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High mobility group box protein 1 and white matter injury following traumatic brain injury: perspectives on mechanisms and therapeutic strategies

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Traumatic brain injury (TBI) is a major cause of morbidity and mortality worldwide. Despite significant medical advances over recent decades, many survivors of TBI develop long term neuro-cognitive deficits. Previously, only moderate and severe injuries were thought to account for the devastating consequences of TBI. However, there is increasing evidence that even milder injuries may result in problematic lifelong cognitive and affective disturbances. TBI is typically characterized by an an acute physical injury followed by a protracted innate neuro-inflammatory response. These reponses, mediated via neuronal, astrocyte and microglial cells, amongst others, and may result in widespread neuronal death and a micro-environment that is not conducive to brain repair (Manivannan et al., 2021). Whilst the primary physical injury often evades intervention from a medical perspective, the subsequent neuro-inflammatory response offers a potential therapeutic target. Nonetheless, effective pharmacological strategies continue to elude clinicians and scientists due to the complex underlying pathogenesis and difficulties of modelling such a heterogeneous disease. However, the majority of research to date has focused on investigating the effects of post-traumatic neuro-inflammation on grey matter injury rather than the consequences upon white matter (WM), which contributes greatly to cognitive dysfunction across many neurological diseases (Filly and Kelly, 2018). Herein, we will briefly discuss: (i) high mobility group box protein 1 (HMGB1) as a potential therapeutic target; (ii) the relevance of WM injury in TBI and current understanding of WM repair following injury; and (iii) perspectives on how HMGB1 may play a role.

Post-traumatic neuro-inflammation offers several possible therapeutic targets, but a thorough understanding is required to avoid negating the beneficial effects of endogenous cytokine release. Amongst 'damage associated molecular patterns' proteins that are released following injury, HMGB1 is of particular interest in the context of TBI. This is due to its dual role in mediating neuro-inflammation post-injury, but also in neurogenesis during normal development (Manivannan et al., 2021). Under physiological conditions it is a ubiquitous, non-histone DNA binding protein that is involved in co-ordinating gene transcription. Following injury, HMGB1 is actively secreted by glial cells and passively released by necrotic neurons, and acts predominantly on three target receptors: receptor for advanced glycation end products (RAGE) and toll-like receptors (TLR) 2 and 4 (Manivannan et al., 2021). Downstream signaling cascade culminates in activation of nuclear factor kappa-light-chain-enhancer of activated B cells, which upregulates pro-inflammatory gene expression including tumor necrosis factor-α, inerleukin (IL)-6, IL-10, and IFN-gamma. This positive feedback cycle amplifies the neuro-inflammatory response, which is associated with an unsupportive environment for endogenous neural regeneration. Indeed, using post-natal rodent cortical neural stem/ progenitor cell (NSPC) cultures, we demonstrated that the HMGB1-RAGE axis was responsible for the anti-neurogenic effect of traumatic injury-condition medium (Manivannan et al., 2020). Also, early evidence from *in vivo* animal models of TBI demonstrate improved outcomes with HMGB1 antagonism (Manivannan et al., 2021).

WM tracts span neuroanatomically disparate regions of the brain, facilitating connectivity and the formation of large-scale networks. They are composed of axons, enveloped in myelin sheaths provided by oligodendrocytes (OL), astrocytes in close contact at nodes of Ranvier, and blood vessels. Due to its high metabolic rate, WM is particularly vulnerable to the detrimental effects of neuro-inflammation, excitotoxicity, and oxidative stress that follow TBI (Braun et al., 2017). WM damage after TBI has been characterized in experimental animal models. OL loss has been demonstrated by reduced $CC1^+$ and increased $CC1^+$/Caspase 3^+ immunoreactivity in multiple WM regions at several time points following injury (Flygt et al., 2013; Dent et al., 2015). Increased proliferation of OL precursor cells and potential remyelination at later time points following injury have also been shown (Flygt et al., 2013; Dent et al., 2015; Sullivan et al., 2017). Data from immunohistochemical analysis of post mortem human subjects with severe TBI corroborates these findings (Flygt et al., 2016). However, persistent ongoing loss of OL up to 3 months post controlled cortical impact injury in mice suggests that long term myelination remains impaired, with depletion of a finite pool of OPCs (Dent et al., 2015). However, it is possible that neuronal death and axonal die-back could remove the substrate for remyelination, which may at least in part explain the loss staining for myelin markers following controlled cortical impact studies (Flygt et al., 2013; Dent et al., 2015; Sullivan et al., 2017). This emphasizes the importance of identifying therapeutic strategies in order to provide a supportive environment for remyelination post-TBI.

From a pathophysiological perspective, WM injury involves varying combinations of axonal and oligodendrocyte injury (Armstrong et al., 2016) including: (i) axonal damage with an intact myelin sheath; (ii) demyelination with an intact axon; (iii) axon and OL damage with subsequent neuronal death; and (iv) neuronal death with subsequent OL degeneration due to loss of axon-oligodendrocyte interaction (**Figure 1A**). The former two types of injury offer the potential for meaningful recovery through remyelination or axonal regrowth, provided that OL integrity is maintained. The exact relationship between post-traumatic neuroinflammation and WM injury is an active area of research. However, co-localization of activated microglia and WM damage across mouse, rat, non-human primate, and human models of TBI at several time points led to the hypothesis that chronic microglial activation results in OL damage and subsequent demyelination (Kou et al., 2014). In addition, other neurological diseases may help direct future TBI research into therapeutic targets for WM repair. For example, experimental studies in multiple sclerosis demonstrate that chronic inflammation can prevent remyelination through several mechanisms including hindrance of oligodendrocyte progenitor cell (OPC) recruitment, inhibition of OL differentiation, and disruption of axon-OPC communication (Lubetzki et al., 2020). There is existing evidence for remyelination via two key mechanisms: (i) OPC recruitment, either from existing pools or via neural stem/ progenitor cells (NSPC), and differentiation into oligodendrocytes; or (ii) generation of new myelin sheaths from existing oligodendrocytes. Whilst both mechanisms play a role in repair, inter-species differences have been noted, with a greater reliance of remyelination on endogenous OPCs differentiating, migrating and maturing in functional OLs in rodents, and more reliance upon pre-existing OLs to remyelinate injured axons in humans.

What are the possible mechanisms by which HMGB1 may affect WM repair? Using an *in vitro* needle scratch injury model, we demonstrated that HMGB1 mediated an anti-proliferative effect on $NG2^+$ OPCs in rodent cortical NSPC cultures via a TLR2/4 receptor dependent mechanism (Ved et al., 2021). This suggests that HMGB1 may impede WM repair by inhibiting the OPC proliferation, but further studies are required to identify the implications for remyelination and neurological recovery (**Figure 1B**). Since HMGB1 is physiologicaly involved in NSPC

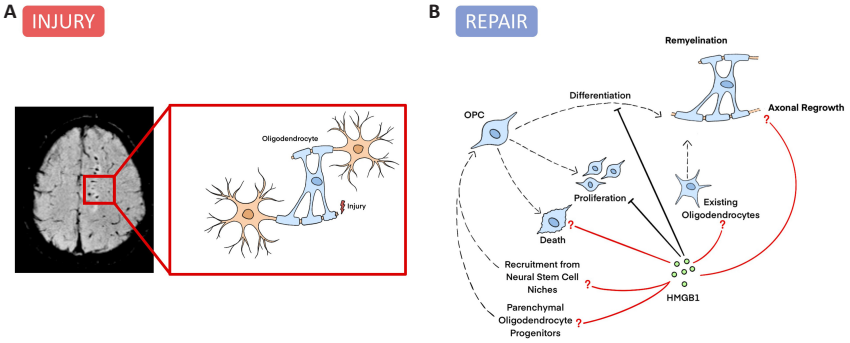

Figure 1 | **Schematic presentation outlining the response of oligodendrocytes and their progenitors to injury.**
(A) depicts diffuse axonal injury as appears on susceptibility weighted MRI (left) and injury to the neurone-oligodendrocyte unit (right). (B) Summarizes the numerous actions HMGB1 may have upon oligodendrocyte progenitor cell (OPC) and mature oligodenedrocyte following neurotrauma.

development and migration, it may also be involved in generation of OPC from existing stem cell niches. Whether HMGB1 is involved in OPC generation and, if so, whether this role changes depending on physiological or pathological conditions remains to be determined. Also, given that pre-existing oligodendrocytes may play a more important role in remyelination in humans, the effects of HMGB1 on fully differentiated cells should be clarified. This could happen via direct mechanisms, since there is evidence that OL express TLR2 and RAGE. Indirect mechanisms should also be explored, as HMGB1 interaction with immune cells, both resident within the central nervous system and peripherally within the blood stream, may influence oligodendrocyte maturation and survival. Similarly, modulation of the neurovascular unit is thought to indirectly affect OL maturation. HMGB1 also promotes an increase in permeability of the brain-blood-barrier via increased astrocytic aquaporin-4 (AQP-4) expression and microglial activation, and this may have an impact remyelination as well. Since HMGB1 is implicated in increased blood-brain-barrier permeability via increased astrocytic AQP-4 expression and microglial activation, (Manivannan et al., 2021) this may also affect remyelination. Aside from its potential effects on remyelination, it is also possible that HMGB1 could promote axonal growth in instances of traumatic axonal injury with an intact myelin sheath (**Figure 1B**). Indeed, the early description of 'amphoterin', the original name given to HMGB1, was of a protein that promoted neurite outgrowth and axonal growth (Manivannan et al., 2020, 2021).

Drawing on evidence from other neurological diseases and the role of HMGB1 signaling across different physiological and pathological contexts points toward its potential as a therapeutic target for WM repair following TBI. However, several unanswered questions remain regarding aspects of HMGB1 signaling and traumatic WM injury. Facets of HMGB1 signaling that must be clarified include the influence of isoforms, local concentration, target receptors, and heterocomplex formation (Manivannan et al., 2021). Three different HMGB1 isoforms exist, based on redox status: disulfide HMGB1, fully reduced HMGB1, and oxidized HMGB1. Post-injury neuro-inflammation is thought to be mediated by disulfide HMGB1 and fully reduced HMGB1 but the role of oxidized HMGB1 remains less clear. Identifying the actions of each isoform is essential to avoid the negation of potentially beneficial effects. The actions of HMBG1 seem to depend on both concentration and context. This is demonstrated by the promotion of NSPC migration at lower concentrations and by the neurotoxic effects that become apparent at higher concentrations post-injury. Total inhibition of HMGB1, could, therefore, negatively impact the prospects of repair. Whether such a dual role exists with respect to axonal regrowth, OL survival, or OPC recruitment remains to be determined. For the same reason, HMGB1 signaling should be targeted in a receptor-specific manner. RAGE, TLR-2, and TLR-4 receptor expression on OPCs and oligodendrocytes, and the downstream effects of activation should be better described. In addition, indirect effects mediated by necrotic neurons and glial cells should also be taken into account. Finally, HMGB1 is renowned for its ability to form heterocomplexes with other cytokines, which may result in amplification of the neuro-inflammatory response or, on the contrary, support protective anti-inflammatory effects.

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