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Neuroinflammation in the Evolution of Secondary Injury, Repair, and Chronic Neurodegeneration after Traumatic Brain Injury

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Abstract

The "silent epidemic" of traumatic brain injury (TBI) has been placed in the spotlight following investigations and popular press coverage of athletes and returning soldiers with single and

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DECLARATION OF INTERESTS

The authors declare no competing interests

repetitive injuries; however, treatments to improve the outcome for patients with TBI across the spectrum from mild to severe TBI are lacking. Neuroinflammation may cause acute secondary injury after TBI, and it has been linked to chronic neurodegenerative diseases. Despite these findings, anti-inflammatory agents have failed to improve outcomes in clinical trials. We therefore propose in this review a new framework for future exploration of targeted immunomodulation after TBI that incorporates factors such as the time from injury, mechanism of injury, and secondary insults in considering potential treatment options. Structured around the dynamics of the immune response to TBI – from initial triggers to chronic neuroinflammation – the ability of soluble and cellular inflammatory mediators to promote repair and regeneration versus secondary injury and neurodegeneration is highlighted, with knowledge from human studies explicitly defined throughout this review. Recent advances in neuroimmunology and TBI-responsive neuroinflammation are incorporated, including inflammasomes, mechanisms of microglial polarization, and glymphatic clearance. In addition, we identify throughout this review where these findings may offer novel therapeutic targets for translational and clinical research, incorporate evidence from other brain injury models, and identify outstanding questions in the field.

Keywords

traumatic brain injury; inflammation; secondary brain injury; repair; neurodegeneration; microglial activation

I. INTRODUCTION AND OVERVIEW

The Centers for Disease Control estimates 1.7 million people suffer traumatic brain injury (TBI) in the United States each year and 5.3 million are living with TBI-related disability.[1] This may grossly underestimate the scope of the epidemic, particularly for mild TBI (mTBI)[2], and globally the incidence of TBI appears to be increasing.[1] TBI and mTBI are “signature injuries” of the wars in Iraq and Afghanistan, primarily due to blast exposure from conventional and improvised explosive devices, and can similarly represent consequences of civilian terrorist attacks. In addition, TBI has now been linked to post-traumatic stress disorder, memory deficits, chronic traumatic encephalopathy (CTE), and chronic neuroinflammation.[3]

The inflammatory reaction to TBI was thought to occur solely through peripheral immune mediators entering via a disturbed blood brain barrier (BBB); it is now recognized as a robust and complex interaction between central and peripheral cellular and soluble components influenced by patient age, sex, mechanism of injury (focal, diffuse, blast), degree of injury (mild, repetitive mild, severe), secondary insults (hypoxemia, hypotension), therapeutic interventions, and genetic variability. TBI leads to early resident microglial activation and peripheral neutrophil recruitment, followed later by infiltration of lymphocytes and monocyte-derived macrophages.[4] Simultaneously, pro- and anti-inflammatory cytokines vie to promote and terminate the post-traumatic neuroinflammatory response, and chemokine signaling results in the activation and recruitment of immune cells towards the lesion.[5–9]

This post-traumatic inflammation may be beneficial, by promoting clearance of debris and regeneration, and/or harmful, mediating neuronal death and progressive neurodegeneration (Figure 1). Several multicenter clinical trials have been conducted with therapies shown in pre-clinical and single center trials to have beneficial anti-inflammatory effects. Unfortunately, each trial failed to show benefit; several therapies were deleterious.[10–17] We therefore propose in this review a new framework to guide future preclinical and clinical trials to optimize the immune response to TBI:

1. Limit the acute pro-inflammatory response to the level needed for clearance of debris and danger signals.
2. Promote an anti-inflammatory and pro-regenerative immune phenotype.
3. Prevent the development of chronic neuroinflammation and return to normal function.

Using this framework, we review the dynamics of the immune response to TBI, progressing from initiation of acute inflammation by danger signals and early inflammatory mediators, to subacute inflammation occurring days to weeks after injury, and lastly to chronically activated elements of the immune system which may remain active for months to years and have been linked to the development of traumatic encephalopathies. Mechanisms that balance pro-inflammatory and pro-reparative immune activation are discussed, as well as potential for therapies to promote beneficial aspects of inflammation. We discuss recent discoveries in immunology and our current understanding of the role these processes and systems may play in the immune response to TBI. Acknowledging the limitations of TBI models[18,19], we incorporate a comprehensive review of what is known from human studies over the past two decades of TBI research; though, notably, limited human data are available of mTBI. Lastly, considering the current knowledge of post-traumatic neuroinflammation we propose new areas for advancing translational and clinical research.

II. ACUTE AND SUBACUTE NEUROINFLAMMATION

A. Triggers–DAMPs, Mitochondrial stress, Excitotoxicity, Vascular Injury

Cellular membrane disruption as a result of primary mechanical insult or secondary injury causes release of damage associated molecular patterns (DAMPs) capable of triggering and amplifying neuroinflammation (Table 1). Examples include DNA and RNA, high mobility group box 1 (HMGB1), S-100 proteins, adenosine triphosphate, uric acid, lysophospholipids, and lipid peroxidation-derived carbonyl adducts of proteins, among others.[4,20,21] In response, tumor necrosis factor (TNF)α, interleukin (IL)-6 and IL-1β are up-regulated rapidly by local glial cells and infiltrating immune cells[22] and represent early effectors that drive post-traumatic neuroinflammation (Table 2).

The dual nature of inflammation was demonstrated in experimental models investigating the role of TNFα and inducible nitric oxide synthase (iNOS) after TBI. TNFα is linked to brain edema, BBB disruption, and recruitment of leukocytes.[9] However, TNFα$^{-/-}$ mice had impaired motor function and larger lesions at 4 weeks after injury, despite showing early neuroprotection.[23] Similarly, although TBI increased iNOS expression in the brain with

multiple pro-inflammatory and neurotoxic effects, genetic or chemical iNOS blockade resulted in significantly worsened spatial memory 2–3 weeks after injury.[24]

Cell death via programmed necrosis, such as necroptosis through TNFα mediated RIP kinase activation,[25,26] may lead to a vicious cycle of necrosis→membrane disruption→ DAMP release→necrosis and amplification of inflammation. The prototypical DAMP, HMGB1, is increased in cerebrospinal fluid (CSF) of patients after severe TBI and is associated with elevated intracranial pressure (ICP) in adults and poor outcome in children.[27,28] HMGB1 is a structural DNA-binding protein that regulates transcription by stabilizing nucleosomes under normal conditions.[28] It can be released from cells by membrane disruption or actively secreted by monocytes/macrophages and signals through receptor for advanced glycation end products and toll like receptor 2 (TLR2)/TLR4 receptors to increase production and release of cytokines.[25]

One mechanism of cytokine production triggered by DAMPs is via activation of the inflammasome complex. Binding to intracellular pattern recognition receptors such as the NOD-like receptor containing an N-terminal pyrin domain (NLRP) family or absent in melanoma (AIM) leads to auto-activation of caspase-1 and processing of pro-IL-1β and pro-IL-18 to their active forms.[29,30] Relatively few inflammasome complexes are expressed in the brain: NLRP1 and AIM2 in neurons,[31–33] NLRP3 in astrocytes[33] and microglia are present in both mice and humans.[34–36] In patients, NLRP1 and caspase-1 are increased in the CSF after severe TBI and are associated with unfavorable outcomes.[33] In mice, neutralization of the NLRP1 and NLRP3 inflammasomes attenuated IL-1β processing and reduced lesion volume.[31,32] Inflammasome-dependent cytokine production also contributes to disease progression in mouse models of multiple sclerosis, Alzheimer's disease, and amyotrophic lateral sclerosis.[34,37,38] However, it remains unclear which inflammasome complexes are the primary producers of IL-1β and IL-18 after TBI, and whether neurons, microglia or astrocytes are the key cellular mediators of inflammasome-mediated tissue damage.

Concurrent with the release of DAMPs, a massive increase in extracellular glutamate (and other excitatory amino acids)[39–41] may occur and lead to excitotoxic neuronal injury via activation of neuronal glutamate receptors, such as N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, followed by Ca^{++}-dependent degeneration.[42] Elegant interactions between inflammatory mediators and glutamate signaling have been demonstrated in mice, including: 1) TNFα and IL-1β mediated changes in cell surface expression, distribution, and function of NMDA and AMPA receptors, 2) NMDA receptor induction of inflammatory gene expression, and 3) TNFα and IL-1β mediated reduction in astrocytic glutamate transporters resulting in impaired glutamate clearance from the synaptic cleft.[43] NMDA receptor blockade is therefore an attractive therapeutic strategy, however, antagonists have failed in clinical TBI trials due in part to a limited therapeutic window, off-target neurotoxicity, and as a result of inhibiting normal synaptic function and plasticity.[44] In response to TBI and glutamate toxicity, high levels of the endogenous neuroprotectant adenosine is produced from breakdown of adenosine triphosphate and mRNA.[45] Activation of the adenosine A1 receptor after TBI has anti-excitotoxic[46] and anti-inflammatory effects in mice,[47] however systemic

administration of adenosine to patients may result in bradycardia and hypotension. A variety of adenosine related strategies are being actively investigated to mitigate excitoxicity and various facets of acute and chronic neuroinflammation.[48]

Mitochondrial dysfunction and reactive oxygen species (ROS) generation, caused by direct and indirect injury after TBI, has also been identified as a trigger of neuroinflammation.[49] Translocation of the phospholipid cardiolipin from the inner to outer mitochondrial membrane, shown to occur after experimental TBI, tags damaged mitochondria for mitophagy but may also be a final pathway for inflammasome activation.[50,51] Failure of mitophagy and resultant cell death can lead to release of mitochondrial DAMPs as reported for mitochondrial DNA after TBI in children.[52] These mitochondrial danger signals produce local and systemic responses by the interaction with receptors on immune cells: mitochondrial DNA by TLR9 on dendritic cells and N-formyl peptides by formyl peptide receptor-1 on neutrophils.[53] Membranes with mitochondrial cardiolipins on their surface are engulfed via cluster of differentiation 36 (CD36)-dependent phagocytosis.[54] There is a paucity of data regarding CD36-mediated inflammatory response after TBI, however, CD36 plays a beneficial role in neurological outcome in patients with intracranial hemorrhage.[55]

In addition to inflammatory triggers released from neurons and glia, trauma to the vasculature can lead to leakage of blood components into the cerebral parenchyma, including complement and the potent neurotoxin and immune modulator cell-free hemoglobin (fHb).[56] Complement factors have been detected in brain tissue[57] and CSF[58] of patients within hours of severe TBI, and have been found to correlate with BBB permeability. Although required for normal wound healing, evidence from murine models[59–61] suggests acute dysregulation of complement may cause secondary injury after TBI (for review:[62]). Inhibition of alternative complement pathway[63] or the membrane attack complex[64,65] in a weight-drop model of TBI in mice reduced neurodegeneration and axonal loss, and improved neurologic outcomes. Also released into contused areas of brain, fHb-haptoglobin complexes are cleared by CD163 receptors on microglia and macrophages with resultant differentiation to an anti-inflammatory phenotype.[66] If the haptoglobin-CD163 pathway is dysfunctional or is outcompeted by the amount of fHb present, fHb and its breakdown products heme and iron can induce direct neuronal toxicity by generating ROS and scavenging nitric oxide (NO).[67] This pathway is implicated in the development of post-traumatic epilepsy in rodents.[68] CSF levels of soluble CD163 and the iron-binding protein ferritin are increased after TBI in children and correlate with injury severity and unfavorable outcome.[69] Thus, inflammation triggered by fHb and its degradation products could be therapeutic targets after TBI.

Several of the biochemical and molecular mechanisms of secondary injury listed above have been reported in blast-induced mTBI. Characterized by axonal, periventricular, and hippocampal neuronal injury, blast-induced mTBI is associated with cytokine and chemokine release, adenosine production (likely from mRNA breakdown), and activation of microglia.[70,71] Promising neuroprotective effects were demonstrated with the anti-inflammatory drug minocycline in this model.[72]

B. Cellular mechanisms regulating acute neuroinflammation following TBI

i. Dynamics of cellular response to TBI—The first circulating immune cells to infiltrate the CNS after trauma are neutrophils, which typically peak in mice within 24–48 hours before rapidly declining.[73,74] Diapedesis between endothelial cells is dependent on binding of integrins to vascular adhesion molecules, and within 4 hours of experimental TBI the expression of neutrophilic vascular adhesion molecules endothelial (E)-selectin (CD62E) and intracellular adhesion molecule-1 (CD54) is increased on endothelium of the injured hemisphere.[75] Administration to mice of antibody to the CD11d/CD18 integrin, located on cell surface of neutrophils and monocytes, reduced leukocyte infiltration to the CNS as well as the systemic inflammatory response to TBI (Box 1).[76,77] Chemokine gradients are established (e.g. C-C motif chemokine ligand 2 [CCL2]) that attract monocytes from the circulation to injured brain where they differentiate into macrophage subpopulations distinguished by relative cell-surface expression of the chemokine receptors, C-C motif chemokine receptor 2 (CCR2) and CX3CR1 (inflammatory monocytes: $CD11b^{+}CD45^{hi}CCR2^{+}Ly6C^{hi}$ | patrolling monocytes: $CD11b^{+}CD45^{hi}CX3CR1^{+}$)[78]. Chemokines and their receptors play several crucial roles in response to TBI, and the reader is referred to several excellent reviews on this topic: [8,9]. Studies of monocyte infiltration in mice have demonstrated accumulation within the lesion through 3 days post-injury.[79,80] Dendritic cells (DCs), T lymphocytes and natural killer cells are similarly recruited during this period,[81] but at lower numbers.

Box 1

Systemic Inflammatory Response Syndrome and the Compensatory Anti-Inflammatory Response

In this review we focus primarily on the neuroinflammatory response to TBI. A systemic inflammatory response syndrome (SIRS) and compensatory anti-inflammatory response have also been described after isolated TBI that may increase risk of nosocomial infection or multiple organ dysfunction. Via the sympathetic and parasympathetic nervous system, glymphatic and lymphatic clearance, hypothalamic-pituitary-adrenal axis, and disrupted blood-brain-barrier, there are several pathways for CNS injury to affect the peripheral immune response. In addition, therapeutic agents routinely used in neurocritical care such as sedatives, antiepileptics, and hyperosmolar agents may affect peripheral immune function. Age appears to be an important factor, for example early neutrophilia in adults is associated with significantly greater oxidative burst activity[219] whereas neutrophils in children with TBI have significantly reduced ROS generation.[220] Perhaps the most important factor influencing the characteristics of the systemic inflammatory response to TBI is time from injury. Although few studies have carefully studied the time course of peripheral immune function, there appears to be a marked immunosuppressed state at ~ 1 week out from TBI that corresponds to the time of peak nosocomial infection rate. For excellent review and future directions, the reader is referred to: [221].

Concurrently within the CNS, astrocytes, a vital regulator of CNS inflammation, undergo reactive astrogliosis characterized by morphological and functional adaptations including

up-regulation of GFAP and production of cytokines and chemokines that further recruit and activate immune cells (for review: [82,83]). YKL-40, a marker of reactive astrocytes, is significantly elevated in the CSF of adults with severe TBI on day 2 and peaked on day 4 post-injury (Table 3). Microglia undergo a similar transformation in morphology and function with an initial peak approximately 7 days post-injury.[66,84,85]

ii. Pro and anti-inflammatory roles of microglia—The concept of post-traumatic neuroinflammation as a "double-edged sword"[86], with both beneficial and injurious effects, has recently been expanded to include the function of microglia.[87,88] Similar to peripheral macrophages, microglia respond to changes in their microenvironment to become polarized along an activation spectrum ranging from classical M1-like to alternative M2-like (Figure 2).[89] This concept has evolved from the canonical M1/M2 subset classification to reflect mixed-phenotypes and the functional plasticity of tissue macrophages / microglia to changes in the microenvironment. Stimulation by DAMPs, free radicals, or pro-inflammatory cytokines such as interferon-(IFN)γ induce a M1-like phenotype characterized by production of pro-inflammatory cytokines (e.g. IL-1β, TNFα), chemokines (e.g. CCL2, CXCL9), ROS generation, and reduced phagocytic activity.[89–91] Although M1-like 'pro-inflammatory' cells are often presumed to be harmful, a well-regulated M1-like response may be neuroprotective after TBI. An exaggerated or prolonged M1-like response, however, can lead to secondary brain injury and drive a self-propagating hyperinflammatory state.[92,93] The M2a-like 'alternative' phenotype[89–91], in response to IL-4 and IL-13 stimulation, is associated with production of anti-inflammatory cytokines and increased phagocytic activity.[90,91] The M2c-like 'deactivated' phenotype occurs in response to IL-10, glucocorticoids, or uptake of apoptotic cells regulates tissue repair and remodeling.[90,91] Lastly, the M2b-like 'intermediate' phenotype is stimulated by immune complex exposure or TLR ligands[89–91] and has both pro- (IL-1, IL-6, TNFα) and anti-inflammatory (IL-10) effects.[90,91] The degree to which microglia assume a particular phenotype (or multiple phenotypes) is dependent upon these and other changes in the lesion microenvironment driving complex intracellular signaling pathways, influenced by genetic and epigenetic factors, that may offer additional opportunities for therapeutic intervetion.[91,94]

Microglial polarization has been shown to vary over time and between different TBI models. In mice, activated microglia demonstrate a bimodal increase after focal contusion with an initial M2-like peak at 7 days followed by an M1-like peak at 21–28 days; though, the bulk of activated microglia have mixed M1-/M2-like activation markers.[81,84,93] In diffuse brain injury, M1-/M2-like polarization dynamics are strikingly different, likely due to altered cellular immune responses that include reduced neutrophil infiltration and restricted macrophage/microglial accumulation to white matter regions that incurred greatest damage. Diffuse brain injury results in transient increases in IL-1β, TNFα, and CD14 expression in the cortex and hippocampus of mice as early as 4 hours post-injury that return to baseline by 72 hours.[95] In addition, iNOS+/Arginase1+ microglia/macrophages are also increased at 24 hours post-injury,[96] indicating that diffuse injury also up-regulates mixed M1- and M2-like activation markers. However, the functional role of M1-/M2-like phenotypes in axonal injury and repair following diffuse brain injury remains to be elucidated.

iii. Links to adaptive immune response—The adaptive immune response mediated by T cells and B cells can strongly influence microglia phenotype and function, but the role of the adaptive immune system after TBI remains rather unclear. T cells infiltrate injured tissues after CNS injury and sequestration of lymphocytes in lymph nodes by FTY720 administration results in reduced inflammation and better recovery in rodents with spinal cord injury.[97–99] However, FTY720 also acts directly on CNS cells complicating data interpretation. On the other hand, mice deficient in T cells (due to deficiency of RAG or MHCII genes) have worse outcomes in CNS injury models,[100,101] suggesting a dominant neuroprotective effect of T cells.

Somewhat counter-intuitively, activation of autoimmune T cells in mice that provoke myelin- targeted encephalitis provides protection from secondary neurodegeneration in CNS injury, coined ‘protective autoimmunity’.[101–104] One mechanism for this protection may be T cell production of neurotrophic factors that act on neurons and astrocytes to promote survival and repair.[105,106] T cells are required for normal CNS development, as mice deficient in T cells show cognitive and behavioral developmental abnormalities, suggesting that T cells also contribute to the development and perhaps maintenance of the healthy brain.[107] As well as regulating the M1/M2-like balance, T cell-produced IL-4 protects neurons through potentiation of neurotrophin signaling.[100] Unusually, IL-4-mediated T cell protection of injured CNS tissue does not appear to require antigen-specific receptor activation of T cells, and neurons directly induce IL-4.[100] The IL-1 family alarmin IL-33 is released from damaged cells, and is also neuroprotective after CNS injury in mice.[108] IL-33 is known to act on Th2 cells that produce IL-4;[109] hence IL-33 may provide a link between CNS injury and activation of IL-4 production. An IL-33-responsive population of tissue-resident regulatory T cells has been identified in muscle[110,111] and gut of mice,[112] and contributes to resolution of inflammation and wound repair in those tissues. It is intriguing to speculate that a similar IL-33-Treg axis could operate in human brain after TBI.

Specific mechanisms of T cell mediated protection versus damage need to be precisely targeted to produce benefit. The methods used to invoke a CNS injury-protective autoimmune response are also used to induce the rodent model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE). Th17 cells, named because of their production of IL-17 along with other pro-inflammatory cytokines, are thought to drive inflammatory demyelination of the spinal cord in EAE.[113] Th17 cells and other ‘type 17’ T cells have been associated with myriad autoimmune and inflammatory conditions[114] but have not yet been investigated in TBI. Type-17 responses are promoted by cytokines known to be released after TBI in humans, particularly IL-1β, and induce CXCL8 and neutrophil recruitment. In ischemic stroke, harmful IL-17 is largely produced by ‘type-17’ γδT cells that rapidly infiltrate the injured brain.[115] These cells are strongly influenced by the remote gut environment, as antibiotic-induced dysbiosis of gut microbial flora resulted in protection from stroke that could be linked to reduced frequencies of IL-17^{+} γδT cells.[116] The profound impact of the gut microbiome on peripheral tissue immune responses, including the CNS, is a recurring theme in immunology.[117–119] CNS-gut communication may also influence cognition, mood and anxiety.[120,121] It is thus possible that antibiotic

administration or changes in diet associated with intensive care unit hospitalization after severe TBI could inadvertently alter this gut microbiome-brain-inflammation axis.

Lymphatic drainage of body tissues regulates interstitial fluid and removal of waste products. The lymphatics also support immune surveillance by carrying macromolecules and activated dendritic cells bearing foreign antigens to local lymph nodes where they can be presented to activate the adaptive immune response. This may represent a critical step, since naïve T and B cells typically circulate through lymph nodes via blood and efferent lymphatics but do not enter non-lymphoid tissues until primed. Until recently, the brain was considered an immune privileged site with lack of lymphatic drainage supporting the blinding of the peripheral immune system to ongoing events in that tissue. However, adaptive immune responses are primed and recruited following CNS injury, and waste products must be rapidly cleared from this highly metabolic organ. Technological advances in imaging that allowed for interrogation of brain drainage in closed skull systems have resolved these paradoxes. Two most likely intercepting systems that drain brain tissue have been delineated in mice. The ‘glymphatic system’, describes the astrocyte-regulated convective bulk flow of CSF from the paravascular space through interstitial fluid in an arterial-venous direction.[122,123] This flow allows for rapid entry of small molecules, and perhaps more importantly for fluid drainage and clearance of metabolites, soluble proteins and waste products including beta amyloid from the brain interstitial space.[122,124,125] Glymphatic flow is greatly increased during sleep, associated with increased brain interstitial space volume; this can be partly attributed to mechanical mechanisms as lateral posture in awake mice replicated the increased flow compared to upright posture.[126,127] The second CNS clearance system consists of lymphatics that line the dural sinuses and meningeal arteries.[128,129] These vessels have classical lymphatic architecture and drain to the deep cervical lymph nodes—providing a direct conduit between the brain and the peripheral immune system. They also contain immune cells and macromolecules, mimicking peripheral lymphatics. Brain lymphatics include populations of T cells and B cells,[129] which have presumably migrated through and surveyed the brain tissue.

TBI impairs the glymphatic system drainage in rodent models,[130] resulting in accumulation of damage and waste products such as Tau,[124] and providing a potential link between injury-induced disruption of glymphatic drainage and development of CTE. Inflammatory astrocyte activation may amplify the effects of mechanical damage on glymphatic flow after TBI. Effects of TBI on brain lymphatic drainage to deep cervical lymph nodes have not yet been investigated, but one could envision that TBI would readily alter the associated lymph vessels. Accumulation of waste products due to impaired lymphatic drainage might trigger neuroinflammation by activating pattern recognition receptors on microglia. The interaction between altered lymphatic drainage and neuroinflammation and ensuing long-term consequences therefore warrants further investigation.

C. The impact of secondary insults on the acute inflammatory response to TBI

A critical determinant of outcome after TBI, particularly in severe TBI, is the presence of a concurrent secondary insult such as polytrauma, hypotension, and/or hypoxemia. With severe TBI, secondary insults occur in as many as two-thirds of victims.[131] These insults are

frequently hemorrhagic in nature, compromising perfusion and oxygen delivery to the injured brain.[132] Analysis of >2000 patients with severe TBI revealed a mortality rate of 72% for combined injury vs. 46% for TBI alone.[133]

Despite the importance of polytrauma and secondary insults in TBI, there has been little study of their impact on the cerebral or systemic inflammatory responses in both pre-clinical and clinical investigations. Although one might anticipate that polytrauma and/or secondary insults, by superimposing tissue hypoxemia and/or ischemia onto the traumatic insult, would amplify the local inflammatory response in brain, surprisingly that has not been observed. Instead, both pre-clinical and clinical studies have revealed that second insults shift the cytokine response to a more anti-inflammatory phenotype, amplifying the IL-10 response. Shein et al.[134] studied the impact of a brief period of severe hemorrhagic shock (HS) after controlled cortical impact TBI in mice. The combination of TBI plus HS led to nearly 100-fold and 30-fold increases in serum IL-10 levels vs. TBI or HS alone. Also, six pro-inflammatory cytokines and chemokines, namely IP-10, TNFα, CXCL1, CCL2, CCL3, and CCL11 were all increased in serum after TBI alone but not after TBI plus HS. In addition, animals with combined TBI and HS had lower serum IL-6 vs. TBI alone. However, despite worsening of both long term behavioral and histological outcomes by HS after TBI,[135] the local cytokine and chemokine responses in brain were not appreciably altered.

Clinical data, in general, parallel these pre-clinical findings. Relative to patients with TBI alone, patients with TBI plus polytrauma have increased serum concentration of the anti-inflammatory agents IL-10, IL-1ra, an sTNFr-I and no change in pro-inflammatory cytokines IL-1β and TNFα.[136] Kumar et al.[137] carried out a trajectory analysis of cytokines and adhesion molecules in serum and CSF after severe TBI in 114 adults. Again, serum IL-10 levels were higher among individuals with TBI plus polytrauma versus isolated TBI. It remains unclear, however, if all types of secondary insults produce a similar shift to an anti-inflammatory phenotype, or whether HS confers a unique effect.

Indeed, several forms of peripheral injury such as skeletal fracture or hepatic contusion may increase the circulation of pro-inflammatory cytokines in patients. To address the effect of peripheral injuries on TBI outcomes, combined models that incorporate long-bone fracture have increased in use.[138,139] Shultz et al.[140] reported findings on a mouse model of tibia fracture plus diffuse brain injury in which mice with combined injury exhibited increased anxiety-related behavior and brain atrophy. Associated with these outcomes, the combined injury group had evidence of increased astrogliosis, neutrophil infiltration, and brain tissue IL-1β relative to mice with isolated fracture or TBI. Similarly, the systemic administration of pro-inflammatory mediators IL-1β[141] and lipopolysaccharide[142] in rodent models of diffuse TBI exacerbate the neuroinflammatory response, result in larger contusion volume, and worsen behavioral outcomes. Whether this was mediated directly via binding to receptors on microglia and astrocytes, or through effects such as hypotension or hyperthermia may confound the results of these studies.

Finally, there has been limited study of the impact of secondary insults in mTBI. Titus et al.[143] reported that a brief period of imposed hyperthermia to 39°C beginning 15 min before and continued for 4 hours after mild fluid percussion injury (FPI) in rats, produced cognitive

deficits despite the use of an injury level that was otherwise devoid of cognitive deficits. Cooling back to normothermia at 15 min after TBI prevented development of the deficits. Amplification of neuroinflammation by hyperthermia was implicated. Given the prevalence of concussions during the summer months in training camps for sports such as football, this observation if translated to humans could be important.

D. Inflammation-mediated neurogenesis, gliogenesis, and angiogenesis

Neuronal death after TBI may be mitigated by an increase in neuronal progenitor cell (NPC) proliferation, migration to injured brain regions, differentiation to neurons, and integration into neural networks.[144] Similar to the dual effects of inflammation on secondary brain injury, experimental evidence suggests that inflammatory mediators are a key component of neurogenesis and may support or hinder NPCs at multiple steps. For example, microglia stimulated to an M1-like phenotype with LPS reduce adult hippocampal neurogenesis in the mouse,[145] an effect similarly seen with pro-inflammatory cytokines TNFα, IL-1β, and IL-6.[146] This was reversed by treatment with minocycline[145] or indomethacin.[147] In contrast, M2-like microglia, stimulated by IL-4 or low-dose IFN-γ, release neurotrophins such as insulin-like growth factor-1 (IGF-1) and induce neurogenesis.[148] This effect was seen in mice with addition of a running wheel to the cage after TBI, simulating clinical rehabilitation, which reduced M1-like microglial activation and was associated with increased production of IGF-1 (as well as IL-10 and brain-derived neurotrophic factor) to increase neurogenesis and improve cognitive outcomes.[149] NPCs also express chemokine receptors, such as CCR2 and CXCR4, and chemokines may direct their migration to the area of injury.[150]

Brain atrophy increases over time after TBI,[151–157] and yet most survivors of TBI will show a temporally linked degree of functional recovery.[158] This recovery represents brain plasticity and reorganization, in addition to recovery of function of existing neuronal pathways. Although data from TBI models are limited, experiments modeling other forms of brain injury suggest neural-immune interactions may be critical to forming and strengthening new synaptic connections.[159–161] The degree of activation and the local inflammatory milieu likely define whether any particular cytokine or inflammatory cell type benefits or disrupts brain plasticity. For example, in a GFAP-IL-6 transgenic mouse model, over-expression of IL-6 caused a significant reduction in long-term potentiation (LTP) in the hippocampus.[159] However, when anti-IL-6 is used to block basal levels of IL-6 signaling, there is a significant prolongation of LTP and improved long-term memory.[162] Elevated IL-1β also impairs LTP but surprisingly promotes neurite outgrowth and is synergistic with neurotrophin-3.[163] In rats subjected to repetitive mTBI, activation of microglia was associated with inability to induce LTP, attenuated NMDA-mediated signal, and impaired memory–these effects were not seen after single mTBI.[164]

Microglial polarization and the local inflammatory milieu may also influence repair through activation of angiogenesis and gliogenesis. Although evidence from TBI models is scant, experimental autoimmune and hypoxic/ischemic brain injury suggest M1-like microglia impair oligodendrogensis, oligodendrocyte maturation and viability by a TNFα-dependent mechanism.[94,165] In contrast, M2-like microglia or conditioned media from M2-like

microglial culture promote oligodendrogenesis and remyelination in these models. M2-like microglia may also, via production of pro-angiogenic cytokines (e.g. TGFβ) and growth factors, induce angiogenesis and vascular repair. Whether these findings translate to recovery from TBI in humans is unknown, however treatment with pro-angiogenic growth factors released by M2-like microglia is neuroprotective in mice and promotes neurogenesis and angiogenesis in experimental TBI.[166]

E. Clinical experience of agents impacting acute neuroinflammation

A key question arises, is TBI-responsive neuroinflammation a clinically relevant therapeutic target (Figure 3)? As described above, numerous pre-clinical studies suggest this is the case. However, few clinical trials of therapies primarily targeting inflammation have been reported. Corticosteroids, surprisingly, have been the least successful anti-inflammatory class of drugs in TBI (Table 3). Despite the potent effects on suppressing inflammation, high dose methylprednisolone (5 mg/kg/day),[167] "megadose" dexamethasone (100 mg),[168] "ultrahigh dose" dexamethasone (2.3 g),[169] the aminosteroid tirilazad,[170] and a trial of hydrocortisone and fludrocortisone (primary outcome hospital acquired pneumonia)[171] all failed to demonstrate benefits on neurological outcome. Off-target effects with systemic administration of corticosteroids likely impact outcomes, and in the case of tirilazad, limited brain exposure may have been a confounder.[172]

Other anti-inflammatory strategies have been evaluated in humans including testing of the bradykinin B2 receptor antagonist Anatibant, which produced a trend toward worse outcome in 228 patients enrolled.[173] A provocative randomized controlled trial (RCT) used recombinant human granulocyte colony stimulating factor (G-CSF) to enhance the cellular inflammatory response.[174] Although the primary outcome in this study was the incidence of nosocomial infection, and the number of patients was low, no differences in mortality or hospital length of stay were detected between G-CSF treated and placebo groups, nor was there a difference in primary outcome. Minocycline has shown promise in a phase II trial in patients with spinal cord injury;[175] however, it has not been reported in human TBI and pre-clinical data are equivocal.[176] Of note, all of the studies targeting inflammation after TBI have been performed in adult patients. Given studies showing an association between heightened inflammation and younger age in children with severe TBI, anti-neuroinflammatory strategies may be more impactful in the developing brain.[69,177]

Inferences can also be made extrapolating from clinical trials using multi-faceted therapies with anti-inflammatory consequences. The anti-inflammatory effects of hypothermia were touted as one of its main modes of efficacy.[178] Disappointingly, despite single center studies in adults with severe TBI showing reduction of IL-1β in CSF,[179] multicenter RCTs of therapeutic hypothermia after TBI have failed to show benefit in adults[180,181] or children.[11,12] Progesterone, which blunted the neuroinflammatory response to trauma in mice, was evaluated in two large multicenter RCTs[13,14] of adults with moderate-severe TBI and failed to show benefit in 6-month GOS or mortality.

These clinical studies suggest that non-selective attenuation of the inflammatory response early after severe TBI is not beneficial, or possibly detrimental. The existing literature lacks studies using targeted, single pathway anti-inflammatory strategies in humans, and more

personalized approaches that individualize treatments to genotype, inflammatory biomarkers, timing and duration of therapy, patient age and sex. Identification of specific patient subsets, for example the study by Diamond et al.[182] that identified an IL-1β gene variant associated with risk of post-traumatic epilepsy, to target enrollment criteria for clinical studies may favorably influence their success.

Furthermore, it is unclear whether inhibiting inflammation after mild or repetitive mild TBI acutely may prevent chronic sequelae such as CTE. Raising the key questions, does a single exposure, or multiple exposures, to TBI prime the brain for chronic neuroinflammation, and accordingly would impacting the inflammatory response early after mTBI, or targeting the immune response late, represent clinically relevant approaches? Finally, from a therapeutic perspective, given the aforementioned benefit of strategies mimicking rehabilitation on neuroinflammation neurogenesis and cognitive outcome, it is possible that optimal enhancement of beneficial aspects of neuroinflammation, rather than inhibiting detrimental effects, could represent a more successful avenue for future clinical investigation.

III. CHRONIC NEUROINFLAMMATION

A. Chronic neuroinflammation after TBI: innocent bystander or driver of pathology?

As discussed, after TBI an acute inflammatory response is elicited that one might expect would resolve to a resting state, prepared for the next inflammatory trigger. However, in a subset of patients, chronic neuroinflammation may develop and last for years after injury.[183–186] The proportion of patients in whom chronic inflammation will develop, the dominant triggers and intracellular pathways propagating inflammation, and genetic susceptibilities to chronic inflammation are under active investigation.

An examination of autopsy specimens from patients surviving >1 year after TBI, and in cases up to 18 years, revealed a significant increase in amoeboid microglia in subcortical white matter tracts versus control tissue.[184,187] Activated microglia were observed in 28% of the autopsies and was associated with thinning of the corpus callosum.[184,187] These findings are supported by positron emission tomography (PET) imaging studies using translocator protein (TSPO) ligands, which likely bind activated microglia, to examine chronic neuroinflammation in TBI survivors.[185,188] In one study, diffuse [^{11}C]R-PK11195 (TSPO ligand) binding was found in adults with moderate to severe injury up to 17 years later in areas remote to the trauma including thalamus, putamen, and the occipital cortex.[185] Inflammation in the thalamus was associated with more severe cognitive impairments.[185] The 2nd generation TPSO ligand [^{11}C]DPA-713 was used to study retired National Football League (NFL) players with self-reported history of career concussions. The supramarginal gyrus and right amygdala exhibited ligand binding to levels greater than seen in age matched controls.[188] Serum cytokines may also demonstrate a chronic immune activation state after TBI. For example, a prospective TBI biomarker study reported chronically elevated expression of TNFα in serum after TBI and association of increased TNFα with unfavorable long-term neuropsychiatric outcomes.[189]

Experimental studies substantiate the clinical evidence of a chronic inflammatory state after TBI, and indicate underlying molecular mechanisms and potential therapeutic

strategies.[164,190–194] Chronic microglial activation with cell surface markers MHC II, CD68, and NADPH oxidase (NOX2) is seen one year after moderate to severe contusion.[92] These markers would indicate M1-like phenotype, with pro-inflammatory cytokine production and reduced phagocytic activity that would be less effective at protective functions such as Aβ clearance. Over the course of the year, mice demonstrate progressive neurodegeneration with enlarging lesion volume, persistent oxidative stress, demyelination, and cognitive impairments. Chronic neuroinflammation is also observed in several repetitive mTBI models. Like the acute inflammatory response to mild repetitive injury, microglia are characteristically localized to white matter tracts and may be seen in those regions bordering degenerating axons with associated neurobehavioral changes 12–18 months after injury.[164,191,195–198]

There is interest, therefore, in the development of pharmacologic and non-pharmacologic approaches to reduce chronic neuroinflammation after TBI as a therapeutic strategy, greatly expanding the window for targeted interventions. This concept has been examined in pre-clinical studies, for example, with the selective metabotropic glutamate receptor 5 agonist (RS)-2-chloro-5-hydroxyphenylglycine (CHPG), previously shown to reduce microglial activation[199] and improve functional recovery[200] when given acutely after trauma. Mice given CHPG at 1 month after focal brain injury had improved neurological recovery, decreased neuroinflammation, arrested lesion expansion, sparing of white matter, and reduced neurodegeneration at 4 months.[201] Similarly, although with a more generalized anti-inflammatory approach, administration of the phosphodiesterase inhibitor ibudilast on days 30–34 after FPI in rats reduced anxiety-like behavior and gliosis at 6 months.[202] Exercise regimens that simulate physical rehabilitation may modulate neuroinflammation and promote release of neurotrophic factors after TBI. Piao et al.[149] found that a 4-week treatment with voluntary exercise attenuated IL-1β gene expression and chronic microglial activation, increased production of IL-10 and neurotrophic factors, improved behavioral outcomes, and reduced lesion volume.[149] Importantly, the authors compared two start dates for the exercise regimen which was only effective if delayed to 5 weeks after injury and was potentially pro-inflammatory when initiated at 1 week post-injury. Thus, accumulating pre-clinical research indicates that chronic neuroinflammation and related neurodegeneration can be treated weeks after TBI, which suggests exciting potential for clinical translation of delayed anti-inflammatory therapies.

B. Progressive neurodegeneration following single and repetitive brain trauma

Accelerated neurodegeneration and CTE may occur following single or repetitive TBI, as has been reported in cases of athletes[203] and military personnel[3] with high incidence of head trauma and concussion.[204] TBI increases the risk of developing dementia in some patients, specifically non-Alzheimer's dementia, years after the initial injury.[205–209] Recently, the role of chronic inflammation in the pathophysiology of neurodegenerative disorders has attracted considerable attention[210,211] and led investigators to speculate about the role of post-traumatic neuroinflammation in mediating neurodegeneration, non-Alzheimer's dementias, and CTE. Cases of CTE associated with repetitive mTBI have also shown activated microglia in perivascular regions of subcortical white matter and throughout the brain as the disease progresses.[3,212,213] To date, however, comprehensive studies of neuroinflammation

in patients with CTE have yet to be reported. Webster et al.[214] investigated an early intervention with progesterone to prevent neurodegeneration after repetitive mTBI in rodents. Animals were administered three mild diffuse injuries, each separated by 5 days, and randomized to vehicle or progesterone for 15 days after the first injury. At 12-weeks post-treatment, a chronic time point in rodent TBI models, progesterone-treated rats exhibited improved neurocognitive outcomes, reduced brain atrophy, and attenuated neuroinflammation compared to repetitive mTBI plus vehicle. Based on these promising data, additional preclinical studies are warranted.

It remains unclear whether persistent inflammation initiates the characteristic neuropathology – formation of neurofibrillary tangles, phosphorylated TAR DNA-binding protein 43 (TDP-43) accumulation, and Aβ deposition – and should be targeted. Or, if accumulation of these abnormal proteins triggers the inflammatory response, though perhaps one ill-suited to restore normal function. Further research is required to advance our understanding of critical mechanisms underlying the chronic pathologies of TBI, including chronic neuroinflammation, and their relationship to development of neurodegenerative disease (Figure 4). Advances in clinical TBI neuroimaging, including use of selective PET ligands for amyloid,[215,216] tau,[217,218] and neuroinflammation,[185,188] may clarify the mechanisms driving chronic neurodegeneration after TBI, and provide opportunities to develop targeted therapies for the long-term sequelae.

IV. CONCLUDING REMARKS

Advances in our understanding of TBI-responsive neuroinflammation have led to exciting new questions (Box 2), identified new therapeutic targets and expanded the time frame in which to consider treating. Clinical trials with therapies modulating inflammation after TBI are in their infancy – even in severe TBI – and therapies targeting neuroinflammation after mTBI in patients are completely unexplored. Nevertheless, thus far it appears that treating all patients with TBI using a broad-acting anti-inflammatory agent has not shown benefit in RCTs. Clearly, there is a need to define inflammatory phenotypes of our patients based on injury characteristics such as patient age, sex, genetic predisposition, presence or absence of secondary insults, and serum / CSF / imaging biomarkers. Such an approach will allow us to answer questions posited in our initial framework, including: how to target inflammation for clearance of debris, who will benefit from therapies to promote reparative aspects of inflammation, and when should therapies targeting chronic inflammation be initiated. This approach should be combined with enhanced pre-clinical trials, which 1) incorporate multiple injury models, injury severities, and secondary insults 2) define clinically-relevant therapeutic window(s) and treatment durations 3) expand outcomes to examine both harmful and protective aspects of inflammation and include acute- and chronic endpoints. Coupled with important new trials design strategies such as adaptive design, the result of this approach will be clinical trials targeting specific patients with personalized immunomodulatory treatments that we hope will reduce secondary injury, enhance repair, and improve patient outcomes.

Box 2

Outstanding Research Questions / Unmet Needs

- What is the level of acute inflammation needed for clearance of debris? How can it be determined?
- Does the M1-/M2-like paradigm translate to human brain injury? Are there injury severity or regional differences in phenotype?
- Can autoreactive adaptive immune responses be harnessed for benefit in TBI? Do Th17/IL-17 adaptive responses contribute to neurodegeneration in TBI?
- Does the extent of damage to the CNS lymphatic drainage systems following TBI play a role in defining the magnitude of long-term neuroinflammation?
- What mechanisms prime reactivity of glia acutely after TBI and sustain their immune activation for weeks, months and years? Will delayed interventions that modulate chronic microglial activation be effective for treatment?
- How can we determine that the reparative processes are no longer beneficial and how should we facilitate return of the inflammatory process to a normal state?
- Can therapeutic trials be targeted to inflammatory phenotypes or biomarkers?
- Imaging biomarkers: There is an urgent need to identify new stable and selective PET ligands or MRI based methods to image neuroinflammation.

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Review Criteria

For review of human data on TBI neuroinflammation, we searched PubMed for articles published in English from January 1950 to March 2016 using the following query: “traumatic brain injury” or “closed head injury” or “closed-head injury” or “head trauma” AND ((Case Reports[ptyp] OR Clinical Study[ptyp] OR Clinical Trial[ptyp] OR Clinical Trial, Phase I[ptyp] OR Clinical Trial, Phase II[ptyp] OR Clinical Trial, Phase III[ptyp] OR Clinical Trial, Phase IV[ptyp] OR Controlled Clinical Trial[ptyp] OR Comparative Study[ptyp] OR Meta-Analysis[ptyp] OR Multicenter Study[ptyp] OR Randomized Controlled Trial[ptyp]) AND Humans[Mesh]). We selected articles reporting clinical findings of neuroinflammation in human traumatic brain injury. Reference lists were used, as well as these authors’ expertise, for inclusion of additional relevant studies.

Key Points

- Traumatic brain injury is a significant public health issue that is increasing in global incidence and recognition in popular press, particularly for mild, repetitive, and blast injuries.
- Neuroinflammation, triggered by release of endogenous danger signals and innate immune activation, is crucial to recovery after traumatic brain injury; however, a dysregulated immune response may lead to secondary injury.
- The activity of microglia and infiltrating macrophages and adaptive immune cells is driven by extracellular injury signals and intracellular molecular pathways that may represent novel therapeutic targets.
- Design of preclinical and clinical trials studying immunomodulatory interventions should account for changes in neuroinflammation that occur over time, between injury type / severity / secondary injury, and across patient characteristics such as age, sex, and genetic variability.
- Chronic neuroinflammation, which may develop and last for years after traumatic brain injury, is being investigated as a link to accelerated neurodegeneration and chronic traumatic encephalopathy.

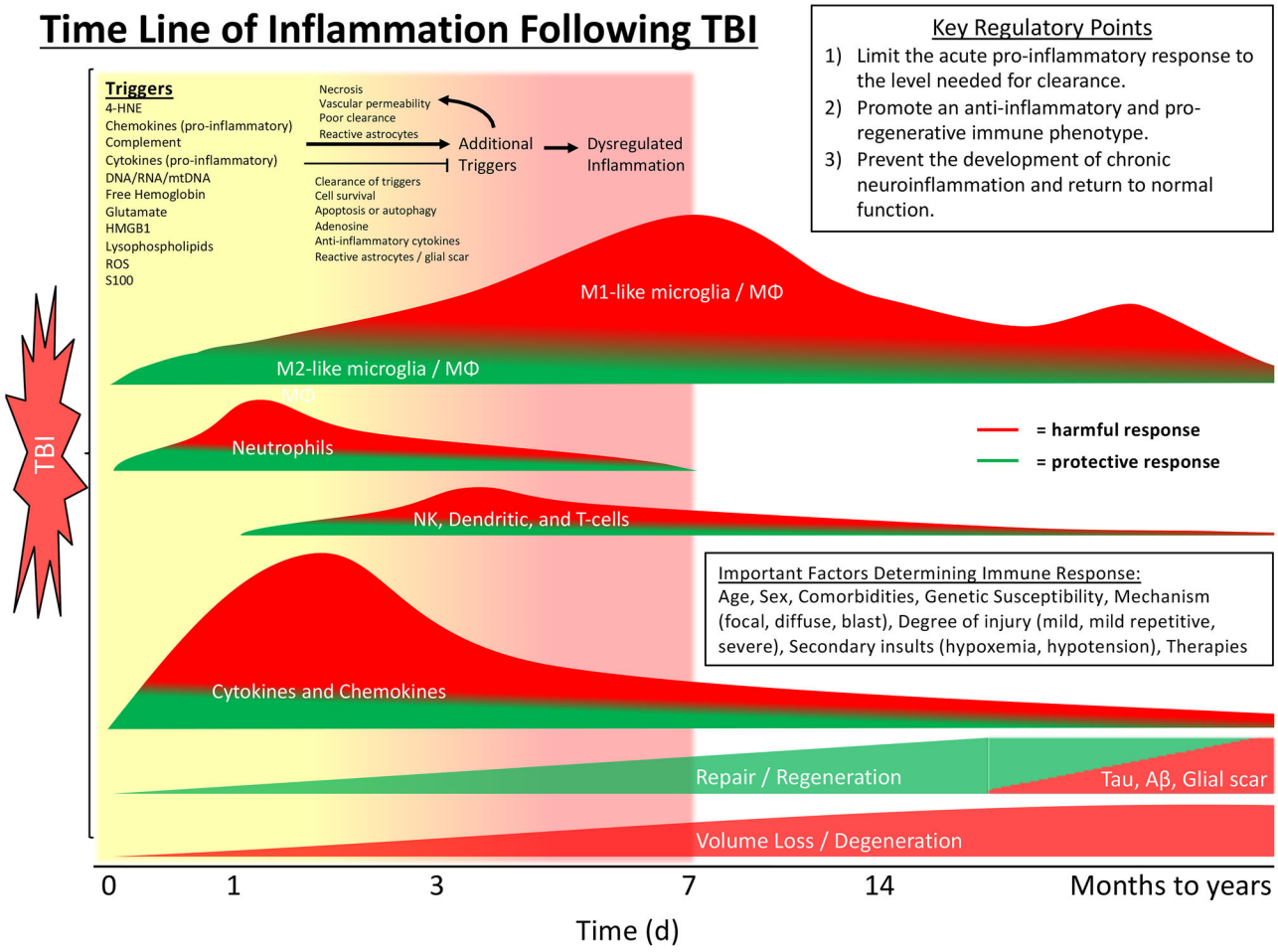

Figure 1. Overview of Neuroinflammation after TBI

Primary mechanical injury to the CNS may cause cell membrane disruption, vascular rupture, and BBB damage followed by secondary reactions involving ionic imbalance, release of excitatory amino acids, calcium overload, and mitochondrial dysfunction - ultimately culminating in cell death pathways. Primary and secondary injury lead to release of DAMPs, cytokines, chemokines, activation of microglia and astrocytes, and recruitment of circulating immune cells. These immune responses largely overlap temporally. The inflammatory response is crucial to clearance of debris, repair, and regeneration after TBI. However, dysregulated inflammation can lead to additional acute and chronic brain injury. Abbreviations: CNS, central nervous system; BBB, blood brain barrier; DAMP, damage-associated molecular pattern; TBI, traumatic brain injury

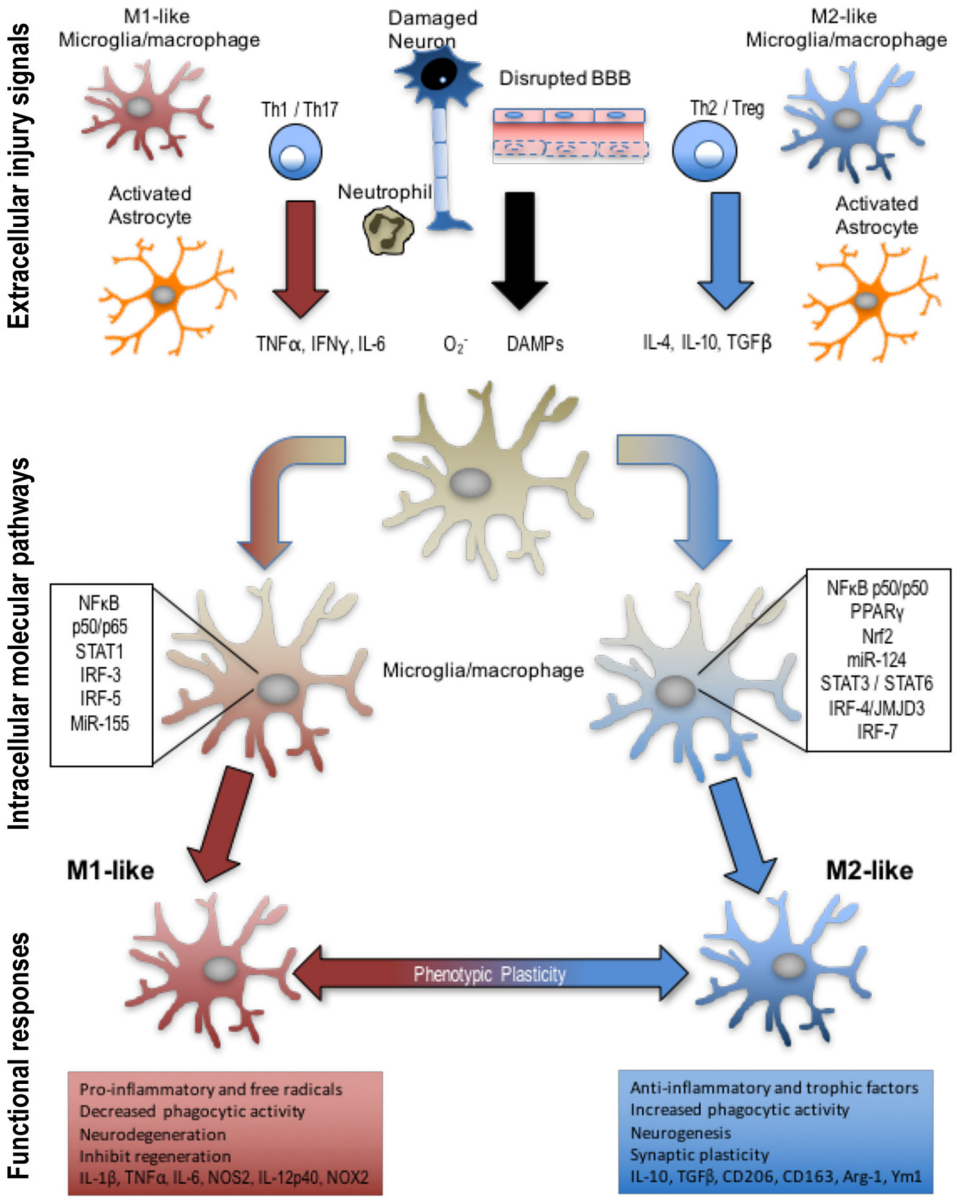

Figure 2. Extracellular injury signals and intracellular molecular pathways control polarization of microglia and macrophages following TBI

Molecular signals from injured tissue can drive phenotypic and functional responses in microglia/macrophages after TBI. DAMPs released by injured neurons, pro-inflammatory or oxidative mediators released by infiltrating immune cells including TNFα, IFNγ, IL-6, and O^{2-} can polarize cells towards an M1-like phenotype. M1-like populations are characterized by expression of proteins such as IL-1β, TNFα, IL-6, NOS2, IL-12p40, and NOX2. Molecular pathways that regulate the M1-phenotype include STAT1, IRF-3/5, NFκB p50/p65 and miR-155, among others. M1-like microglia and macrophages release pro-inflammatory factors and free radicals that promote chronic neuroinflammation, oxidative stress and neurodegeneration, and inhibit regeneration. In response to anti-inflammatory and neurotrophic signals microglia and macrophages can be polarized towards an M2-like

phenotype, characterized by expression of proteins such as CD206, CD163, Arginase 1, FCγR, Ym1, IL-10, and TGFβ. Molecular pathways that regulate M2-like phenotypic transitions include STAT6/3, IRF-4/7, NF-κB p50/p50, Nrf2 and miR-124, among others. M2-like microglia and macrophages release anti-inflammatory and trophic factors that resolve inflammation. They also have increased phagocytic activity, and improve brain repair by modulating neurogenesis, axonal regeneration, synaptic plasticity, and angiogenesis. Microglia and macrophages demonstrate significant plasticity and can switch between M1- and M2-like phenotypes. Moreover, it is recognized that following TBI they present mixed phenotypes during the acute phase post-injury, and transitions to an M1-like dominant phenotype in the chronic phase after TBI.

Abbreviations: TBI, traumatic brain injury; DAMP, damage-associated molecular pattern; TNF, tumor necrosis factor; IFN, interferon; IL, interleukin; NOS, nitric oxide synthase; NOX, nicotinamide adenine dinucleotide phosphate oxidase; STAT, signal transducer and activator of transcription; IRF, interferon regulatory factor; CD, cluster of differentiation; TGF, transforming growth factor; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells

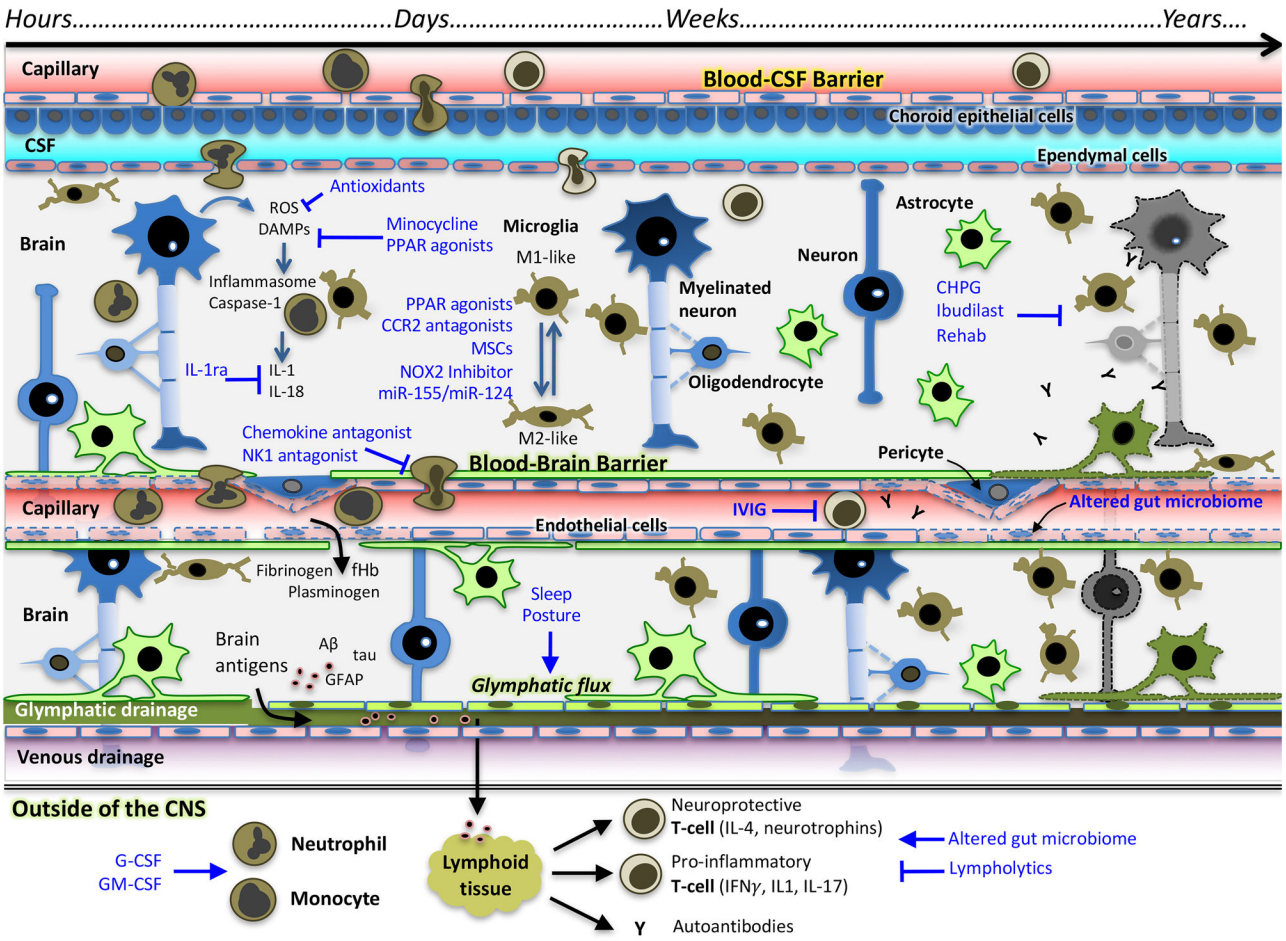

Figure 3. Novel therapies for TBI targeting inflammation at different time points from injury

Therapies targeting TBI-responsive inflammation may be effective at different time points depending on the therapeutic target(s). Similarly, design of pre-clinical and clinical trials of anti-inflammatory agents should note that inflammation causing secondary injury at one time-point may be protective at others. Initially, inflammation triggered by release of DAMPs and ROS generation can be blocked through the use of antioxidants, minocycline, and PPAR agonists, among others. Inflammasome activation will cause release of IL-1β, the action of which can be inhibited at IL-1 receptors with IL-1ra (Anakinra). Over the next several hours-days, invasion of CNS by circulating immune cells will contribute to neuroinflammation, and this process can be inhibited by therapies such as NK1 antagonism and chemokine antagonists. Microglial polarization to M2-like phenotype has been shown to be neuroprotective. M1-like phenotype, which peaks ~7 days from injury, is proinflammatory and associated with secondary injury. The M2-like phenotype can be promoted by MSC, PPAR agonists, and CCR2 antagonists, among other possibilities. The adaptive immune response peaks days after injury. T-cells must be primed to enter CNS – this may be inhibited by therapies such as IVIG. Additionally, alterations in gut microbiome may affect the relative number of pro- and anti-inflammatory T-lymphocytes. Glymphatic clearance may be impaired after TBI, which may lead to impaired clearance of pro-inflammatory mediators. Investigations are ongoing to determine ways to improve

glymphatic flow, however it has been shown to be maximized during sleep. Chronic microglial activation may develop and lead to chronic neurodegeneration, encephalopathy, and dementia. Activation of the mGluR5 on microglia, such as with CHPG, attenuates M1-like microglial activation. Rehabilitation and exercise have also been shown to reduce M1-like microglial activation.

Abbreviations: TBI, traumatic brain injury; CSF, cerebrospinal fluid; ROS, reactive oxygen species; DAMP, damage-associated molecular pattern; BBB, blood brain barrier; PPAR, peroxisome proliferator-activated receptor; IL, interleukin; MSC, mesenchymal stem cell; CHPG, (RS)-2-Chloro-5-hydroxyphenylglycine; IVIG, intravenous immunoglobulin; IFN, interferon; fHb, free hemoglobin; GFAP, glial fibrillary acidic protein

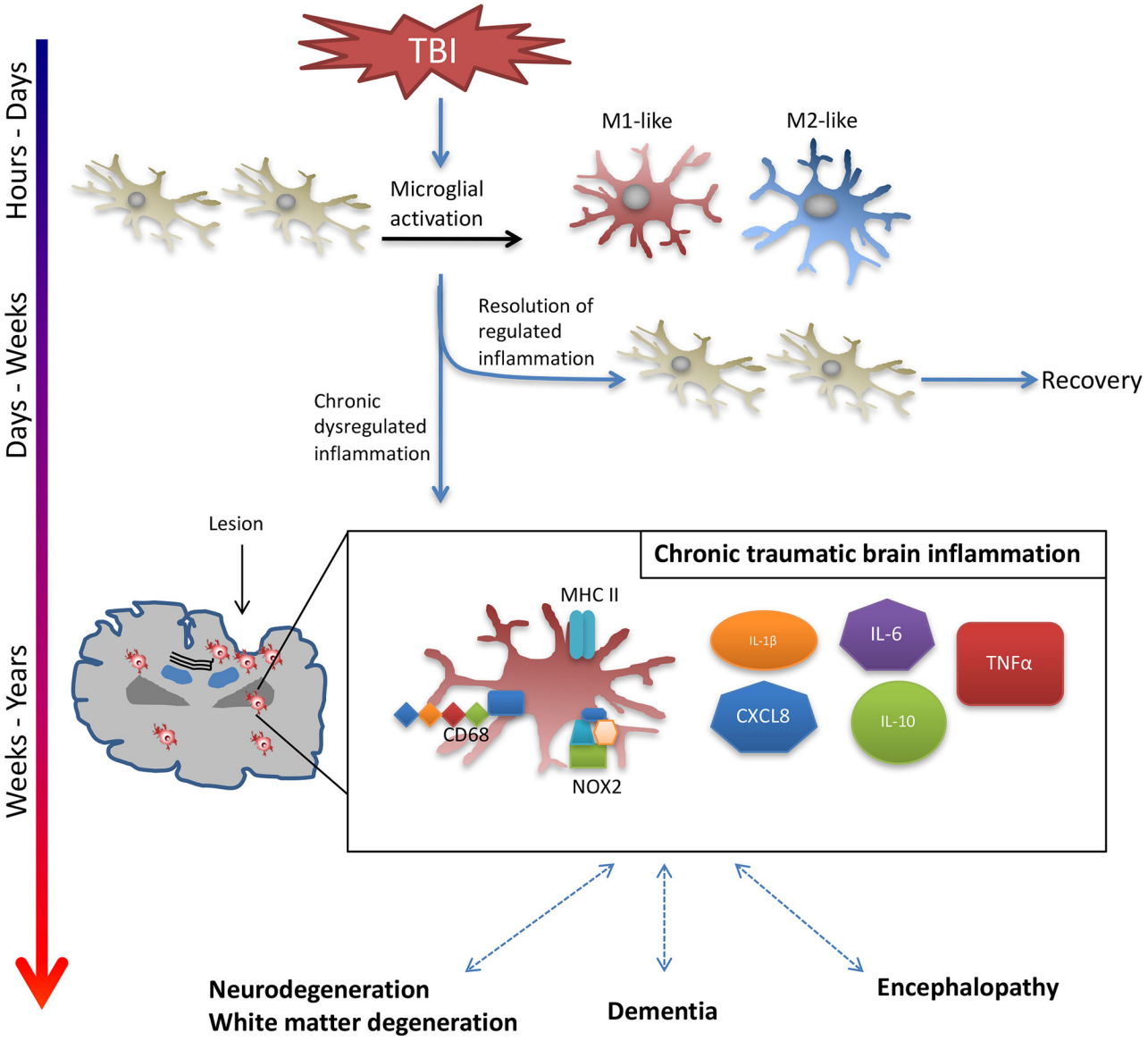

Figure 4. Chronic neuroinflammation contributes to chronic neurodegeneration, dementias, and encephalopathy after TBI

Neuroinflammation and microglial activation are key mediators of repair and recovery from TBI. However, recent clinical and laboratory data have shown that TBI can cause persistent neuroinflammation and microglial activation, in some cases lasting many years, and lead to chronic neurodegeneration, dementia, and encephalopathy. Prospective studies of TBI biomarkers in adults with severe TBI have shown that serum levels of IL-1β, IL-6, CXCL8, IL-10, and TNFα are chronically increased. Experiments in animal models have demonstrated persistently increased numbers of microglia expressing MHC II, CD68, and NOX2 at the margins of the lesion and in the thalamus at 1-year post-injury associated with oxidative stress and white matter disruption. These inflammatory findings have been correlated with chronic neurodegeneration, the development of dementia, and

encephalopathies – which may subsequently cause additional inflammation in a self-perpetuating deleterious feedback mechanism.

Abbreviations: TBI, traumatic brain injury; IL, interleukin; CD, cluster of differentiation; TNF, tumor necrosis factor

Table 1

Human Studies of Neuroinflammation after TBI: Triggers / Brakes

Inflammatory mediator	Tissue / Fluid	Time Course	Association with clinical outcome(s)	Other	Ref
Triggers / Brakes					
Adenosine	CSF ECF	Increased within hours of injury Rapidly decline over 12–24h	Pediatric: No association with outcome after severe TBI Adult: Higher level in patients that died after severe TBI	A1AR gene variants associated with post-traumatic epilepsy Increased CSF adenosine associated with jugular vein desaturation	222–224
Complement	CSF	Peak day 1 post-injury, decline day 2–7	Adult: Increased MAC level associated with BBB dysfunction in severe TBI	C3 and Factor B increased relative to controls	58,225
	Tissue	Increased in tissue resected 2–82h post-injury	Unknown	C3-mRNA also detected in penumbra, suggesting a contribution of local synthesis	57
Glutamate	CSF ECF	Multiple courses described Most common peak day 1, decline day 2–3	Pediatric: Higher level associated with poor 6mo-GOS in severe TBI Adult: Increase in ECF glutamate associated with poor outcomes	Increased glutamate associated with age < 4 and child abuse Hourly levels not affected by transient hemodynamic or ICP change	41,226–228
HMGB1	CSF	No change over time	Pediatric: Higher level associated with unfavorable 6 month GOS in severe TBI Adults: Increased level associated with high ICP	Not associated with age or mechanism of injury	27,28
	Tissue	Translocated to cytoplasm of cells in contused area at 30min – 1d Localized to cytoplasm of phagocytic microglia at 2–20d	Unknown	HMGB1 receptor, RAGE, expression also increased in contused area in phagocytic microglia	229
NLRP1	CSF	Unknown	Adult: Higher level in patients with unfavorable 5 month GOS in moderate to severe TBI	Adaptor protein ASC also increased in CSF of TBI patients	230
Caspase-1	CSF	Unknown	Adult: Higher level in patients with unfavorable 5mo-GOS in moderate to severe TBI	Identified by p20 subunit suggests activated form of enzyme	230
	Tissue	Pro-caspase-1 is cleaved to active form on day 1	Unknown		231
mtDNA	CSF	Peak day 1, declined on day 3, though still above control	Pediatric: Higher levels associated with unfavorable 6mo- GOS in severe TBI	Positively correlated with HMGB1 level	52

Abbreviations: TBI – traumatic brain injury; CSF – Cerebrospinal fluid; ECF – Extracellular fluid; A1AR – adenosine receptor A1; MAC – membrane attack complex; ICP – intracranial pressure; HMGB1 – high mobility group box 1; RAGE – receptor for advanced glycation endproducts; NLRP1 – NACHT, LRR and PYD domains-containing protein 1; GOS – Glasgow Outcome Scale; mtDNA – mitochondria DNA; BBB – blood brain barrier; GCS – Glasgow Coma Scale

Table 2

Human Studies of Neuroinflammation after TBI: Cytokines / Chemokines

Inflammatory mediator	Tissue / Fluid	Time Course	Association with clinical outcome(s)	Other	Ref
Cytokines / Chemokines					
TNFα	CSF ECF	Peaks early on day 1 Prolonged elevation in patients with hypoxia	Mixed results Most studies show no association with outcome Higher 6h level may be associated with ICP and outcome	Soluble TNF receptor levels peak later (day 4–9) TNF allele variants associated with clinical outcome	5,7,136,189,232–237
	Tissue	Increased above control within 17min of injury	Unknown	4-fold increase in mRNA within 17 min	6
IFN-γ	Tissue	Increased above control within 17min of injury	Unknown	Behind IL-6, was second highest cytokine concentration measured	6
IL-1β	CSF ECF	Peak day 1–2, decrease day 2–4 IL-1ra consistently much higher than IL-1β	Pediatric: Mixed results – no correlation with outcome vs. worse outcome Adults: Mixed results – no correlation with outcome vs. worse outcome and elevated ICP Adults: High ECF IL-1ra and IL-1ra/IL-1β ratio associated with good outcome	IL-1RN (IL-1ra gene) polymorphisms associated with cerebral hemorrhage after TBI Principle component analysis of microdialysis data shows close covariance with TNFα	136,237–244
	Tissue	Increased above control 6–122h after injury	Unknown	5-fold increase in mRNA at 6–122h	6
IL-6	CSF ECF	Marked increase after TBI Peak day 1, decline day 2–3	Pediatric: Mixed results – no correlation with outcome vs. worse outcome Adults: Higher CSF or ECF level associated with favorable GOS	Pediatric: 2-fold greater in children with intermittent vs. continuous CSF drainage Adults: Associated with NGF level. CSF added to astrocyte culture induced NGF production, blocked by anti-IL6 antibody	237–239,243,245–251
	Tissue	Increased above control within 17min of injury	No relationship to ICP, brain oxygenation, edema	20-fold increase in mRNA levels at 6–122h	6,243
IL-10	CSF ECF	Peak day 1, decline day 2–3 May have second or third peak of lower magnitude Later peak in ECF, day 4–6	Pediatric: Higher level associated with mortality in severe TBI Adults: Mixed results – no correlation with outcome vs. worse outcome	Higher level associated with age < 4y No change relative to control in contused tissue	5,6,136,237,242,243,245,248,252
IL-12p70	CSF ECF	Increased day 2–3 Peak day 3–5	Pediatric: No association with outcome	35-fold greater in ECF than plasma	237,238
GM-CSF	Tissue	Increased above control 6–122h after injury	Unknown	Expression in CSF prolonged by hypoxia	6,253
TGF-β	CSF	Peak day 1, gradually decrease over 21 days	Adult: No associated with outcome	Associated with BBB permeability	254

Inflammatory mediator	Tissue / Fluid	Time Course	Association with clinical outcome(s)	Other	Ref
CCL2 (MCP-1)	CSF ECF	Peak day 1, decrease and plateau by day 4 but remains elevated relative to controls through day 10	Unknown	10-fold higher in ECF relative to plasma	237,255,256
	Tissue	mRNA detected 3h – 15d after injury	Unknown	Chemokine most consistently and strongly expressed mRNA in evacuated contusion	256
CCL3 (MIP1α)	CSF ECF	Increased day 1–3, no clear peak	Pediatric: Not associated with outcome	No association with age, gender, GCS	237,238
	Tissue	mRNA detected 3h – 15d after injury	Unknown	Intermediate levels of mRNA detected	256
CXCL8 (IL-8)	CSF ECF	Peak day 1. Marked decline day 2–3. Above control up to 108h after injury	Pediatric: Higher level strongly associated with mortality Adult: Higher level associated with BBB permeability, but not mortality	No association with age, gender, or GCS 10 to 20-fold higher in CSF and ECF relative to plasma	237,238,248,257
	Tissue	mRNA detected 3h-15d after injury	Unknown	139-fold increase in mRNA at 6–122h	6,256

Abbreviations: TBI – traumatic brain injury; CSF – Cerebrospinal fluid; ECF – Extracellular fluid; TNF – tumor necrosis factor; IFN – interferon; IL – interleukin; NGF – nerve growth factor; GOS – Glasgow Outcome Scale; TGF – transforming growth factor; BBB – blood brain barrier; CCL – C-C motif chemokine ligand; CXCL – C-X-C motif ligand; GCS – Glasgow Coma Scale

Table 3

Human Studies of Neuroinflammation after TBI: Cellular Mediators

Inflammatory mediator	Tissue / Fluid	Time Course	Association with clinical outcome(s)	Other	Ref
Cellular mediators					
Microglia	Tissue (path)	Proliferating microglia observed after 72h Peak 3mo Extensive amoeboid CR3/43 and CD68 immunoreactive cells seen in ~25% of cases surviving >2wk and up to years after TBI, particularly with DAI	Unknown	Associated with ongoing white matter degeneration. May be influenced by IL-1 genotype	6,183,184,187,258
	Tissue (image)	Increased [^{11}C]DPA -713 binding to TPSO 24–42yr since NFL play Increased [^{11}C](ROPK11195 binding to TPSO in thalami, putamen, occipital cortex, and internal capsule years after moderate to severe TBI	Adults: Higher PK binding associated with more severe cognitive impairment	Observed atrophy of hippocampus	185,188
Astrocytes	CSF	YKL-40 (reactive astrocytes) elevated day 1, peak day 4	Adults: Trend to association with outcome	Associated with CSF IL-1β, TNFα, and CRP	259
	Tissue	Increased GFAP in ipsilateral and contralateral cortex at 6–122h	Unknown	Anti-GFAP antibodies detectable in serum	6,260

Abbreviations: TBI – traumatic brain injury; CD – cluster of differentiation; DAI – diffuse axonal injury; IL – interleukin; TPSO – translocator protein; NFL – National Football League; CSF – Cerebrospinal fluid; TNF – tumor necrosis factor; CRP – C-reactive protein; GFAP – glial fibrillary acidic protein

Table 4

Human Studies of Neuroinflammation after TBI: Selected Clinical Trials

Clinical Trials								
Therapy	**Effects on Inflammation**	**Study / Design**	**N**	**Dose**	**Primary Outcome**	**Secondary Outcomes**	**Comments**	**Refs**
Anatibant	Block bradykinin signaling, prevent BBB disruption	"Brain Trial" Multicenter RCT	228 adults with GCS ≤ 12	Low (10mg load, 5mg/d), Mid (20mg load, 10mg/d), High (30mg load, 15mg/d) vs. placebo	No difference in incidence of serious adverse events	Trend towards harm in discharge GCS, DRS, and HIREOS	Recruitment paused due to DSMB concerns Terminated due to withdrawal of funding	173
Cyclosporin A	Reduces T-cell counts and activation	Single center RCT	38 adults with GCS ≤ 8	5mg/kg over 24h vs. 10mg/kg over 48h vs. placebo	No difference in T-cell counts in blood	No difference in incidence of infection	Reduced lymphocyte count on admission associated with worse outcome and increased respiratory infections	261
Dexanabinol	Inhibitor of TNFα NMDA antagonism	Multicenter RCT	861 adults with ICP monitoring, GCS motor 2–5	Single 150mg dose vs. placebo, within 6h of injury	No difference in GOS-E at 6mo	No difference in adverse events		262
Erythropoietin	Decreases production of pro-inflammatory cytokines and chemokines IL-1 and TNF block EPO production	"EPO-TBI" Multicenter RCT	606 adults with moderate and severe groups - GCS 9–12 and GCS ≤ 8	40,000IU weekly x 3wk vs. placebo	No difference in GOS-E at 6mo	No difference in mortality or DVT	Mortality reduced in patients without mass lesions, no increase in good outcome Many patients did not receive full course	16
		Multicenter RCT	200 adults with TBI, unable to follow commands	500IU/kg q24h x 3 doses or 500IU/kg x 1 dose then weekly x 2wk vs. placebo	No difference in GOS-E at 6mo	No difference in mortality, ARDS, or infection	Original dosing regimen (daily x 3 doses) stopped by FDA due to safety concern (higher mortality in stroke trial)	15
G-CSF	Stimulate stem cells to produce granulocytes	Multicenter RCT	61 adults GCS ≤ 8 expected to require mechanical ventilation > 3d	75μg/d vs. 300μg/d x 10d vs. placebo	Dose-dependent increase in neutrophil count	No difference in mortality, LOS, or nosocomial infection Significant decrease in bacteremia incidence	Adverse events similar between groups Included patients with cerebral hemorrhage as well as TBI	174
Hypertonic saline	Improve T-cell function Reduced TNF-α and IL-10	Multicenter RCT	1331 adults with severe TBI	250mL bolus of 7.5% saline / 6% dextran 70 vs. 7.5% saline vs. 0.9%	No difference in GOS-E at 6mo	No difference in survival at 28d	Terminated early for futility	263

Clinical Trials								
Therapy	**Effects on Inflammation**	**Study / Design**	**N**	**Dose**	**Primary Outcome**	**Secondary Outcomes**	**Comments**	**Refs**
				saline initiated pre-hospital				
Hypothermia	Humoral and cellular immune response is temperature dependent Decreased neutrophil accumulation in CNS Decreased IL-1β, possibly by reduction in temperature-dependent Caspase-1 activity	"Cool Kids" Multicenter RCT	77 children with GCS ≤ 8	32–33°C vs. 36.5–37.5°C for 48–72h	No difference in mortality at 3mo	No adverse events	Terminated early for futility	264
		Multicenter RCT	225 children with GCS ≤ 8	32.5°C vs. 37°C for 24h	No difference in 6mo PCPC score	No difference in mortality	Trend to increased mortality and significantly higher incidence of hypotension and vasoactive agent use during rewarming (+0.5°C q2h)	11
		"NABIS: H II" Multicenter RCT	97 adults with GCS 4–8 Enrolled within 2.5h of injury	32–34°C vs. 35.5 – 37°C for 72h	No difference in 6mo outcome	No difference in mortality	Terminated early for futility Improved outcomes in patients with evacuated hematoma treated with hypothermia	181
		Eurotherm3235 Multicenter RCT	387 adults with severe TBI and ICP > 20mmHg despite stage 1 treatments	Cooled to 32–35°C followed by stage 2 if ICP remained high vs. stage 2 treatments alone	Lower GOS-E in hypothermia group	Stage 3 treatments (coma, craniectomy) were required in more patients of the control group	Terminated early for safety concerns	180
Anakinra	Block IL-1 signal transduction	Single Center RCT	20 adults with GCS ≤ 8	100mg S.Q. q24h x 5 doses	Increased IL-1ra in CNS ECF within 6h	PCA of 42 cytokine multiplex demonstrated separation between treatment and placebo groups	Subsequent study showed patients receiving rIL-1ra had cytokines biasing to M1-like microglial phenotype Control patients were relatively biased to M2-like phenotype	265,266
Probiotics	Modify lymphocyte polarization prior to CNS infiltration	Single Center RCT	52 adults with GCS 5–8	10^9 bacteria for 21d vs. placebo	No difference in 28d mortality	On day 21, treatment group had higher IFNγ and lower IL-10 and IL-4	Trend to decrease late VAP Unclear what effects might be in CNS	267
Statins	Inhibit expression of vascular adhesion molecules and chemokines to reduce leukocyte infiltration of CNS	Single Center RCT	21 adults with GCS 9–13	Rosuvastatin 20mg/d x 10d vs placebo	Modest decrease in amnesia and disorientation time	No difference in disability at 3mo	Subsequent study showed reduction in plasma TNFα at 72h	268,269

Clinical Trials								
Therapy	**Effects on Inflammation**	**Study / Design**	**N**	**Dose**	**Primary Outcome**	**Secondary Outcomes**	**Comments**	**Refs**
	Associated with reductions in pro-inflammatory cytokines							
Steroids	Inhibit leukocyte activation and infiltration Modulate cytokine release Progesterone decreased upregulation of IL-1β, TNFα, and complement factors 3 and 5 Reduce M1-like microglial activation	"CRASH" Multicenter RCT	10,008 adults with GCS ≤ 14	Methylprednisolone Load: 2g over 1h Maintenance: 0.4g/h x 48h vs placebo	Higher risk of mortality at 2wk in steroid group	Higher risk of mortality at 6mo in steroid group	Terminated early for safety concerns	167
		Multicenter RCT	161 adults with TBI and coma	Dexamethasone 100mg vs placebo	No difference in survival	No difference in 6mo outcome		168
		Multicenter RCT	163 adults with GCS 9–12 and 957 adults with GCS 4–8	Tirilazad 10mg/kg within 4h of injury and q6 x 5d vs placebo	No difference in 6mo GOS	No difference in mortality	Significant differences in pretreatment hypotension and hypoxia related to inter-center variation	170
		"Corti-TC" Multicenter RCT	336 adults with GCS ≤ 8	Hydrocortisone (200mg/day, tapered) + Fludrocortisone (50μg/day) x 10d vs. placebo	No difference in incidence of hospital acquired pneumonia	No change when analyzed according to presence or absence of adrenal insufficiency	Study may have been underpowered due to lower than expected incidence of hospital-acquired pneumonia	171
		"ProTECT III" Multicenter RCT	882 adults with GCS 4–12	Progesterone Infusion started within 4h of injury, duration 96h	No difference in 6mo-GOS	No difference in mortality	Terminated for futility	14
		"SyNAPSe" Multicenter RCT	1195 adults with GCS ≤ 8	Progesterone 0.71mg/kg load then 0.5mg/kg/h infusion for 119h	No difference in 6mo-GOS	No difference in mortality		13

Abbreviations: TBI – traumatic brain injury; BBB – blood brain barrier; RCT – randomized controlled trial; GCS – Glasgow coma scale; DRS – Disability Rating Scale; HIREOS – head injury related early outcomes score; DSMB – data safety monitoring board; TNF – tumor necrosis factor; NMDA – N-methyl-D-aspartate; ICP – intracranial pressure; IL – interleukin; EPO – erythropoietin; DVT – deep venous thrombosis; ARDS – acute respiratory distress syndrome; LOS – length of stay; PCPC – pediatric cerebral performance category; CNS – central nervous system; ECF – extracellular fluid; PCA – principal components analysis; GABA – gamma-aminobutyric acid; IFN – interferon; VAP – ventilator associated pneumonia