrocytes to ensure proper re-myelination after injury (Susarla et al., 2014; Scheller et al., 2017). Furthermore, some contexts could promote NG2-glia to differentiate into astrocyte-like cells (Dean et al., 2023). Taken together, this evidence shows that all members of the neurovascular unit, composed of neurons, endothelial cells, smooth muscle cells, pericytes, oligodendrocytes, astrocytes, and microglia, show alterations after TBI (Dinet et al., 2019).

Reactive Astrogliosis and Molecular Mechanisms in Traumatic Brain Injury

Definition and characteristics of reactive astrogliosis

Astrocytes exhibit a generic response known as reactive astrogliosis against injury to the CNS, both in acute or chronic pathological states. This phenomenon involves a plethora of molecular, biochemical, transcriptomic, and morphological changes that have the potential to alter astrocyte functions (Burda and Sofroniew, 2014; Pekny and Pekna, 2016). As previously mentioned, astrocytes play a crucial role in maintaining brain homeostasis. In response to brain injury, these astrocytes transform into reactive astrocytes that can impact surrounding cells either beneficially or detrimentally (Zamanian et al., 2012; Escartin et al., 2021; Yu et al., 2021).

Among the characteristic changes observed in reactive astrocytes, morphological alterations stand out. These alterations are associated with changes in gene expression, resulting in a reversible increase in the levels of astroglial cytoskeletal proteins, such as glial fibrillary acidic protein (GFAP). Recently, according to the morphological changes that undergo reactive astrocytes, researchers have proposed classifying reactive astrogliosis into two distinct forms: isomorphic and anisomorphic astrogliosis. In isomorphic astrogliosis, a reversible process, astrocytes become hypertrophic but remain within their individual territorial domains to adapt to local requirements without extensive migration. In contrast, anisomorphic astrogliosis involves more dynamic changes, and astrocytes proliferate and migrate toward the site of the lesion. There, they reorient and extend their long processes, forming the glial scar around the lesional core (Verkhratsky et al., 2021; Zheng et al., 2022; Cieri et al., 2023). Recent studies reveal that the proliferative astrocyte response in TBI is limited and predominantly occurs in juxtavascular astrocytes (Frik et al., 2018).

Astrogliosis is not an all-or-nothing process; rather, it occurs along a continuous gradient of progressive alterations over time. Furthermore, it does not manifest uniformly but constitutes a highly heterogeneous phenomenon dependent on context (Giovannoni and Quintana, 2020). Understanding these diverse astrocytic responses is crucial for developing targeted therapies to enhance tissue repair and minimize damage after CNS injuries.

Astrocyte activation in response to TBI

Primary traumatic injury to the central nervous system CNS directly damages the tissue; triggering a cascade of events mediated by various pathways that include the local and systemic neuroinflammatory response. In this context, microglia-astrocyte crosstalk plays a crucial role in initiating the inflammatory response after TBI, which progressively contributes to neurological deterioration (Karve et al., 2016; Yang et al., 2020).

During functional interaction microglia-astrocytes, microglial cells, being part of the innate immune system, are dispersed throughout the brain, acting as sentinels. They constantly survey and detect any modifications in their environment. This surveillance occurs through several families of generic receptors collectively called pattern recognition receptors (PRRs), which are expressed on the microglial cell membrane (Janeway, 1992). PRR receptors can recognize pathogen-associated molecular patterns, which are molecules derived from pathogens (such as bacterial lipopolysaccharide) not found in host cells. Consequently, innate immune cells, including microglia, respond to pathogens by activating specific signaling pathways (Karve et al., 2016; Li and Wu, 2021). Thus, microglial cells are the first responders to disruptions in CNS homeostasis and appear to be quicker in their response to pathogens. Additionally, the innate immune system can be activated by endogenous molecules known as DAMPs. These DAMPs bind to PRRs and initiate downstream signaling. DAMPs are released into the extracellular milieu by damaged or stressed cells, serving as alarm signals for tissue damage. Some examples of DAMPs include nucleic acids, ATP, the amphoterin protein (high mobility group box-1, HMGB-1), histone proteins, and heat shock proteins, among others. Consequently, they are recognized by PRRs, leading to innate immune activation and subsequent inflammation (Li and Wu, 2021).

In addition to the well-known function of microglia as resident professional immune cells in the CNS, astrocytes have become key players in the CNS innate immune response. Reactive astrocytes also express PRRs, through which they detect pathogen-associated molecular patterns or DAMPs, initiating the innate immune response. Following TBI or other acute brain damage, DAMP release efficiently activates reactive astrogliosis (Auzmendi et al., 2020; Cieri et al., 2023). Overactivation of PRR-dependent pathways can lead to pathological remodeling, characterized by a loss of homeostatic functions and a gain of proinflammatory functions that contribute to neurodegeneration (Villarreal et al., 2021). This conversion to a proinflammatory neurodegenerative astroglial profile depends on cooperation with microglia, activation of Toll-like receptors, and downstream nuclear factor kappa B (NF-κB) transcription factor (Liddelow et al., 2017; Rosciszewski et al., 2018; Auzmendi et al., 2020). Once the local innate immune system is activated, an inflammatory response ensues through the secretion of cytokines and chemokines. Some of these cytokines subsequently trigger the adaptive (specific) immune response by communicating with T cells (Li and Wu, 2021).

Pathological remodeling of reactive astrocytes following TBI

Reactive astrocytes are critical regulators of brain injury and recovery in various neuroinflammatory disorders and they play an important role in neuroinflammation (Kolliker-Frers et al., 2021). Pioneering transcriptomic studies have demonstrated that over 1000 genes alter their expression levels in reactive astrocytes. Approximately 50% of these genes are specific to the type of injury and can give rise to astrocyte phenotypes that may have opposing effects, beneficial or detrimental, depending on the triggering damage (Zamanian et al., 2012). Reactive astrocytes with a predominance of neuroprotective functions express high levels of neurotrophic factors and proteins that promote synaptogenesis, among others (Zamanian et al., 2012; Liddelow et al., 2017). Furthermore, reactive astrocytes that lose their capacity for homeostatic regulation and exhibit a gain in neuroinflammatory functions positively regulate proinflammatory pathways, components of the antigen presentation pathway (major histocompatibility complex class I), genes associated with interferon response, and complement pathways, among others (Zamanian et al., 2012; Liddelow et al., 2017; Rosciszewski et al., 2018, 2019; Cieri et al., 2023). Currently, it is understood that reactive astrocytes are not polarized into two functional states, neuroprotective and neurotoxic, but rather exist across a broad spectrum of intermediate phenotypes between these two functional states (Escartin et al., 2021).

After TBI, astrocytes rapidly become reactive, and astrogliosis propagates from the injury core to distant tissues, affecting distant areas from the initial injury (Cieri et al., 2023). This characteristic radial propagation of reactive gliosis is also observed in other types of acute injuries, such as focal ischemia and epileptic seizures (Rossi et al., 2013; Auzmendi et al., 2020). Additionally, the expression of astroglial homeostatic proteins, such as glutamine synthetase, is severely impaired in the vicinity of the TBI core (Cieri et al., 2023). Furthermore, exacerbating inflammation with peripheral lipopolysaccharide leads to further downregulation of astroglial homeostatic genes and increased expression of pro-inflammatory genes (Cieri et al., 2023).

Numerous reports have demonstrated astrocyte reactivity following TBI and have suggested that reactive astrocytes can exert both positive and negative effects on neuronal survival and regeneration. This controversy can be just the tip of the iceberg, as astroglial heterogeneity likely underlies these opposing effects (Ramos, 2016). Another alternative explanation arises from reports showing that acute astrocyte activation can promote neuronal regeneration after brain injury (Beard et al., 2021), while prolonged astrocyte activation may negatively impact neuronal survival (Li and Wu, 2021).

Traumatic injury, as well as stroke, infection, and neurodegenerative diseases of the CNS, trigger a series of pathophysiological processes that lead to the formation of a complex structure, known as, the glial scar. This glial/fibrotic scar arises from interactions between reactive astrocytes and microglia, in cooperation with other cell types. Specifically, astrocytes and NG2 oligodendrocyte progenitor cells form a cord around the epicenter of the inflammatory lesion, while macrophages and fibroblasts are sequestered within the lesion core to prevent inflammation propagation beyond the injury site (Tran et al., 2022).

Currently, it is widely accepted that the glial scar plays a dual role, both protective and detrimental, in functional recovery following CNS injury. On one hand, during the acute stage, this structure is essential for limiting secondary damage and resolving inflammatory processes after focal or extensive CNS injury. On the other hand, in the chronic phase, glial scar formation is associated with substantial changes in tissue reorganization and structural alterations, such as extracellular matrix remodeling. This remodeling occurs due to the deposition of chondroitin sulfate proteoglycans, which are partly secreted by reactive astrocytes. These chondroitin sulfate proteoglycans act as inhibitory molecules for axonal regeneration, effectively creating a biochemical and physical barrier to functional recovery (Sofroniew and Vinters, 2010; Anderson et al., 2016; Milich et al., 2021; Tran et al., 2022). Furthermore, reactive astrocytes can secrete molecules that promote inflammation by enhancing interactions with microglial cells and increasing BBB permeability. This, in turn, can lead to oxidative stress, which may be detrimental to neuronal functionality and survival (Sofroniew and Vinters, 2010). While it is essential to restrict immune cell migration into the brain parenchyma, long-term glial scar formation has been associated with cognitive impairment observed in rodents exposed to experimental TBI and in human patients (Wardhana

et al., 2023). Therefore, mitigating scar formation or limiting the glial scar stability in the long term could represent an interesting therapeutic target, as discussed in the next section of this review.

Sex differences in TBI and the astroglial response

Several studies have shown that women have a better recovery after TBI, in this context, in a recent work the authors have found worse long-term results in males compared to females, in terms of a greater injury volume and neuronal degeneration in males one month after TBI. This is associated with a greater inflammatory response and peripheral leukocyte infiltration in men compared to women (Bahader et al., 2024). Likewise, a larger lesion size in males has been observed in rodent experimental models of TBI by controlled cortical impact (CCI) or fluid percussion injury (FPI) (Spani et al., 2018). It has been proposed that, at the cellular level, astrocytes might contribute to some of the sex difference outcomes observed in patients and animal models, for example, they could facilitate a greater infiltration of immune cells to the site of the injury in males than in females, or it has even been reported that astrocytes from females would be more resistant to oxidative stress than male astrocytes (Munoz-Ballester and Robel, 2023).

Concerning the role of astrocytes in sex differences in TBI, the topic is just starting to be studied in detail since most studies were originally performed in males. However, it is accepted that astrocytes and microglia have sex-related differences defined both at the chromosomal level but also due to circulating hormones (Chowen and García-Segura, 2021). In a clinical trial looking for biomarkers, Sass et al. (2021) showed that women suffering from TBI presented large levels of circulating soluble GFAP in comparison to males and that levels positively correlated with role limitations due to emotional problems, while males did not show correlation. In fact, women are reported to be more vulnerable to post-traumatic symptoms like severe disability, persistent vegetative state, and mortality than males across the age groups (Kim et al., 2019). As recently proposed by Chowen and Garcia-Segura (2021), not only the differences in astrocytic response to acute brain injury, but also the astroglial ability (and different strategies) of male and female astrocytes to recover homeostasis are probably the key to the observed differences in male and female astrocytes. Sex differences in astroglial GFAP expression after TBI have been reported but they are not fully conclusive as recently discussed by Muñoz-Ballester and Robel (2023). More precisely, GFAP overexpression after TBI seems to be the present evidence pointing towards sex differences in the ability of astrocytes to manage metabolic pathways, both intrinsically derived from their chromosomal load but also derived from the effect of circulating sex hormones. In addition, microglia also have different responses to injury in males and females (Villa et al., 2018). Thus, astroglial sex differences may not be only determined by their intrinsic differences but also modified by the bidirectional communication with microgliocytes. Results from the middle cerebral artery occlusion stroke model have shown a more robust astroglial response in young males compared to females, as well as a more significative increase in microglial CD11b (Morrison and Filosa, 2017) that is likely to modify the astroglial response.

There is a male bias in preclinical research since primarily male animals have been used in basic and translational science. However, the incidence of TBI is increasingly independent of sex due to accidental falls and increasing female participation in high-risk activities or increasing TBI in women due to intimate partner violence. Thus, it is critical attention to potential sex differences in the design of preclinical TBI trials to better predict human outcomes (Spani et al., 2018). A greater understanding of the pathophysiology in both sexes is essential to finding an effective therapy for the treatment of traumatic brain injury. In particular, astrocytes are key elements in these sex differences, and they seem to be intrinsically different between males and females, but also astrocytes are profoundly affected by circulating hormones and the interaction with microglia. Detailed studies about the signals and pathways that determine differential astroglial response after TBI are only in their infancy.

Therapeutic Approaches Targeting Astrocytes and Glial Scar Formation after Traumatic Brain Injury

Current therapeutic strategies for TBI

As we mentioned previously, after traumatic injury, initial damage results from mechanical damage that affects all brain cells and recruits professional immune cells from the periphery. Consequently, TBI triggers an inflammatory response within minutes, followed by a cascade of events leading to delayed secondary damage characterized by neuronal loss and further brain damage. Therefore, cellular and molecular events that lead to neuroinflammation and propagate the initial damage but are triggered delayed after the initial injury may provide a therapeutic window to prevent or treat secondary injury (Tani et al., 2022). In concordance, it has been shown that therapies based on the administration of drugs that attenuate neuroinflammation, reduce reactive gliosis, and are beneficial in post-TBI functional recovery, among which we can highlight statins, cannabinoids, progesterone, beta blockers, and cerebrolysin (Gruenbaum et al., 2016).

Statins have been demonstrated to exert anti-inflammatory effects, in the context of acute brain injury. It has been shown that treatment with simvastatin one day after TBI in rats attenuates the reactivity of astrocytes and microglia at 1 and 3 days post-TBI and suppresses the secretion of proinflammatory cytokines. Therefore, treatment with simvastatin inhibits secondary inflammation triggered from the injured core and reduces the propagation of tissue damage, which leads to better recovery of neurological function post-TBI (Li et al., 2009).

Cannabinoid analogs, such as dexanabinol, have been shown highly neuroprotective properties, after TBI both in preclinical studies in animal models and in clinical trials, which have been reported to reduce inflammation, glutamate neurotoxicity, and free radical damage (reviewed in Gruenbaum et al., 2016). Additionally, endocannabinoids, such as 2-arachidonoylglycerolare, are molecules produced by the body that act as neurotransmitters and exhibit significant anti-inflammatory effects. It has been demonstrated that increased levels of 2-arachidonoylglycerol in astrocytes, through the inhibition or elimination of the enzyme responsible for metabolizing this endocannabinoid (monoacylglycerol lipase), exerted a neuroprotective effect that led to improvement in cognitive and synaptic functions after TBI (Loane, 2022).

On the other hand, progesterone treatment after TBI has been shown to modulate astroglial AQP4 expression levels in a time-dependent and region-specific manner associated with a reduction in cerebral edema in post-TBI rats (Guo et al., 2006). Also, the administration of progesterone has shown other beneficial effects such as reduced neuroinflammation, and oxidative stress by positively regulating antioxidant enzymes and reactive oxygen species scavengers, and it has also been

reported to induce less neuronal excitotoxicity and apoptosis after TBI, both in studies with animals as well as in initial clinical trials (reviewed in Jarrahi et al., 2020). Despite promising findings in experimental models, although progesterone administration improved the clinical outcomes of severe TBI patients within 3 months post-TBI, however, did not show significant long-term benefits at 6 months post-injury (Pan et al., 2019). In relation to these findings, progesterone has been evaluated by two large-scale clinical trials: SyNAPSe (Skolnick et al., 2014) and ProTECT III (Wright et al., 2014), but did not demonstrate clinical benefit in patient mortality and functional outcomes at 6 months post-TBI. These failures could be attributed to a variety of factors, including mechanistic and physiological differences in animal systems, heterogeneity in the severity of lesions in patients, and/or problems with the dose and duration of progesterone treatment, therefore, more evaluations would be necessary for its use as a therapeutic for TBI (reviewed in Tani et al., 2022).

It has been proposed that the severity of TBI would be related to the levels of catecholamines in the blood, being higher with greater severity. The immediate release of catecholamines after TBI triggers inflammation and apoptosis (Ko et al., 2016). In fact, beta blockers that inhibit catecholamine interaction with beta-adrenergic receptors are usually administered to patients hospitalized with TBI (Ley et al., 2018; Ding et al., 2021) and they have been shown to reduce mortality when administered early (Ko et al., 2016). Particularly, one of the beta blockers that have shown beneficial results in the treatment of TBI is propranolol, since it has been reported to induce less leukocyte mobilization and vascular permeability, as well as reduce brain edema, associated with better functional neurological recovery post-TBI (Lopez et al., 2022). The findings on the leukocyte infiltrate could be related to the results shown in a very recent work where differential gene expression changes impacting the bone marrow are observed after 1 month of short exposure to propranolol post-TBI (Smith et al., 2023).

Cerebrolysin (CBL) is a mixture of neuropeptides purified from the porcine brain that has shown neuroprotective and neurorestorative properties both *in vitro* and *in vivo*. CBL is approved for use in patients diagnosed with TBI based on the reported beneficial effects on the clinical outcome. CBL has been reported to be anti-inflammatory by reducing reactive oxygen species production and the levels of inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, IL-6, and NF-κB. CBL has also been shown to decrease brain edema by alleviating BBB permeability and reducing apoptosis, leading to improved neurological scores after TBI (Lu et al., 2022). Mechanistically, CBL seems to have beneficial effects after TBI by modulating the TLR2 and TLR4 signaling pathways in mice (Lu et al., 2022). Furthermore, it has been reported that CBL treatment reduces astrogliosis and axonal injury and promotes neurogenesis, associated with improved cognitive and sensorimotor function recovery in rats with mild TBI (Zhang et al., 2019b). Likewise, it has been shown that CBL treatment also induces a reduction in astrogliosis at the site of the injury and an increase in neuronal viability after spinal cord injury (Fouad et al., 2023).

Although the neuroprotective effect of these compounds has been demonstrated, more evaluation is necessary to test the dose-response, duration, and route of administration to optimize the therapeutic window in which the treatment protocols are efficient.

Potential interventions to specifically modulate astroglial response to TBI

As explained above, astrocytes play essential roles in maintaining brain homeostasis and supporting neuronal function. However, in response to TBI, astrocytes can undergo substantial changes and adopt various phenotypes, including a proinflammatory/neurodegenerative profile. Thus, they can actively contribute to the progression of the pathological process due to the neuroinflammatory responses (He et al., 2024). For this reason, specific modulation of the astroglial responses is considered a promising therapeutic strategy to improve TBI-induced brain dysfunction. Currently, various therapeutic strategies are being investigated to modulate astrocytic reactivity, among the most recent and promising experimental therapies are, for example, the administration of extracellular vesicles, such as exosomes, containing microRNAs (miRNAs, miRs) or long non-coding RNA (lncRNA), viral vectors, as well as repurposing drugs that have been tested in other pathologies and demonstrated beneficial effects on astroglial population.

miRNAs

MicroRNAs are small noncoding RNAs that function as posttranscriptional regulators of gene expression. They play a crucial role in the regulation of neuroinflammation (Korotkov et al., 2020). Specifically, in animal models of TBI, various transcriptomic studies have revealed changes in the gene expression of miRNAs involved in regulating post-TBI brain inflammation in the cerebral cortex and hippocampus (Korotkov et al., 2020).

MiR-155 and miR-142 have been found to be upregulated in the perilesional cortex 2 weeks after TBI. In the human perilesional cortex, miR-155 is most prominently expressed by reactive astrocytes, while miR-142 is predominantly expressed by microglia, macrophages, and lymphocytes. It has been reported that human astrocytes adopt a proinflammatory phenotype and overexpress miR-155 under proinflammatory stimuli. Furthermore, the expression of miR-142 in immune cells enhances the conversion toward the proinflammatory astroglial profile. Consequently, in astrocytes it promotes the activation of pro-inflammatory signaling pathways associated with the NF-κB and activator protein 1 transcription factors, leading to the induction of astroglial miR-155 (Korotkov et al., 2020). In relation to this, the inhibition of miR-155 attenuated the overexpression of matrix metalloproteinase 3 after stimulation with the pro-inflammatory cytokine IL-1β in cultured human fetal astrocytes (Korotkov et al., 2018). Furthermore, in the context of TBI, immediate inhibition of miR-155 reduced neuroinflammatory markers, such as TNF-α and IL-1β, and improved neurological recovery at both 1 and 7 days after a CCI model in mice (Henry et al., 2019). In conclusion, these miRNAs may participate in the secondary injury post-TBI through astroglial proinflammatory phenotype conversion (Korotkov et al., 2020), and thus, their specific downregulation in astrocytes emerges as a tempting strategy to reduce pro-inflammarory cascades in reactive astrocytes after TBI.

Furthermore, a single microRNA can induce different effects depending on the acute or chronic phase post-TBI. Specifically, miRNA-9-5p levels are significantly increased in brain tissues after TBI. On one hand, the upregulation of miRNA-9-5p contributes to the recovery of neurological function by significantly alleviating apoptosis, neuroinflammation, and BBB damage in rats. This effect is achieved by activating the Hedgehog pathway and inhibiting the NF-κB/MMP-9 pathway during the acute phase after TBI (Wu et al., 2020). In contrast, downregulating miR-9-

5p during the chronic phase after TBI contributes to neurological function recovery. This effect is achieved by promoting astrocyte proliferation and increasing the release of astrocyte-derived neurotrophic factors around the injured brain tissues after TBI (Wu et al., 2021). Briefly, this is because miR-9-5p was identified as a post-transcriptional modulator of thrombospondin 2 (Thbs-2). Consequently, blocking miRNA-9-5p promotes the expression of Thbs-2 protein in astrocytes and, in turn, increases the concentration of Thbs-2 protein between neurons. Thbs-2 promotes the activation of the Notch pathway in neurons, leading to the activation of the ERK and AKT pathways, which enhances the expression of neuronal synapse proteins, including post-synaptic density protein 95 and synaptotagmin (Wu et al., 2020). Furthermore, it has been reported that reactive astrocytes in a neuroinflammatory condition facilitate astrocytogenesis of neural stem/precursor cells through activation of the JAK-STAT pathway. Another miRNA, the miR-17-92 cluster, represses the expression of multiple proteins in the JAK-STAT pathway. When transplanted neural stem/precursor cells are exposed to a neuroinflammatory condition caused by brain injury, miR-17-92 may repress astrocytogenesis while enhancing the number of generated neurons. This effect leads to improved motor coordination in brain-injured mice (Mao et al., 2016).

In healthy and diseased brains, exosomes loaded with miRNA play a crucial role in cellular communication between astrocytes and microglia, making them relevant in post-TBI recovery (Qian et al., 2024). Specifically, astrocyte-derived exosomes are essential for protecting neurons from oxidative stress and promoting regeneration and repair in TBI and related diseases. These exosomes are rich in miRNAs and can deliver miRNAs from host cells to target cells, further modulating recipient cell function. Notably, in the context of TBI, exosomes have been isolated from astrocytes after stimulation with TBI brain extract. Specifically, astrocyte-derived exosomes containing miR-148a-3p significantly improved the neurological severity score and attenuated brain injury by reducing the area of brain injury and the degree of brain edema (Qian et al., 2024). Additionally, astrocyte-derived exosomal miR-148a-3p regulates microglial phenotype by promoting the conversion of microglia to the anti-inflammatory M2 phenotype, reduces neuroinflammation by inhibiting the NF-κB pathway, and restores neurological function in TBI (Qian et al., 2024). In another study, similar effects were observed with astrocytes-derived exosomes enriched with miR-873a-5p. These exosomes inhibited neuroinflammation by modulating microglia phenotypes after TBI and improved neurological deficits following a CCI mouse model. The mechanism also involved inhibiting the NF-κB signaling pathway (Long et al., 2020). These findings highlight the critical role of astrocytes-derived exosomes and their miRNA cargo in TBI recovery and neuroinflammation modulation.

Furthermore, several microRNAs have been reported to be involved in modulating neuroinflammation following TBI. For instance, miR-200b has been shown to modulate microglia-mediated neuroinflammation in TBI. When miR-200b is downregulated, it leads to increased inflammatory responses through the cJun/MAPK signaling pathway (Jadhav et al., 2014). Additionally, miR-21-5p has been reported to be elevated in neurons and microglia after TBI. Notably, neuron-derived exosomes containing miR-21-5p have been observed to be phagocytosed by microglia, inducing proinflammatory microglia polarization (Yin et al., 2020). A comprehensive analysis of different types of noncoding RNAs that regulate neuroinflammation across various pathologies has been recently reviewed (Yang et al., 2023).

Conflicting, and sometimes opposite, effects for the same miRNAs observed in the experimental models of TBI and cell cultures reinforce the idea that specificity in cell types must be achieved in the delivery system to obtain the effects on the desired cell type. For this purpose, exosomes derived from specific cell types or glia-targeted nanoparticles (Murta et al., 2019) may serve as a cell-specific vehicle for miRNA delivery to astrocytes or other cell types.

lncRNAs

In general, non-coding RNAs, such as microRNAs, participate in and promote pathophysiological changes in reactive astrocytes during brain injury (Ye and Yuan, 2023). Additionally, lncRNAs also play an important role in modulating post-TBI inflammation. It has been demonstrated that TBI can induce changes in the expression levels of lncRNAs *in vivo* (Beylerli et al., 2023). Specifically, alterations in the expression of 823 lncRNAs were observed, with 667 being overexpressed and 156 downregulated in the cortex after TBI in mice (Beylerli et al., 2023).

Particularly in the context of TBI, it has been discovered that the lncRNA Gm4419 plays a role in promoting astrocyte apoptosis induced by trauma. This occurs through the up-regulation of the expression of the inflammatory cytokine TNF-α. Overexpression of Gm4419 in injury-treated astrocytes leads to increased protein levels of TNF-α, Bax, cleaved caspase-3, and cleaved caspase-9. Simultaneously, it results in decreased levels of Bcl-2 and CyclinD1, ultimately contributing to astroglial apoptosis. To understand the role of Gm4419 at the molecular level, it is essential to recognize that the post-transcriptional regulation of TNF-α also involves another non-coding RNA, a microRNA called miR-466l. MiR-466l targets the TNF-α transcript, leading to its degradation and inhibition of translation. Mechanistically, it has been proposed that the Gm4419 transcript acts as a sponge for miR-466l, effectively competing for its binding. Consequently, Gm4419 could up-regulate TNF-α expression, contributing to both inflammatory damage and astrocyte apoptosis after TBI (Yu et al., 2017).

Furthermore, another lncRNA called Malat1 has shown a high protective effect against TBI-induced astrocyte swelling and brain edema by regulating the IL-6, NF-κB, and AQP4 expression. It has been found that Malat1 is downregulated in astrocytes after an FPI model *in vitro*. Similarly, the Malat1 expression was reduced in the border zone of brain edema, while both AQP4 and NF-κB levels were significantly increased after an FPI model *in vivo*. On the contrary, a protective effect has been reported by the overexpression of Malat1 since it induces an attenuated astrocyte swelling, meanwhile reduces IL-6, NF-κB, and AQP4 expression in astrocytes (Zhang et al., 2019a).

Just as we have described extracellular vesicles or exosomes loaded with microRNAs that are important in modulating the response to TBI, lncRNAs that are also involved in this regulation are currently being widely studied. In particular, astrocyte-derived extracellular vesicles carrying a lncRNA called NKILA, due to its interaction with NF-κB, have been associated with the inflammatory response following TBI. This is because NKILA can inhibit the NF-κB signaling pathway. The upregulation of NKILA in astrocyte-derived EVs increases neuron proliferation and decreases neuron apoptosis, thereby promoting brain recovery after TBI *in vivo*. Thus, astrocyte-derived EVs enriched with NKILA may serve as a promising new target for the development of therapeutic strategies for TBI (He et al., 2021).

In addition, other studies have identified significant roles of lncRNAs in regulating the inflammatory response of microglial

cells after TBI. Specifically, researchers have evaluated the function of HOTAIR, an lncRNA that is upregulated in TBI models and microglial cell cultures treated with lipopolysaccharide. HOTAIR acts as a molecular sponge for a microRNA called miR-136-5p, which negatively regulates the AKT2-NF-κB signaling pathway (Duan et al., 2018). Additionally, the upregulation of HOTAIR also enhances the expression of MYD88. Conversely, blocking HOTAIR leads to reduced MYD88 expression, resulting in the suppression of microglial activation and decreased secretion of inflammatory mediators such as TNF-α, IL-1β, and IL-6 (Cheng et al., 2021).

In summary, it is well-established that non-coding RNAs (ncRNAs) play a crucial role in various pathologies. They regulate gene expression in astrocytes and microglia, impacting multiple signaling pathways associated with neuroinflammation. Modulating these ncRNAs (either through overexpression or inhibition) allows astrocytes and microglia to reverse the phenotype proinflammatory-neurodegenerative. Delivery of these lncRNA encounters the same difficulties as for miRNA and the design of these carriers is of utmost importance to achieve specific effects on pro-inflammatory astrocytes.

Viral vectors

Currently, researchers have assessed the use of genetic constructs based on adeno-associated virus vectors to modulate astrocyte activity. They have also examined practical applications of optogenetic techniques, which involve using various genetically encoded opsins to manipulate astroglial function (Salmina et al., 2021). However, the diverse subtypes of astrocytic cells pose challenges in designing an optimal viral vector for specific experimental goals. Consequently, variations in transduction efficiency occur across different brain regions and species (Borodinova et al., 2021).

Furthermore, understanding the characteristics of different vectors is crucial for optimizing gene therapy for traumatic brain injuries (Negro-Demontel et al., 2014). In this context, a study has compared the use of lentiviral vectors with several modular protein-based nanovectors as therapeutic tools for TBI, using a CCI model (Negro-Demontel et al., 2014). Although both viral vectors did not show toxicity after TBI, on the one hand, the nanovectors showed stable and lower green fluorescent protein transgene expression from 1 to 14 days post-TBI, while the lentiviral vectors showed expression of the transgene at 1 day after TBI and increased expression at 14 days (Negro-Demontel et al., 2014). While, in the long-term post-TBI, in another more recent work the researchers showed that the intracerebral administration of integrative-deficient lentiviral vectors or the non-viral HNRK recombinant modular protein/DNA nanovector did not alter the microglial or astroglial reactivity at 3 months post-CCI (Blanco-Ocampo et al., 2020). Furthermore, these vectors induce per se reduced short-term neurological deficits and diminished brain tissue loss at 3 months after injury. These gene therapy tools may be potential and promising short- and long-term post-TBI therapeutic strategies (Blanco-Ocampo et al., 2020).

Specifically in astrocytes, it has been shown that by using adenovirus-associated viruses the overexpression of GJA1-20K enhances the expression of functional connexin 43 in astrocytes. Connexin 43 (encoded by GJA1) participates in the formation of connexin hemichannels in gap junction, but GJA1-20k is one of the highest expressed isoforms, which has been proven to modulate the microtubule-dependent mitochondrial transportation (Czyzewski et al., 2024). Overexpressing GJA1-20k specifically in astrocytes by using adenovirus-associated viruses promoted the viability and recovery of neurons in the TBI context, through regulating their mitochondrial function. This gene therapy approach promoted the recovery of neurite and cognition of rats in a FPI model of TBI (Ren et al., 2020).

In another work, the impact of activating cyclic AMP response element-binding protein (CREB) signaling specifically in reactive astrocytes after a model of focal traumatic brain injury has been evaluated. The researchers used a bitransgenic mouse model where a constitutively active CREB (VP16-CREB) was selectively expressed in astrocytes. VP16 is a herpes-virus-derived strong transcriptional activator that when fused to the DNA binding domain of CREB produces a chimeric protein that drives transcription from CRE-driven promoters in a constitutive manner. By using this strategy of constitutively activating CRE in astrocytes in the context of TBI, they observed reduced neuronal death and damage in the cerebral cortex after TBI, reduced macrophage infiltration, and preserved mitochondrial function. Therefore, enhancing CREB activity in reactive astrocytes emerges as a novel therapy for acute brain injury (Pardo et al., 2016).

Additionally, in a recent work, the impact of silencing the *AQP4* gene using lentiviral vectors in a rat model of TBI has been explored. AQP4 silencing alleviated brain edema and neurological deficits and it also reduced neuronal apoptosis and inhibited astrocyte reactivity. Silencing AQP4 using shRNA in the TBI rat model led to improved outcomes, suggesting its potential as a neuroprotective strategy post-TBI (Li et al., 2022b).

Repurposed drugs and natural compounds

Given the lack of effective neuroprotective strategies to reduce mortality in patients with TBI, current therapeutic approaches have shifted focus toward mitigating secondary injury triggered by the inflammatory cascade following the primary injury (Czyzewski et al., 2024). In this context, heightened reactivity of astrocytes with a proinflammatory profile exacerbates the inflammatory cascade and adversely impacts disease prognosis. Recognizing the diverse roles of astrocytes in CNS injury, various efforts have been made to enhance their beneficial effects or mitigate their detrimental impact. Currently, several candidate drugs have been proposed to target different aspects of astroglial reactivity, including inhibiting exaggerated proinflammatory responses, reducing edema formation, and toxicity, and promoting neuronal protection (Yu et al., 2021). Interestingly, some of these drugs are already effective in treating various pathologies and can be repurposed due to their demonstrated benefits in the context of TBI pathology (Ghiam et al., 2021). By repurposing old drugs, the time required to identify effective drugs and advance them to clinical trials could be significantly reduced, some of these drugs under investigation will be discussed at the end of this section.

Among the drugs aimed at reducing excessive astroglial reactivity and inflammation in TBI, one notable candidate is N-docosahexaenoylethanolamine (synaptamide). Synaptamide is an endogenous endocannabinoid-like metabolite synthesized from docosahexaenoic acid. It possesses neuroprotective, synaptogenic, neuritogenic, and anti-inflammatory properties within the nervous system. Notably, synaptamide can reverse cognitive deficits caused by mild TBI and also mitigate post-traumatic neuroinflammation (Ponomarenko et al., 2021). Furthermore, synaptamide decreases the reactivity of GFAP- and S100-positive astrocytes during the acute (1 day) phase of mild TBI. Additionally, it reduces the expression of proinflammatory markers and exhibits antioxidant effects both acutely (1 day) and during the chronic (7 days) phases of TBI.

Remarkably, synaptamide also stimulates the secretion of the neurotrophin brain-derived neurotrophic factor at 7 days after TBI (Ponomarenko et al., 2022).

Not being a repurposed drug, but an endogenous molecule of extended clinical use, melatonin induces a significant increase in neuronal activity and inhibits astroglial reactivity in the medial prefrontal cortex and hippocampus after trauma. Consequently, melatonin attenuates cognitive decline when administered during early pathological stages, but not in the late (chronic) stages following TBI (Cao et al., 2021). Additionally, another study has demonstrated that melatonin treatment following TBI reduces astrocyte reactivity and neuronal cell apoptosis in the rat brain cortex (Babaee et al., 2015).

Recent research highlights that the DAMP named HMGB1 is a key molecule in various CNS disorders, including TBI, Alzheimer's disease, Parkinson's disease, multiple sclerosis, and epilepsy (Paudel et al., 2018). Specifically after TBI, HMGB1 binds to transmembrane receptors, including TLR2, TLR4, and RAGE, leading to the expression of inflammatory cytokines in microglia and astrocytes (Rosciszewski et al., 2019). This cascade may contribute to the development or exacerbation of secondary brain injury (Paudel et al., 2018). Therefore, HMGB1 is believed to play a critical role in promoting inflammation and exacerbating damage following TBI. In fact, in primary co-cultures of glial cells and neurons, exposure to HMGB-1 has been shown to induce dendrite loss and neurodegeneration through a crosstalk mechanism involving astrocytes and microglia, mediated by NF-κB activation (Rosciszewski et al., 2019). Various strategies have been employed to target HMGB1, including the use of anti-HMGB1 monoclonal antibodies, BoxA HMGB1 antagonist, and natural inhibitors like glycyrrhizin. Glycyrrhizin downregulates the HMGB1-(TLR4/RAGE)-NF-κB inflammatory pathway, resulting in the attenuation of brain edema and improvement of motor function in TBI rats (Jarrahi et al., 2020). Similarly, HMGB1 inhibition with glycyrrhizin has been associated with improved locomotor function and reduced edema, accompanied by a downregulation of astrocyte reactivity and AQP4 expression in spinal cord injury rats (Sun et al., 2019). The modulation of DAMP-activated pathways in brain injuries appears crucial for mitigating secondary lesions and represents a potential target for future therapeutic interventions (Czyzewski et al., 2024).

Other natural compounds used in experimental TBI are flavonoids. These molecules are polyphenolic compounds found in a wide variety of plants and have been evaluated as potential therapeutic alternatives for treating CNS pathologies, including TBI. Their anti-inflammatory, antioxidant, and immunomodulatory properties of flavonoids make them promising candidates (de Amorim et al., 2020). Specifically, research has demonstrated that the flavonoid agathisflavone reduces the expression of GFAP protein and mitigates astrocyte hypertrophy in an *in vitro* scratch wound model of TBI using primary neuroglial cell cultures from rat cerebral cortex. Notably, agathisflavone also enhances neuronal survival and promotes neurite growth at the site of the scratch wound injury. Additionally, it induces the expression of neurotrophic factors, such as nerve growth factor and glial cell line-derived neurotrophic factor, which are associated with a neuroprotective profile in glial cells (de Amorim et al., 2020; Yu et al., 2021).

Repurposed drugs like minocycline, an Food and Drug Administration–approved second-generation tetracycline antibiotic, have gained recognition for their neuroprotective and anti-inflammatory properties in both experimental and clinical studies related to CNS diseases, including TBI (Ghiam et al., 2021). Notably, minocycline anti-inflammatory effects involve the modulation of microglial cells, leading to reduced secretion of proinflammatory cytokines and chemokines, matrix metalloproteinases, and nitric oxide. Additionally, minocycline has demonstrated benefits such as reducing edema, apoptosis, and preserving BBB function (Koulaeinejad et al., 2019). Additionally, astroglial cell markers, such as S100B, are elevated in serum after TBI and correlate with patient prognosis. Notably, acute TBI patients treated with minocycline exhibit lower S100B levels in serum and improved GCS scores (Koulaeinejad et al., 2019). However, it is essential to consider that long-term minocycline treatment in a human study involving 15 patients more than 6 months post moderate/severe TBI resulted in increased neurodegeneration, as indicated by elevated plasma neurofilament light chain levels. To address this, a proposed approach involves combining minocycline neuroprotective properties with N-acetylcysteine. In a TBI model, this combination improved memory, cognition, and repaired white matter damage by promoting the survival of oligodendrocytes (Haber et al., 2018; Sangobowale et al., 2018).

Phenserine (PHEN), an anti-acetylcholinesterase agent initially developed for Alzheimer's disease treatment, has garnered attention for its neuroprotective and anti-inflammatory properties in both experimental and clinical studies related to TBI (Ghiam et al., 2021). Specifically, PHEN anti-acetylcholinesterase activity has demonstrated efficacy in reducing neuroinflammation, alleviating amyloid deposition, preventing apoptosis, and mitigating secondary injury associated with TBI. At the molecular level, treatment with PHEN appears to promote neuronal survival by increasing levels of brain-derived neurotrophic factor and B-cell lymphoma 2 (Bcl-2), while simultaneously decreasing activated-caspase 3, amyloid precursor protein, and GFAP astroglial expression, potentially mediated through the ERK signaling pathway (Chang et al., 2017). Additionally, PHEN has shown positive effects in reducing intracranial pressure, as measured by lateral ventricle size, and contusion volume post-TBI. These findings highlight the potential of PHEN as a therapeutic candidate for TBI management (Hsueh et al., 2019) that seems to regulate astrocytes, probably in an indirect manner.

Similarly, Maraviroc, a C–C chemokine receptor type 5 antagonist, is commonly used as part of antiretroviral therapy for HIV infection. However, it has also emerged as a novel therapeutic approach for treating neuroinflammatory diseases. Specifically, the pharmacotherapeutic effects of Maraviroc have been evaluated in a CCI model of TBI. The results demonstrated its neuroprotective effects, leading to improvements in cognitive function and motor abilities. These assessments were conducted using the neurological severity score, rotarod test, Morris water maze test, and measurements of lesion volume (Liu et al., 2023a). One of the mechanisms underlying Maraviroc efficacy is its ability to reduce the conversion of reactive proinflammatory-neurodegenerative astrocytes ($GFAP^+C3^+$). Furthermore, Maraviroc protects against TBI by suppressing NLRP3 inflammasome activation, modulating microglial polarization from M1 to M2, reducing neutrophil and macrophage infiltration, and inhibiting the release of proinflammatory factors after TBI (Liu et al., 2023a).

Other recent studies have explored the use of various classes of anti-inflammatory drugs to regulate astroglial reactivity and mitigate neuroinflammation. These drug categories include non-steroidal anti-inflammatory drugs, which inhibit cyclooxygenase enzymes; glucocorticoids; and disease-modifying anti-rheumatic

drugs, each with distinct mechanisms of action (Madeira et al., 2015). While most of these drugs have been extensively studied for their role in peripheral inflammation inhibition, it is also recognized that they have the potential to alleviate astrocyte reactivity and subsequent neuroinflammation by impeding neurotoxicity mediated by human microglia and astrocytes (He et al., 2024).

Gold thiol compounds have demonstrated effectiveness in reducing peripheral inflammation, with auranofin being a notable example. Auranofin is commonly used to treat inflammation associated with rheumatoid arthritis. Additionally, research has shown that auranofin inhibits neurotoxicity mediated by human microglia and astrocytes. This effect is attributed to auranofin ability to inhibit NF-κB activation, reduce TNF-α production and secretion, and upregulate heme oxygenase-1, an anti-inflammatory and neuroprotective enzyme in astrocytes (Madeira et al., 2015).

Dextromethorphan, also known as d-3-methoxy-17-methylmorphinan, is an antitussive agent commonly used in cough medicines. Dextromethorphan has demonstrated the ability to inhibit peripheral production and secretion of several inflammatory mediators. Additionally, it reduces glia-mediated neuroinflammation and may have neuroprotective effects (Chechneva et al., 2011).

Rosiglitazone (RSG) is a drug commonly used in the treatment of type 2 diabetes. RSG treatment has been associated with several positive effects, including reduced infarct volume, improved neurological function, and reduced neutrophil accumulation in the brain parenchyma of post-ischemic mice. Additionally, RSG has demonstrated direct neuroprotective activity in models of TBI. Treatment with RSG has also led to enhanced expression of neuroprotective chaperones and antioxidant enzymes in the post-traumatic brain. In addition, it is important to note that RSG treatment also resulted in the expression of proinflammatory genes and apoptotic proteins such as Bax and caspase-3. So, the overall outcome was an improved neuronal survival post-TBI (Yi et al., 2008; Madeira et al., 2015).

Interestingly, other repurposed drugs, such as Enoxacin and Metformin, have been evaluated to reduce neuroinflammatory effects. In the case of Enoxacin, it has been observed to upregulate the pool of anti-inflammatory microRNAs *in vitro* by stimulating the processing activity of Dicer (Felicetti et al., 2020). Furthermore, Metformin exerts anti-inflammatory effects through multiple pathways, including the inhibition of nuclear translocation of the p65 subunit of NF-κB (Feng et al., 2023), but also augments the processing of miRNA pools, in different cell types, including astrocytes. However, further investigation is needed to clarify the specific mechanisms of action for these drugs and their potential use as therapeutic agents in neuroinflammatory CNS pathologies.

AQP4 is expressed in astrocytes and exhibits a polarized localization in their end-feet, playing a crucial role in both edema formation and resolution. Specifically, in the context of TBI, elevated expression of AQP4 in the brain contributes to increased intracranial pressure caused by brain edema, which consistently leads to poor patient prognosis (Ding et al., 2013). Notably, the modulation of AQP4 represents a potential therapeutic target for managing TBI. In this context, Trifluoperazine, a calmodulin inhibitor, is utilized as a therapeutic agent for treating psychiatric disorders and can reduce CNS edema. Recent research has revealed that the antipsychotic agent Trifluoperazine inhibits AQP4 accumulation in astrocytic end-feet following TBI. This inhibition is associated with a reduction in brain edema, the size of the lesion area, and improved functional recovery in rats after TBI. Furthermore, an RNA sequencing analysis identified that the genes and signaling pathways targeted by Trifluoperazine are associated with the inhibition of apoptosis and the inflammatory response induced by TBI (Xing et al., 2023). The repurposed drug Propofol (2,6-diisopropylphenol) is a widely used intravenous anesthetic that has been studied for its neuroprotective roles. Propofol effectively suppresses the expression of AQP4 in astrocytes and significantly reduces brain edema in a CCI model in rats. Additionally, this reduction in brain edema is associated with attenuated expression of IL-1β and TNF-α. In primary astrocyte-enriched cultures, Propofol functions as a dual inhibitor of NF-κB and p38/MAPK pathways. Consequently, it can inhibit the expression of AQP4 induced by the proinflammatory cytokines IL-1β and TNF-α (Ding et al., 2013).

Less explored in TBI, but the interesting pathway to study is the calcium signaling in reactive astrocytes which offers another promising avenue for TBI treatment (reviewed in Czyzewski et al., 2024).

As previously mentioned, drug repurposing offers a fast-tracked pathway for developing effective therapies to treat TBI. The upmentioned small list of candidates (summarized in **Table 2**) presents several repurposed drugs effective on astroglial cell population, that have demonstrated significative efficiency to reduce astroglial pathological pro-inflammatory remodeling.

Strategies to mitigate glial scar formation

As previously described, the glial scar represents a physical barrier that encases damaged tissues and restricts the migration of inflammatory cells from the injury core to the CNS parenchyma (Yu et al., 2021). Different loss-of-function strategies have been employed to prevent the proliferation of reactive astrocytes and thereby mitigate or prevent glial scar formation following stab wound TBI model (Hu et al., 2023) or traumatic spinal cord injury (Wanner et al., 2013). In these studies, as expected, glial scar formation was not observed. Interestingly, but surprisingly, spontaneous axonal regeneration was also absent and, instead, larger lesions and tissue degeneration were observed. Furthermore, these investigations revealed that astroglial cells express multiple molecules that promote axonal growth following the injury model (Anderson et al., 2016). This can be explained by the fact that fibrotic tissues and macrophages within the lesion core act as inhibitory factors for axonal regeneration. Conversely, the glial scar and adjacent reactive astrocytes may contribute to limiting the detrimental effects of fibrotic tissue and macrophages. Additional studies have shown that while regenerating axons avoid dense cellular clusters in fibrotic tissue, they can go through the lesion core associated with astrocytes, which act as permissive bridges for axonal regeneration (Zukor et al., 2013). However, it has been suggested that achieving functional recovery may require not only axonal regeneration but also other essential components such as synaptic reorganization and plasticity on the target field (Zukor et al., 2013; Anderson et al., 2018).

The process of astrogliosis occurs following a CNS injury, leading to a phenotypic change in astrocytes from naive astrocytes into reactive astrocytes. Eventually, these reactive astrocytes transform into scar-forming astrocytes, which can impair axonal regeneration and functional recovery. While this process has traditionally been considered unidirectional and stable, there is currently a proposal for the environment-dependent plasticity of reactive astrogliosis. In other words, reactive astrocytes can revert

Table 2 | **Experimental treatment strategies aimed to target astrocytes**

Therapeutic strategies	Examples	Effect on astrocytes	Observed results in TBI	References
miRNA	miR155	-Attenuates the overexpression of MMP3.	-Reduces neuroinflammatory markers. -Improves neurological recovery.	Korotkov et al., 2018; Henry et al., 2019
	miRNA-9-5p (upregulated in the acute phase of TBI)		-Contributes to the recovery of neurological function. -Alleviates apoptosis, neuroinflammation, and BBB damage. -Activates the Hedgehog pathway. -Inhibits the NFκB/MMP-9 pathway during the acute phase after TBI. -Contributes to neurological function recovery.	Wu et al., 2020
	miRNA-9-5p (downregulated in the chronic phase of TBI)	-Promotes astrocyte proliferation. -Increases the release of astrocyte-derived neurotrophic factors around the injured brain tissues after TBI.		Wu et al., 2021
	miR-17-92 cluster	-Represses astrocytogenesis while enhancing the number of generated neurons.	-Represses the expression of multiple proteins in the JAK-STAT pathway. -Improves motor coordination in brain-injured mice.	Mao et al., 2016
	Astrocyte-derived exosomal miR-148a-3p	-Secretes exosomes containing miR-148a-3p	-Improves the neurological severity score. -Reduces the area of brain injury and the degree of brain edema. -Promotes the conversion of microglia to the anti-inflammatory M2 phenotype. -Inhibits the NF-κB pathway. -Restores neurological function in TBI.	Qian et al., 2024
	Astrocyte-derived exosomal miR-873a-5p	-Secretes exosomes containing miR-148a-3p	-Inhibits neuroinflammation by modulating microglia phenotypes. -Improves neurological deficits. -inhibits NF-κB signaling pathway.	Long et al., 2020
lncRNA	lncGm4419	-Promotes astrocyte apoptosis induced by TBI. -Upregulates TNF-α expression.	-Implications for neuroinflammation and CNS injury.	Yu et al., 2017
	lncMalat1	-Protectives against TBI-induced astrocyte swelling and brain edema. -Regulates IL-6, NFκB, and AQP4 expression.		Zhang et al., 2019a
	Astrocyte-derived EVs enriched with NKILA	-Astrocytes secrete EVs containing NKILA.	-Inhibits the NF-κB signaling pathway. -Increases neuronal proliferation and decreases neuronal apoptosis. -Promotes brain recovery after TBI. -Promising new target for the development of therapeutic strategies for TBI.	He et al., 2021
Viral vectors	Adenovirus-associated viruses that overexpress GJA1-20K	-Overexpresses GJA1-20k, an isoform of connexin 43.	-Promotes viability and recovery of neurons. -Regulates the neuronal mitochondrial function. -Promotes the recovery of neurite and cognition.	Ren et al. 2020; Czyzewski et al., 2024
	Lentiviral vectors with shRNA for AQP4	-AQP4 silencing in astrocytes. -Inhibits astrocyte reactivity.	-Alleviates brain edema. -Reduces neuronal apoptosis. -Improves outcomes neurological.	Li et al., 2022b
Drugs	Synaptamide	-Decreases astroglial reactivity.	-Reduces the expression of proinflammatory markers. -Exhibits antioxidant effects. -Stimulates the secretion of the neurotrophin brain-derived neurotrophic factor.	Ponomarenko et al., 2022
	Glycyrrhizin	-Downregulates astrocyte reactivity and AQP4 expression.	-Downregulates HMGB1-(TLR4/RAGE)-NF-κB inflammatory pathway -Attenuates brain edema. -Improves locomotor function.	Jarrahi et al., 2020
	Melatonin	-Inhibits astroglial reactivity.	-Increases neuronal activity and decreases apoptosis. -Attenuates cognitive decline.	Babaee et al., 2015; Cao et al., 2021
	Agathisflavone	-Reduces the expression of GFAP protein and mitigates astrocyte hypertrophy.	-Enhances the number of neurons and promotes neurite growth. -Induces the expression of neurotrophic factors.	de Amorim et al., 2020; Yu et al., 2021
	Minocycline/Minocycline + NAC	-Reduces S100B levels in serum.	-Modulates microglial cells. -Reduces secretion of proinflammatory cytokines and chemokines, MMPs, and nitric oxide. -Reduces edema, apoptosis, and preserving BBB function. -Improves Glasgow Coma Scale scores.	Haber et al., 2018; Sangobowale et al., 2018
	Phenserine	-Reduces GFAP astroglial expression, potentially mediated through the ERK signaling pathway.	-Promotes neuronal survival -Reduces intracranial pressure and contusion volume post-TBI.	Chang et al., 2017; Hsueh et al., 2019
	Maraviroc	-Reduces the conversion of reactive proinflammatory-neurodegenerative astrocytes.	-Suppress NLRP3 inflammasome activation. -Modulates M2 microglial polarization. -Reduces neutrophil and macrophage infiltration. -Inhibits the release of proinflammatory factors.	Liu et al., 2023a
	Auranofin	-Inhibits neurotoxicity mediated by human microglia and astrocytes. -Inhibits NF-κB activation, reduces TNF-α production and secretion, and upregulates HOX-1.		Madeira et al., 2015
	Trifluoperazine	-Inhibits AQP4 accumulation in astrocytic end-feet.	-Reduces brain edema, the size of the lesion area, and improves functional recovery.	Xing et al., 2023
	Propofol	-Suppresses the expression of AQP4. -Attenuates expression of IL-1β and TNF-α.	-Reduces brain edema.	Ding et al., 2013

AQP4: Aquaporin-4; BBB: blood–brain barrier; CNS: central nervous system; ERK: extracellular signal-regulated kinase; EVs: extracellular vesicles; GFAP: glial fibrillary acidic protein; HMGB1: high mobility group box-1; HOX-1: homeobox A1; IL-1β: interleukin-1β; IL-6: interleukin-6; MMP3: matrix metalloproteinase-3; MMP-9: matrix metalloproteinase-9; NAC: N-acetyl cysteine; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; NLRP3: NLR family pyrin domain containing 3; RAGE: receptor for advanced glycation end products; S100B: S100 calcium-binding protein B; TBI: traumatic brain injury; TLR4: Toll-like receptor 4; TNF-α: tumour necrosis factor alpha.

to a naive state or become scar-forming astrocytes depending on the context (Hara et al., 2017). Studies have demonstrated that scar-forming astrocytes are induced through the interaction of reactive astrocytes and type I collagen expressed during glial scar formation, via the integrin–N-cadherin pathway. Blocking this pathway with antibodies leads to improved axonal regrowth and better functional outcomes (Hara et al., 2017). Therefore, inhibiting the conversion of reactive astrocytes into glial scar–forming astrocytes may represent an ideal treatment for CNS injury. This approach would allow for the restriction of inflammation by reactive astrocytes without compromising secondary damage limitation, while simultaneously inhibiting the formation of the glial scar boundary to alleviate the inhibitory effects of the surrounding environment on axonal regeneration (reviewed in Yu et al., 2021).

The astroglial scar surrounding the injury core is formed in a STAT3-dependent manner. This conclusion is based on reports that STAT3-deficient astroglia fail to form functional scar borders (Wanner et al., 2013). Recently, a study demonstrated that intracranial injection of the kinase inhibitory region of SOCS3 in mice with TBI reduces reactive astrogliosis and inhibits glial scar formation by suppressing the JAK2-STAT3 pathway. Consequently, this intervention leads to greater neuronal survival post-TBI (Cai et al., 2023). Additionally, suppression of JAK2-STAT3 activity also results in a reduction in the proportion of C3/GFAP double-labeled proinflammatory-neurodegenerative pathologically remodeled reactive astrocytes (Cai et al., 2023).

Furthermore, another key regulator for scar formation and maintenance of astrocyte reactivity is the actin-binding protein Drebrin, which becomes upregulated in astrocytes triggered by TBI. At the molecular level, Drebrin facilitates the remodeling of the actin cytoskeleton in astrocytes, allowing for the trafficking of surface proteins involved in astrogliosis and adhesion mediators, such as β1-integrin, which are essential for glial scar formation after TBI (Schiweck et al., 2021). Consistently, Drebrin deficiency leads to defective astrocyte scar formation and excessive neurodegeneration post-TBI (Schiweck et al., 2021). Additionally, the transcription factor Sox2 is essential for astrocyte reactivity in response to cortical TBI, promoting the proliferation of reactive astrocytes and the formation of glial scars after postnatal TBI. Conversely, Sox2-deficient animals exhibited reduced reactive gliosis and improved functional recovery after early postnatal TBI (Liu et al., 2023b).

In addition to gene therapy strategies (i.e. with viral vectors), drugs such as methylprednisolone have also been evaluated. Methylprednisolone is a synthetic glucocorticoid that has already been used clinically due to its neuroprotective and anti-inflammatory effects for treating acute spinal cord injury. It has been reported that methylprednisolone can reduce astrocyte reactivity and downregulate the expression of chondroitin sulfate proteoglycan, thereby promoting neurite outgrowth after injury in adult rats (Liu et al., 2008). If this is also true for TBI and that results in a better outcome remains to be elucidated.

Among other potential therapies targeting astrocytes and glial scar formation, astrocyte transplantation can be mentioned. These astrocytes are obtained from glial precursors by pre-differentiating the precursor cells through exposure to bone morphogenetic protein-4 prior to transplantation. When transplanted into injured rat spinal cords, both rat and human transplanted functional astrocytes promote extensive axonal regeneration, enhance neuronal cell survival, restore tissue structure, and facilitate recovery of behavior to pre-injury levels (Noble et al., 2011). Therefore, astrocyte transplantation could be a promising therapeutic strategy for TBI (reviewed in (Zheng and Wang, 2022)). In the context of cellular therapy, one of the main challenges is the survival and engraftment of transplanted cells following implantation into the host tissue. For this reason, combined therapies involving biomaterials with cell transplantation are being developed to enhance tissue repair processes. These combined approaches aim to improve the cell survival of the graft. Hyaluronic acid hydrogel, known for its favorable biological properties, has garnered interest for its therapeutic effects, particularly after cortical trauma. Notably, the implantation of the hydrogel into the lesioned motor cortex of adult mice facilitated vascularization, reduced glial scar formation around the lesion, and limited the progression of secondary injury. This led to favorable environments for the survival and maturation of the newly generated neurons. These findings suggest a beneficial effect of the biomaterial after TBI (Laine et al., 2022). Although the results of these investigations are preliminary and have not been tested in clinical trial stages, they all constitute important lines of research. A better understanding of the regulation of glial scar formation is essential to develop effective interventions and treatments after TBI.

Challenges and Future Directions

Current limitations in understanding astrocyte biology in TBI

Historically, astrocytes were considered homogenous cell populations. However, recent advances in experimental techniques and our functional understanding of astrocytes have challenged this view. We now recognize that astrocytes exhibit heterogeneity, both in physiological and pathological conditions (Ramos, 2016; Auzmendi et al., 2019; Cieri et al., 2023). This astroglial diversity plays a crucial role in maintaining physiological functions and overall CNS functionality. As well as in astrogliosis, a great diversity of profiles of reactive astrocytes has been evidenced in response to contexts of injury in the CNS (Ramos, 2016; Giovannoni and Quintana, 2020; Cieri et al., 2023).

However, understanding astrocyte biology in TBI remains challenging due to the lack of specific markers depending on the lesion location and distance from the injury core (Cieri et al., 2023). To address this, identifying specific markers for distinct astrocyte subsets becomes crucial. These markers could serve as early indicators of astroglial pathological remodeling and facilitate the way for targeted therapies. In summary, unraveling the heterogeneity of astrocytes and discovering context-specific markers would be essential to adapt therapies to specific astroglial subtypes that allow the development of more effective treatments after CNS injury.

Heterogeneity observed in the astroglial population should also be considered when designing astrocyte-targeted therapies since effects can differ dramatically if different populations are affected. Especially, if the pathologically remodeled population is targeted, therapies should aim to preserve astroglial clusters that sustain neuronal homeostasis.

Potential avenues for novel treatments and interventions

New treatments are emerging as an attractive approach to restorative medicine in CNS pathologies. These approaches are based on either transplanting astrocytes exhibiting desired phenotypes or utilizing stem cells capable of differentiating into astrocytes. Such strategies could offer innovative therapeutic avenues for TBI. In this context, the use of mesenchymal stem cells (MSCs) and genomic editing techniques like CRISPR-Cas9 are promising. These tools enable targeted therapies directed at specific cell types, presenting exciting opportunities for TBI treatment (He et al., 2024).

MSCs have emerged as a compelling therapeutic avenue for CNS diseases and injuries, including spinal cord injury, multiple sclerosis, ischemic stroke, and TBI. Their relative ease of isolation, immunosuppressive properties allowing allogeneic transplantation without immune rejection, and minimal ethical controversies make MSCs promising candidates for clinical utility. However, the *in vivo* therapeutic potency of MSCs presents difficulties, as survival and ability to localize within injured tissue remain limited (Toyoshima et al., 2015). Direct transplantation of MSCs into injured brain tissue during cranial repair operations for TBI patients has demonstrated safety, with no adverse effects. Furthermore, both direct administration to the injured brain tissue and indirect administration via intravenous or intra-arterial injections have shown significant improvements in TBI-induced motor and cognitive deficits in preclinical models (Shahror et al., 2020). Despite these promising outcomes, the adverse microenvironment of injured tissue negatively impacts MSC survival. In summary, MSCs show great therapeutic potential for CNS disorders, therefore researchers are actively seeking strategies to enhance MSC survival and localization within the injured CNS for effective clinical applications.

A promising approach involves genetically modifying MSCs using viral, non-viral, or vector-based methods via CRISPR-Cas9. The goal is to induce overexpression of factors critical for MSC therapeutic effects in the context of TBI. The CRISPR-Cas9 gene editing technique has not only been employed to modify MSCs, but it has also been tested in trials that demonstrated neuroprotective effects in TBI. For instance, targeting different spots in the inflammation pathways involved in reactive astrogliosis, such as NF-κB, using CRISPR is postulated as a promising tool to reverse TBI-induced damage (Swanson et al., 2020).

Conclusion

Astrocytes have a major role in CNS homeostasis and their functions are essential for neuronal survival and synaptic plasticity that underlies, not only brain motor functions but also brain cognitive functions. TBI produces an initial acute mechanical damage that activates rapid neuronal death in the affected region, but the secondary neuroinflammation that occurs in the following days is what seriously affects patients' survival and cognitive recovery. Astrocytes have a fundamental importance in this delayed neuroinflammation since pathological remodeling of reactive astrocytes transforms astrocytes into pathologically remodeled pro-inflammatory cells that have suppressed their homeostatic functions and facilitate neurodegeneration. Experimental data from TBI animal models and tissue cultures have shown that sequential activation of innate immune receptors in microglia and astrocytes as well as the downstream NF-κB/Stat3 pathways are key intracellular events in this process. There are several drugs available that can be repurposed for use in TBI patients, however, cell specificity is a major complication since some pathways (i.e. NF-κB) are also required for neuronal survival. Cellular-specific therapies using cell-targeted drug delivery, and novel technologies like CRISP-Cas or adeno-associated virus gene therapies with specific astroglial promoters emerge as tempting strategies to be explored to limit pathological conversion of reactive astrocytes in TBI.

Author contributions: *MBC and AJR conceived the review, performed the literature search, wrote the manuscript, and approved the final version of the manuscript.*
Conflicts of interest: *The authors declare no conflicts of interest.*
Data availability statement: *Not applicable.*
Open access statement: *This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.*

References

Alam A, Thelin EP, Tajsic T, Khan DZ, Khellaf A, Patani R, Helmy A (2020) Cellular infiltration in traumatic brain injury. J Neuroinflammation 17:328.
Anderson MA, Burda JE, Ren Y, Ao Y, O'Shea TM, Kawaguchi R, Coppola G, Khakh BS, Deming TJ, Sofroniew MV (2016) Astrocyte scar formation aids central nervous system axon regeneration. Nature 532:195-200.
Anderson MA, O'Shea TM, Burda JE, Ao Y, Barlatey SL, Bernstein AM, Kim JH, James ND, Rogers A, Kato B, Wollenberg AL, Kawaguchi R, Coppola G, Wang C, Deming TJ, He Z, Courtine G, Sofroniew MV (2018) Required growth facilitators propel axon regeneration across complete spinal cord injury. Nature 561:396-400.
Auzmendi J, Moffatt L, Ramos AJ (2020) Predicting reactive astrogliosis propagation by bayesian computational modeling: the repeater stations model. Mol Neurobiol 57:879-895.
Babaee A, Eftekhar-Vaghefi SH, Asadi-Shekaari M, Shahrokhi N, Soltani SD, Malekpour-Afshar R, Basiri M (2015) Melatonin treatment reduces astrogliosis and apoptosis in rats with traumatic brain injury. Iran J Basic Med Sci 18:867-872.
Bahader GA, Naghavi F, Alotaibi A, Dehghan A, Swain CC, Burkett JP, Shah ZA (2024) Neurobehavioral and inflammatory responses following traumatic brain injury in male and female mice. Behav Brain Res 456:114711.
Basit RH, Wiseman J, Chowdhury F, Chari DM (2023) Simulating traumatic brain injury in vitro: developing high throughput models to test biomaterial based therapies. Neural Regen Res 18:289-292.
Beard E, Lengacher S, Dias S, Magistretti PJ, Finsterwald C (2021) Astrocytes as key regulators of brain energy metabolism: new therapeutic perspectives. Front Physiol 12:825816.
Beylerli O, Tamrazov R, Gareev I, Ilyasova T, Shumadalova A, Bai Y, Yang B (2023) Role of exosomal ncRNAs in traumatic brain injury. Noncoding RNA Res 8:686-692.
Blanco-Ocampo D, Cawen FA, Alamo-Pindado LA, Negro-Demontel ML, Peluffo H (2020) Safe and neuroprotective vectors for long-term traumatic brain injury gene therapy. Gene Ther 27:96-103.
Bonvento G, Bolaños JP (2021) Astrocyte-neuron metabolic cooperation shapes brain activity. Cell Metab 33:1546-1564.
Borodinova AA, Balaban PM, Bezprozvanny IB, Salmina AB, Vlasova OL (2021) Genetic constructs for the control of astrocytes' activity. Cells 10:1600.
Burda JE, Sofroniew MV (2014) Reactive gliosis and the multicellular response to CNS damage and disease. Neuron 81:229-248.
Burda JE, Bernstein AM, Sofroniew MV (2016) Astrocyte roles in traumatic brain injury. Exp Neurol 275:305-315.
Cai Z, Zhang Z, Zhang L, Tan R, Wang Y, Sun M, Hu X, Ge Q, An J, Lu H (2023) The kinase inhibitory region of SOCS3 attenuates reactive astrogliosis and astroglial scar in mice after traumatic brain injury. J Chem Neuroanat 131:102273.
Cao R, Li L, Zhang W, Lu J, Wang Y, Chen Q, Chen M, Sheng L, Cai K, Xu G (2021) Melatonin attenuates repeated mild traumatic brain injury-induced cognitive deficits by inhibiting astrocyte reactivation. Biochem Biophys Res Commun 580:20-27.
Chang CF, Lai JH, Wu JC, Greig NH, Becker RE, Luo Y, Chen YH, Kang SJ, Chiang YH, Chen KY (2017) (-)-Phenserine inhibits neuronal apoptosis following ischemia/reperfusion injury. Brain Res 1677:118-128.
Chechneva OV, Mayrhofer F, Daugherty DJ, Pleasure DE, Hong JS, Deng W (2011) Low dose dextromethorphan attenuates moderate experimental autoimmune encephalomyelitis by inhibiting NOX2 and reducing peripheral immune cells infiltration in the spinal cord. Neurobiol Dis 44:63-72.
Cheng S, Zhang Y, Chen S, Zhou Y (2021) LncRNA HOTAIR participates in microglia activation and inflammatory factor release by regulating the ubiquitination of MYD88 in traumatic brain injury. J Mol Neurosci 71:169-177.
Chowen JA, Garcia-Segura LM (2021) Role of glial cells in the generation of sex differences in neurodegenerative diseases and brain aging. Mech Ageing Dev 196:111473.
Cieri MB, Villarreal A, Gomez-Cuautle DD, Mailing I, Ramos AJ (2023) Progression of reactive gliosis and astroglial phenotypic changes following stab wound-induced traumatic brain injury in mice. J Neurochem 167:183-203.
Czyzewski W, Mazurek M, Sakwa L, Szymoniuk M, Pham J, Pasierb B, Litak J, Czyzewska E, Turek M, Piotrowski B, Torres K, Rola R (2024) Astroglial cells: emerging therapeutic targets in the management of traumatic brain injury. Cells 13:148.

Datta S, Lin F, Jones LD, Pingle SC, Kesari S, Ashili S (2023) Traumatic brain injury and immunological outcomes: the double-edged killer. Future Sci OA 9:FSO864.

de Amorim VCM, Junior MSO, da Silva AB, David JM, David JPL, de Fatima Dias Costa M, Butt AM, da Silva VDA, Costa SL (2020) Agathisflavone modulates astrocytic responses and increases the population of neurons in an in vitro model of traumatic brain injury. Naunyn Schmiedebergs Arch Pharmacol 393:1921-1930.

Dean T, Ghaemmaghami J, Corso J, Gallo V (2023) The cortical NG2-glia response to traumatic brain injury. Glia 71:1164-1175.

Deshetty UM, Periyasamy P (2023) Potential biomarkers in experimental animal models for traumatic brain injury. J Clin Med 12:3923.

Dinet V, Petry KG, Badaut J (2019) Brain-immune interactions and neuroinflammation after traumatic brain injury. Front Neurosci 13:1178.

Ding H, Liao L, Zheng X, Wang Q, Liu Z, Xu G, Li X, Liu L (2021) Beta-blockers for traumatic brain injury: a systematic review and meta-analysis. J Trauma Acute Care Surg 90:1077-1085.

Ding Z, Zhang J, Xu J, Sheng G, Huang G (2013) Propofol administration modulates AQP-4 expression and brain edema after traumatic brain injury. Cell Biochem Biophys 67:615-622.

Duan C, Liu Y, Li Y, Chen H, Liu X, Chen X, Yue J, Zhou X, Yang J (2018) Sulfasalazine alters microglia phenotype by competing endogenous RNA effect of miR-136-5p and long non-coding RNA HOTAIR in cuprizone-induced demyelination. Biochem Pharmacol 155:110-123.

Ebert SE, Jensen P, Ozenne B, Armand S, Svarer C, Stenbaek DS, Moeller K, Dyssegaard A, Thomsen G, Steinmetz J, Forchhammer BH, Knudsen GM, Pinborg LH (2019) Molecular imaging of neuroinflammation in patients after mild traumatic brain injury: a longitudinal (123) I-CLINDE single photon emission computed tomography study. Eur J Neurol 26:1426-1432.

Escartin C, et al. (2021) Reactive astrocyte nomenclature, definitions, and future directions. Nat Neurosci 24:312-325.

Felicetti T, Cecchetti V, Manfroni G (2020) Modulating microRNA processing: enoxacin, the progenitor of a new class of drugs. J Med Chem 63:12275-12289.

Feng YY, Wang Z, Pang H (2023) Role of metformin in inflammation. Mol Biol Rep 50:789-798.

Fouad S, Elfarrash S, Rizk A, Rezk S, Mosbah E, Saad MA, Awad BI (2023) Cerebrolysin recovers diaphragmatic function and reduces injury-associated astrogliosis following a cervical spinal cord hemi-section injury in rats. Egypt J Neurol Psychiatr Neurosurg 59:116.

Fraunberger E, Esser MJ (2019) Neuro-inflammation in pediatric traumatic brain injury-from mechanisms to inflammatory networks. Brain Sci 9:319.

Freire MAM, Rocha GS, Bittencourt LO, Falcao D, Lima RR, Cavalcanti J (2023) Cellular and molecular pathophysiology of traumatic brain injury: what have we learned so far? Biology (Basel) 12:1139.

Frik J, Merl-Pham J, Plesnila N, Mattugini N, Kjell J, Kraska J, Gomez RM, Hauck SM, Sirko S, Gotz M (2018) Cross-talk between monocyte invasion and astrocyte proliferation regulates scarring in brain injury. EMBO Rep 19:e45294.

Ghiam MK, Patel SD, Hoffer A, Selman WR, Hoffer BJ, Hoffer ME (2021) Drug repurposing in the treatment of traumatic brain injury. Front Neurosci 15:635483.

Giovannoni F, Quintana FJ (2020) The role of astrocytes in CNS inflammation. Trends Immunol 41:805-819.

Gruenbaum SE, Zlotnik A, Gruenbaum BF, Hersey D, Bilotta F (2016) Pharmacologic neuroprotection for functional outcomes after traumatic brain injury: a systematic review of the clinical literature. CNS Drugs 30:791-806.

Guo Q, Sayeed I, Baronne LM, Hoffman SW, Guennoun R, Stein DG (2006) Progesterone administration modulates AQP4 expression and edema after traumatic brain injury in male rats. Exp Neurol 198:469-478.

Haber M, James J, Kim J, Sangobowale M, Irizarry R, Ho J, Nikulina E, Grin'kina NM, Ramadani A, Hartman I, Bergold PJ (2018) Minocycline plus N-acteylcysteine induces remyelination, synergistically protects oligodendrocytes and modifies neuroinflammation in a rat model of mild traumatic brain injury. J Cereb Blood Flow Metab 38:1312-1326.

Hara M, Kobayakawa K, Ohkawa Y, Kumamaru H, Yokota K, Saito T, Kijima K, Yoshizaki S, Harimaya K, Nakashima Y, Okada S (2017) Interaction of reactive astrocytes with type I collagen induces astrocytic scar formation through the integrin-N-cadherin pathway after spinal cord injury. Nat Med 23:818-828.

Hart CG, Karimi-Abdolrezaee S (2021) Recent insights on astrocyte mechanisms in CNS homeostasis, pathology, and repair. J Neurosci Res 99:2427-2462.

Hayashi MK (2018) Structure-function relationship of transporters in the glutamate-glutamine cycle of the central nervous system. Int J Mol Sci 19:1177.

He B, Chen W, Zeng J, Tong W, Zheng P (2021) Long noncoding RNA NKILA transferred by astrocyte-derived extracellular vesicles protects against neuronal injury by upregulating NLRX1 through binding to mir-195 in traumatic brain injury. Aging (Albany NY) 13:8127-8145.

He L, Zhang R, Yang M, Lu M (2024) The role of astrocyte in neuroinflammation in traumatic brain injury. Biochim Biophys Acta Mol Basis Dis 1870:166992.

Henry RJ, Doran SJ, Barrett JP, Meadows VE, Sabirzhanov B, Stoica BA, Loane DJ, Faden AI (2019) Inhibition of miR-155 limits neuroinflammation and improves functional recovery after experimental traumatic brain injury in mice. Neurotherapeutics 16:216-230.

Hsueh SC, Lecca D, Greig NH, Wang JY, Selman W, Hoffer BJ, Miller JP, Chiang YH (2019) (-)-Phenserine ameliorates contusion volume, neuroinflammation, and behavioral impairments induced by traumatic brain injury in mice. Cell Transplant 28:1183-1196.

Hu X, Li S, Shi Z, Lin WJ, Yang Y, Li Y, Li H, Xu Y, Zhou M, Tang Y (2023) Partial ablation of astrocytes exacerbates cerebral infiltration of monocytes and neuronal loss after brain stab injury in mice. Cell Mol Neurobiol 43:893-905.

Iadecola C, Nedergaard M (2007) Glial regulation of the cerebral microvasculature. Nat Neurosci 10:1369-1376.

Iliff JJ, Chen MJ, Plog BA, Zeppenfeld DM, Soltero M, Yang L, Singh I, Deane R, Nedergaard M (2014) Impairment of glymphatic pathway function promotes tau pathology after traumatic brain injury. J Neurosci 34:16180-16193.

Jacquens A, Needham EJ, Zanier ER, Degos V, Gressens P, Menon D (2022) Neuro-inflammation modulation and post-traumatic brain injury lesions: from bench to bed-side. Int J Mol Sci 23:11193.

Jadhav SP, Kamath SP, Choolani M, Lu J, Dheen ST (2014) microRNA-200b modulates microglia-mediated neuroinflammation via the cJun/MAPK pathway. J Neurochem 130:388-401.

Janeway CA Jr (1992) The immune system evolved to discriminate infectious nonself from noninfectious self. Immunol Today 13:11-16.

Jarrahi A, Braun M, Ahluwalia M, Gupta RV, Wilson M, Munie S, Ahluwalia P, Vender JR, Vale FL, Dhandapani KM, Vaibhav K (2020) Revisiting traumatic brain injury: from molecular mechanisms to therapeutic interventions. Biomedicines 8:389.

Jassam YN, Izzy S, Whalen M, McGavern DB, El Khoury J (2017) Neuroimmunology of traumatic brain injury: time for a paradigm shift. Neuron 95:1246-1265.

Karthigeyan M, Gupta SK, Salunke P, Dhandapani S, Wankhede LS, Kumar A, Singh A, Sahoo SK, Tripathi M, Gendle C, Singla R, Aggarwal A, Singla N, Mohanty M, Mohindra S, Chhabra R, Tewari MK, Jain K (2021) Head injury care in a low- and middle-income country tertiary trauma center: epidemiology, systemic lacunae, and possible leads. Acta Neurochir (Wien) 163:2919-2930.

Karve IP, Taylor JM, Crack PJ (2016) The contribution of astrocytes and microglia to traumatic brain injury. Br J Pharmacol 173:692-702.

Kim T, Chelluboina B, Chokkalla AK, Vemuganti R (2019) Age and sex differences in the pathophysiology of acute CNS injury. Neurochem Int 127:22-28.

Knox EG, Aburto MR, Clarke G, Cryan JF, O'Driscoll CM (2022) The blood-brain barrier in aging and neurodegeneration. Mol Psychiatry 27:2659-2673.

Ko A, Harada MY, Barmparas G, Thomsen GM, Alban RF, Bloom MB, Chung R, Melo N, Margulies DR, Ley EJ (2016) Early propranolol after traumatic brain injury is associated with lower mortality. J Trauma Acute Care Surg 80:637-642.

Kolliker-Frers R, Udovin L, Otero-Losada M, Kobiec T, Herrera MI, Palacios J, Razzitte G, Capani F (2021) Neuroinflammation: an integrating overview of reactive-neuroimmune cell interactions in health and disease. Mediators Inflamm 2021:9999146.

Korotkov A, Broekaart DWM, van Scheppingen J, Anink JJ, Baayen JC, Idema S, Gorter JA, Aronica E, van Vliet EA (2018) Increased expression of matrix metalloproteinase 3 can be attenuated by inhibition of microRNA-155 in cultured human astrocytes. J Neuroinflammation 15:211.

Korotkov A, Puhakka N, Gupta SD, Vuokila N, Broekaart DWM, Anink JJ, Heiskanen M, Karttunen J, van Scheppingen J, Huitinga I, Mills JD, van Vliet EA, Pitkanen A, Aronica E (2020) Increased expression of miR142 and miR155 in glial and immune cells after traumatic brain injury may contribute to neuroinflammation via astrocyte activation. Brain Pathol 30:897-912.

Koulaeinejad N, Haddadi K, Ehteshami S, Shafizad M, Salehifar E, Emadian O, Ali Mohammadpour R, Ala S (2019) Effects of minocycline on neurological outcomes in patients with acute traumatic brain injury: a pilot study. Iran J Pharm Res 18:1086-1096.

Kundu S, Singh S (2023) What happens in TBI? A wide talk on animal models and future perspective. Curr Neuropharmacol 21:1139-1164.

Laine A, Brot S, Gaillard A (2022) Beneficial effects of hyaluronan-based hydrogel implantation after cortical traumatic injury. Cells 11:3831.

Lefevre-Dognin C, Cogne M, Perdrieau V, Granger A, Heslot C, Azouvi P (2021) Definition and epidemiology of mild traumatic brain injury. Neurochirurgie 67:218-221.
Ley EJ, et al. (2018) Beta blockers in critically ill patients with traumatic brain injury: results from a multicenter, prospective, observational American Association for the Surgery of Trauma study. J Trauma Acute Care Surg 84:234-244.
Li B, Mahmood A, Lu D, Wu H, Xiong Y, Qu C, Chopp M (2009) Simvastatin attenuates microglial cells and astrocyte activation and decreases interleukin-1beta level after traumatic brain injury. Neurosurgery 65:179-185; discussion 185-176.
Li B, Zhang D, Verkhratsky A (2022a) Astrocytes in post-traumatic stress disorder. Neurosci Bull 38:953-965.
Li B, Wei M, Wan X, Chen Z, Liu M, Fan Z, Yang L (2022b) Neuroprotective effects of lentivirus-mediated aquaporin-4 gene silencing in rat model of traumatic brain injury. Neurol Res 44:692-699.
Li D, Wu M (2021) Pattern recognition receptors in health and diseases. Signal Transduct Target Ther 6:291.
Li S, Li F, Fan X, Sun J, Yu Z, Shang H, Dongye S, Yan S, Xiao J, Xin T (2023) Identifying global research trends on inflammation associated with traumatic brain injury by bibliometric and visualized analysis. Heliyon 9:e22807.
Liddelow SA, et al. (2017) Neurotoxic reactive astrocytes are induced by activated microglia. Nature 541:481-487.
Liu WL, Lee YH, Tsai SY, Hsu CY, Sun YY, Yang LY, Tsai SH, Yang WC (2008) Methylprednisolone inhibits the expression of glial fibrillary acidic protein and chondroitin sulfate proteoglycans in reactivated astrocytes. Glia 56:1390-1400.
Liu XL, Sun DD, Zheng MT, Li XT, Niu HH, Zhang L, Zhou ZW, Rong HT, Wang Y, Wang JW, Yang GL, Liu X, Chen FL, Zhou Y, Zhang S, Zhang JN (2023a) Maraviroc promotes recovery from traumatic brain injury in mice by suppression of neuroinflammation and activation of neurotoxic reactive astrocytes. Neural Regen Res 18:141-149.
Liu Y, Hong W, Gong P, Qi G, Wang X, Kang S, Tang H, Qin S (2023b) Specific knockout of Sox2 in astrocytes reduces reactive astrocyte formation and promotes recovery after early postnatal traumatic brain injury in mouse cortex. Glia 71:602-615.
Loane DJ (2022) MAnGLed astrocytes in traumatic brain injury: astrocytic 2-AG metabolism as a new therapeutic target. Brain 145:7-10.
Long X, Yao X, Jiang Q, Yang Y, He X, Tian W, Zhao K, Zhang H (2020) Astrocyte-derived exosomes enriched with miR-873a-5p inhibit neuroinflammation via microglia phenotype modulation after traumatic brain injury. J Neuroinflammation 17:89.
Lopez AJ, ElSaadani M, Jacovides CL, Georges A, Culkin MC, Ahmed S, Kumar MA, Kaplan LJ, Smith DH, Pascual JL (2022) Beta blockade in TBI: Dose-dependent reductions in BBB leukocyte mobilization and permeability in vivo. J Trauma Acute Care Surg 92:781-791.
Lu W, Zhu Z, Shi D, Li X, Luo J, Liao X (2022) Cerebrolysin alleviates early brain injury after traumatic brain injury by inhibiting neuroinflammation and apoptosis via TLR signaling pathway. Acta Cir Bras 37:e370605.
Maas AIR, et al. (2022) Traumatic brain injury: progress and challenges in prevention, clinical care, and research. Lancet Neurol 21:1004-1060.
Madeira JM, Schindler SM, Klegeris A (2015) A new look at auranofin, dextromethorphan and rosiglitazone for reduction of glia-mediated inflammation in neurodegenerative diseases. Neural Regen Res 10:391-393.
Magistretti PJ, Allaman I (2018) Lactate in the brain: from metabolic end-product to signalling molecule. Nat Rev Neurosci 19:235-249.
Mao S, Li X, Wang J, Ding X, Zhang C, Li L (2016) miR-17-92 facilitates neuronal differentiation of transplanted neural stem/precursor cells under neuroinflammatory conditions. J Neuroinflammation 13:208.
Masel BE, DeWitt DS (2010) Traumatic brain injury: a disease process, not an event. J Neurotrauma 27:1529-1540.
Mathiisen TM, Lehre KP, Danbolt NC, Ottersen OP (2010) The perivascular astroglial sheath provides a complete covering of the brain microvessels: an electron microscopic 3D reconstruction. Glia 58:1094-1103.
Michinaga S, Koyama Y (2021) Pathophysiological responses and roles of astrocytes in traumatic brain injury. Int J Mol Sci 22:6418.
Milich LM, Choi JS, Ryan C, Cerqueira SR, Benavides S, Yahn SL, Tsoulfas P, Lee JK (2021) Single-cell analysis of the cellular heterogeneity and interactions in the injured mouse spinal cord. J Exp Med 218:e20210040.
Mira RG, Lira M, Cerpa W (2021) Traumatic brain injury: mechanisms of glial response. Front Physiol 12:740939.
Morrison HW, Filosa JA (2016) Sex differences in astrocyte and microglia responses immediately following middle cerebral artery occlusion in adult mice. Neuroscience 339:85-99.
Muñoz-Ballester C, Robel S (2023) Astrocyte-mediated mechanisms contribute to traumatic brain injury pathology. WIREs Mech Dis 15:e1622.
Muraleedharan R, Gawali MV, Tiwari D, Sukumaran A, Oatman N, Anderson J, Nardini D, Bhuiyan MAN, Tkac I, Ward AL, Kundu M, Waclaw R, Chow LM, Gross C, Rao R, Schirmeier S, Dasgupta B (2020) AMPK-regulated astrocytic lactate shuttle plays a non-cell-autonomous role in neuronal survival. Cell Rep 32:108092.
Muresanu IA, Grad DA, Muresanu DF, Dobran SA, Hapca E, Strilciuc S, Benedek I, Capris D, Popescu BO, Perju-Dumbrava L, Chereches RM (2022) Evaluation of post-traumatic stress disorder (PTSD) and related comorbidities in clinical studies. J Med Life 15:436-442.
Murta V, Schilrreff P, Rosciszewski G, Morilla MJ, Ramos AJ (2018) G5G2.5 core-shell tecto-dendrimer specifically targets reactive glia in brain ischemia. J Neurochem 144:748-760.
Naumenko Y, Yuryshinetz I, Zabenko Y, Pivneva T (2023) Mild traumatic brain injury as a pathological process. Heliyon 9:e18342.
Negro-Demontel ML, Saccardo P, Giacomini C, Yanez-Munoz RJ, Ferrer-Miralles N, Vazquez E, Villaverde A, Peluffo H (2014) Comparative analysis of lentiviral vectors and modular protein nanovectors for traumatic brain injury gene therapy. Mol Ther Methods Clin Dev 1:14047.
Noble M, Davies JE, Mayer-Proschel M, Proschel C, Davies SJ (2011) Precursor cell biology and the development of astrocyte transplantation therapies: lessons from spinal cord injury. Neurotherapeutics 8:677-693.
Noriega-Prieto JA, Araque A (2021) Sensing and regulating synaptic activity by astrocytes at tripartite synapse. Neurochem Res 46:2580-2585.
Pan ZY, Zhao YH, Huang WH, Xiao ZZ, Li ZQ (2019) Effect of progesterone administration on the prognosis of patients with severe traumatic brain injury: a meta-analysis of randomized clinical trials. Drug Des Devel Ther 13:265-273.
Pardo L, Schluter A, Valor LM, Barco A, Giralt M, Golbano A, Hidalgo J, Jia P, Zhao Z, Jove M, Portero-Otin M, Ruiz M, Gimenez-Llort L, Masgrau R, Pujol A, Galea E (2016) Targeted activation of CREB in reactive astrocytes is neuroprotective in focal acute cortical injury. Glia 64:853-874.
Paudel YN, Shaikh MF, Chakraborti A, Kumari Y, Aledo-Serrano A, Aleksovska K, Alvim MKM, Othman I (2018) HMGB1: a common biomarker and potential target for TBI, neuroinflammation, epilepsy, and cognitive dysfunction. Front Neurosci 12:628.
Pekny M, Pekna M (2016) Reactive gliosis in the pathogenesis of CNS diseases. Biochim Biophys Acta 1862:483-491.
Pellerin L, Magistretti PJ (1994) Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. Proc Natl Acad Sci U S A 91:10625-10629.
Pellerin L, Magistretti PJ (2012) Sweet sixteen for ANLS. J Cereb Blood Flow Metab 32:1152-1166.
Petersen A, Soderstrom M, Saha B, Sharma P (2021) Animal models of traumatic brain injury: a review of pathophysiology to biomarkers and treatments. Exp Brain Res 239:2939-2950.
Ponomarenko A, Tyrtyshnaia A, Ivashkevich D, Ermolenko E, Dyuizen I, Manzhulo I (2022) Synaptamide modulates astroglial activity in mild traumatic brain injury. Mar Drugs 20:538.
Ponomarenko AI, Tyrtyshnaia AA, Pislyagin EA, Dyuizen IV, Sultanov RM, Manzhulo IV (2021) N-docosahexaenoylethanolamine reduces neuroinflammation and cognitive impairment after mild traumatic brain injury in rats. Sci Rep 11:756.
Qian Y, Li X, Li G, Liu H, Li Q, Liu X, Zhang Y, He Z, Zhao Y, Fan H (2024) Astrocyte-derived exosomal miR-148a-3p suppresses neuroinflammation and restores neurological function in traumatic brain injury by regulating the microglial phenotype. eNeuro 11:ENEURO.0336-23.2024.
Ramlackhansingh AF, Brooks DJ, Greenwood RJ, Bose SK, Turkheimer FE, Kinnunen KM, Gentleman S, Heckemann RA, Gunanayagam K, Gelosa G, Sharp DJ (2011) Inflammation after trauma: microglial activation and traumatic brain injury. Ann Neurol 70:374-383.
Ramos AJ (2016) Astroglial heterogeneity: merely a neurobiological question? Or an opportunity for neuroprotection and regeneration after brain injury? Neural Regen Res 11:1739-1741.
Ren D, Zheng P, Feng J, Gong Y, Wang Y, Duan J, Zhao L, Deng J, Chen H, Zou S, Hong T, Chen W (2020) Overexpression of astrocytes-specific GJA1-20k enhances the viability and recovery of the neurons in a rat model of traumatic brain injury. ACS Chem Neurosci 11:1643-1650. Erratum in: ACS Chem Neurosci 13:2235.
Ribeiro TN, Delgado-Garcia LM, Porcionatto MA (2021) Notch1 and galectin-3 modulate cortical reactive astrocyte response after brain injury. Front Cell Dev Biol 9:649854.
Rosciszewski G, Cadena V, Murta V, Lukin J, Villarreal A, Roger T, Ramos AJ (2018) Toll-like receptor 4 (TLR4) and triggering receptor expressed on myeloid cells-2 (TREM-2) activation balance astrocyte polarization into a proinflammatory phenotype. Mol Neurobiol 55:3875-3888.
Rosciszewski G, Cadena V, Auzmendi J, Cieri MB, Lukin J, Rossi AR, Murta V, Villarreal A, Reines A, Gomes FCA, Ramos AJ (2019) Detrimental effects of HMGB-1 require microglial-astroglial interaction: implications for the status epilepticus-induced neuroinflammation. Front Cell Neurosci 13:380.

Rossi AR, Angelo MF, Villarreal A, Lukin J, Ramos AJ (2013) Gabapentin administration reduces reactive gliosis and neurodegeneration after pilocarpine-induced status epilepticus. PLoS One 8:e78516.
Salehi A, Zhang JH, Obenaus A (2017) Response of the cerebral vasculature following traumatic brain injury. J Cereb Blood Flow Metab 37:2320-2339.
Salmina AB, Gorina YV, Erofeev AI, Balaban PM, Bezprozvanny IB, Vlasova OL (2021) Optogenetic and chemogenetic modulation of astroglial secretory phenotype. Rev Neurosci 32:459-479.
Sandhu MRS, Gruenbaum BF, Gruenbaum SE, Dhaher R, Deshpande K, Funaro MC, Lee TW, Zaveri HP, Eid T (2021) Astroglial glutamine synthetase and the pathogenesis of mesial temporal lobe epilepsy. Front Neurol 12:665334.
Sangobowale MA, Grin'kina NM, Whitney K, Nikulina E, St Laurent-Ariot K, Ho JS, Bayzan N, Bergold PJ (2018) Minocycline plus N-acetylcysteine reduce behavioral deficits and improve histology with a clinically useful time window. J Neurotrauma 35:907-917.
Sass D, Guedes VA, Smith EG, Vorn R, Devoto C, Edwards KA, Mithani S, Hentig J, Lai C, Wagner C, Dunbar K, Hyde DR, Saligan L, Roy MJ, Gill J (2021) Sex differences in behavioral symptoms and the levels of circulating GFAP, Tau, and NfL in patients with traumatic brain injury. Front Pharmacol 12:746491.
Scheller A, Bai X, Kirchhoff F (2017) The role of the oligodendrocyte lineage in acute brain trauma. Neurochem Res 42:2479-2489.
Schiweck J, Murk K, Ledderose J, Munster-Wandowski A, Ornaghi M, Vida I, Eickholt BJ (2021) Drebrin controls scar formation and astrocyte reactivity upon traumatic brain injury by regulating membrane trafficking. Nat Commun 12:1490.
Shahror RA, Wu CC, Chiang YH, Chen KY (2020) Genetically modified mesenchymal stem cells: the next generation of stem cell-based therapy for TBI. Int J Mol Sci 21:4051.
Shao F, Wang X, Wu H, Wu Q, Zhang J (2022) Microglia and neuroinflammation: crucial pathological mechanisms in traumatic brain injury-induced neurodegeneration. Front Aging Neurosci 14:825086.
Skolnick BE, Maas AI, Narayan RK, van der Hoop RG, MacAllister T, Ward JD, Nelson NR, Stocchetti N (2014) A clinical trial of progesterone for severe traumatic brain injury. N Engl J Med 371:2467-2476.
Smith JA, Nguyen T, Davis BC, Lahiri DK, Hato T, Obukhov AG, White FA (2023) Propranolol treatment during repetitive mild traumatic brain injuries induces transcriptomic changes in the bone marrow of mice. Front Neurosci 17:1219941.
Sofroniew MV, Vinters HV (2010) Astrocytes: biology and pathology. Acta Neuropathol 119:7-35.
Spani CB, Braun DJ, Van Eldik LJ (2018) Sex-related responses after traumatic brain injury: Considerations for preclinical modeling. Front Neuroendocrinol 50:52-66.
Stokum JA, Kurland DB, Gerzanich V, Simard JM (2015) Mechanisms of astrocyte-mediated cerebral edema. Neurochem Res 40:317-328.
Sun L, Li M, Ma X, Zhang L, Song J, Lv C, He Y (2019) Inhibiting high mobility group box-1 reduces early spinal cord edema and attenuates astrocyte activation and aquaporin-4 expression after spinal cord injury in rats. J Neurotrauma 36:421-435.
Susarla BT, Villapol S, Yi JH, Geller HM, Symes AJ (2014) Temporal patterns of cortical proliferation of glial cell populations after traumatic brain injury in mice. ASN Neuro 6:159-170.
Swanson LC, Trujillo EA, Thiede GH, Katzenberger RJ, Shishkova E, Coon JJ, Ganetzky B, Wassarman DA (2020) Survival following traumatic brain injury in drosophila is increased by heterozygosity for a mutation of the NF-kappaB innate immune response transcription factor relish. Genetics 216:1117-1136.
Tani J, Wen YT, Hu CJ, Sung JY (2022) Current and potential pharmacologic therapies for traumatic brain injury. Pharmaceuticals (Basel) 15:838.
Toyoshima A, Yasuhara T, Kameda M, Morimoto J, Takeuchi H, Wang F, Sasaki T, Sasada S, Shinko A, Wakamori T, Okazaki M, Kondo A, Agari T, Borlongan CV, Date I (2015) Intra-arterial transplantation of allogeneic mesenchymal stem cells mounts neuroprotective effects in a transient ischemic stroke model in rats: analyses of therapeutic time window and its mechanisms. PLoS One 10:e0127302.
Tran AP, Warren PM, Silver J (2022) New insights into glial scar formation after spinal cord injury. Cell Tissue Res 387:319-336.
Vandebroek A, Yasui M (2020) Regulation of AQP4 in the central nervous system. Int J Mol Sci 21:1603.
Verisokin AY, Verveyko DV, Postnov DE, Brazhe AR (2021) Modeling of astrocyte networks: toward realistic topology and dynamics. Front Cell Neurosci 15:645068.
Verkhratsky A, Nedergaard M (2018) Physiology of astroglia. Physiol Rev 98:239-389.
Verkhratsky A, Rose CR (2020) Na(+)-dependent transporters: the backbone of astroglial homeostatic function. Cell Calcium 85:102136.
Verkhratsky A, Parpura V, Vardjan N, Zorec R (2019) Physiology of astroglia. Adv Exp Med Biol 1175:45-91.
Verkhratsky A, Li B, Scuderi C, Parpura V (2021) Principles of astrogliopathology. Adv Neurobiol 26:55-73.
Villarreal A, Vidos C, Monteverde Busso M, Cieri MB, Ramos AJ (2021) Pathological neuroinflammatory conversion of reactive astrocytes is induced by microglia and involves chromatin remodeling. Front Pharmacol 12:689346.
Wanner IB, Anderson MA, Song B, Levine J, Fernandez A, Gray-Thompson Z, Ao Y, Sofroniew MV (2013) Glial scar borders are formed by newly proliferated, elongated astrocytes that interact to corral inflammatory and fibrotic cells via STAT3-dependent mechanisms after spinal cord injury. J Neurosci 33:12870-12886.
Wardhana DW, Yudhanto HS, Riawan W, Khotimah H, Permatasari HK, Nazwar TA, Nurdiana N (2023) Modification of the height of a weight drop traumatic brain injury model that causes the formation of glial scar and cognitive impairment in rats. BMC Neurol 23:439.
Wright DW, Yeatts SD, Silbergleit R, Palesch YY, Hertzberg VS, Frankel M, Goldstein FC, Caveney AF, Howlett-Smith H, Bengelink EM, Manley GT, Merck LH, Janis LS, Barsan WG (2014) Very early administration of progesterone for acute traumatic brain injury. N Engl J Med 371:2457-2466.
Wu J, He J, Tian X, Luo Y, Zhong J, Zhang H, Li H, Cen B, Jiang T, Sun X (2020) microRNA-9-5p alleviates blood-brain barrier damage and neuroinflammation after traumatic brain injury. J Neurochem 153:710-726.
Wu J, Li H, He J, Tian X, Luo S, Li J, Li W, Zhong J, Zhang H, Huang Z, Sun X, Jiang T (2021) Downregulation of microRNA-9-5p promotes synaptic remodeling in the chronic phase after traumatic brain injury. Cell Death Dis 12:9.
Xing R, Cheng J, Yu J, Li S, Ma H, Zhao Y (2023) Trifluoperazine reduces apoptosis and inflammatory responses in traumatic brain injury by preventing the accumulation of Aquaporin4 on the surface of brain cells. Int J Med Sci 20:797-809.
Yang L, Zhou Y, Jia H, Qi Y, Tu S, Shao A (2020) Affective immunology: the crosstalk between microglia and astrocytes plays key role? Front Immunol 11:1818.
Yang R, Yang B, Liu W, Tan C, Chen H, Wang X (2023) Emerging role of non-coding RNAs in neuroinflammation mediated by microglia and astrocytes. J Neuroinflammation 20:173.
Ye Z, Yuan T (2023) Role and therapeutic potential of non-coding RNAs in astrocytes during neonatal brain injury. ResearchGate doi:10.20944/preprints202312.0927.v1 [Preprints].
Yi JH, Park SW, Brooks N, Lang BT, Vemuganti R (2008) PPARgamma agonist rosiglitazone is neuroprotective after traumatic brain injury via anti-inflammatory and anti-oxidative mechanisms. Brain Res 1244:164-172.
Yin Z, Han Z, Hu T, Zhang S, Ge X, Huang S, Wang L, Yu J, Li W, Wang Y, Li D, Zhao J, Zuo Y, Li Y, Kong X, Chen F, Lei P (2020) Neuron-derived exosomes with high miR-21-5p expression promoted polarization of M1 microglia in culture. Brain Behav Immun 83:270-282.
Yu G, Zhang Y, Ning B (2021) Reactive astrocytes in central nervous system injury: subgroup and potential therapy. Front Cell Neurosci 15:792764.
Yu Y, Cao F, Ran Q, Wang F (2017) Long non-coding RNA Gm4419 promotes trauma-induced astrocyte apoptosis by targeting tumor necrosis factor alpha. Biochem Biophys Res Commun 491:478-485.
Zamanian JL, Xu L, Foo LC, Nouri N, Zhou L, Giffard RG, Barres BA (2012) Genomic analysis of reactive astrogliosis. J Neurosci 32:6391-6410.
Zargari M, Meyer LJ, Riess ML, Li Z, Barajas MB (2023) P188 therapy in in vitro models of traumatic brain injury. Int J Mol Sci 24:3334.
Zhang Y, Wang J, Wei J, Wu R, Cai H (2019a) Overexpression of long noncoding RNA Malat1 ameliorates traumatic brain injury induced brain edema by inhibiting AQP4 and the NF-kappaB/IL-6 pathway. J Cell Biochem 120:17584-17592.
Zhang Y, Chopp M, Zhang ZG, Zhang L, Lu M, Zhang T, Winter S, Doppler E, Brandstaetter H, Mahmood A, Xiong Y (2019b) Cerebrolysin reduces astrogliosis and axonal injury and enhances neurogenesis in rats after closed head injury. Neurorehabil Neural Repair 33:15-26.
Zhao Q, Zhang J, Li H, Xie F (2023) Models of traumatic brain injury-highlights and drawbacks. Front Neurol 14:1151660.
Zheng RZ, Lee KY, Qi ZX, Wang Z, Xu ZY, Wu XH, Mao Y (2022) Neuroinflammation following traumatic brain injury: take it seriously or not. Front Immunol 13:855701.
Zheng X, Wang W (2022) Astrocyte transplantation for repairing the injured spinal cord. J Biomed Res 36:312-320.
Zhou J, Liu T, Guo H, Cui H, Li P, Feng D, Hu E, Huang Q, Yang A, Luo J, Tang T, Wang Y (2018) Lactate potentiates angiogenesis and neurogenesis in experimental intracerebral hemorrhage. Exp Mol Med 50:1-12.
Zukor K, Belin S, Wang C, Keelan N, Wang X, He Z (2013) Short hairpin RNA against PTEN enhances regenerative growth of corticospinal tract axons after spinal cord injury. J Neurosci 33:15350-15361.

C-Editors: Zhao M, Liu WJ, Qiu Y; T-Editor: Jia Y