Inflammation and Neuroprotection in Traumatic Brain Injury

Kara N. Corps, DVM, DACVP; Theodore L. Roth, MS; Dorian B. McGavern, PhD

**IMPORTANCE** Traumatic brain injury (TBI) is a significant public health concern that affects individuals in all demographics. With increasing interest in the medical and public communities, understanding the inflammatory mechanisms that drive the pathologic and consequent cognitive outcomes can inform future research and clinical decisions for patients with TBI.

**OBJECTIVES** To review known inflammatory mechanisms in TBI and to highlight clinical trials and neuroprotective therapeutic manipulations of pathologic and inflammatory mechanisms of TBI.

**EVIDENCE REVIEW** We searched articles in PubMed published between 1960 and August 1, 2014, using the following keywords: traumatic brain injury, sterile injury, inflammation, astrocytes, microglia, monocytes, macrophages, neutrophils, T cells, reactive oxygen species, alarmins, danger-associated molecular patterns, purinergic receptors, neuroprotection, and clinical trials. Previous clinical trials or therapeutic studies that involved manipulation of the discussed mechanisms were considered for inclusion. The final list of selected studies was assembled based on novelty and direct relevance to the primary focus of this review.

**FINDINGS** Traumatic brain injury is a diverse group of sterile injuries induced by primary and secondary mechanisms that give rise to cell death, inflammation, and neurologic dysfunction in patients of all demographics. Pathogenesis is driven by complex, interacting mechanisms that include reactive oxygen species, ion channel and gap junction signaling, purinergic receptor signaling, excitotoxic neurotransmitter signaling, perturbations in calcium homeostasis, and damage-associated molecular pattern molecules, among others. Central nervous system resident and peripherally derived inflammatory cells respond to TBI and can provide neuroprotection or participate in maladaptive secondary injury reactions. The exact contribution of inflammatory cells to a TBI lesion is dictated by their anatomical positioning as well as the local cues to which they are exposed.

**CONCLUSIONS AND RELEVANCE** The mechanisms that drive TBI lesion development as well as those that promote repair are exceedingly complex and often superimposed. Because pathogenic mechanisms can diversify over time or even differ based on the injury type, it is important that neuroprotective therapeutics be developed and administered with these variables in mind. Due to its complexity, TBI has proven particularly challenging to treat; however, a number of promising therapeutic approaches are now under pre-clinical development, and recent clinical trials have even yielded a few successes. Given the worldwide impact of TBI on the human population, it is imperative that research remains active in this area and that we continue to develop therapeutics to improve outcome in afflicted patients.
Traumatic brain injury (TBI) is a diverse group of brain injuries that vary in cause, severity, pathogenesis, and clinical outcome. As public awareness of TBI and its consequences increases, there is a growing need to understand the underlying mechanisms and develop therapeutic interventions. Within the United States alone, nearly 2 million people sustain a TBI annually, contributing to one-third of all injury-related deaths. Individuals from all nations and demographics are affected, including athletes, military troops, and individuals with unintentional injuries. Traumatic brain injury is a significant cause of mortality in children and young adults, and the incidence in older individuals has increased with the average life span. Mild TBI (mTBI) is the most frequent type diagnosed, typically resulting in post-TBI survival. Traumatic brain injury is suspected to contribute to a variety of chronic degenerative processes, including chronic traumatic encephalopathy, Alzheimer disease, and Parkinson disease. Traumatic brain injury is initiated by the application of mechanical force to the head, which can occur with or without loss of consciousness. This then triggers a series of cerebral events that depend in part on the nature and location of the injury. A major challenge associated with treating patients with TBI is the diverse pathologic and pathogenic mechanisms that become operational after injuries. For example, TBI often promotes disruption of blood-brain barrier (BBB) integrity and the neurovascular unit, which can result in vascular leakage, edema, hemorrhage, and hypoxia. Other pathologic mechanisms include cell death within the meninges and brain parenchyma, stretching and tearing of axonal fibers, and disruptions at the junctions between white and gray matter, stemming from rotational forces that cause shearing injuries. All these primary pathologic mechanisms are accompanied by cellular and molecular cascades leading to inflammation and additional cell death. This review focuses on our current understanding of the sterile immune reaction to TBI and some clinical successes in treating patients with TBI.

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### Sterile Immune Reaction to TBI

Central nervous system (CNS) resident and peripherally derived inflammatory cells respond quickly to brain injuries and can even participate in the repair process. These responses are commonly referred to as sterile immune reactions. A previous study found that the inflammatory gene expression profile is comparable between mTBI and severe TBI, suggesting a common response to both forms of injury. The acute cellular reaction to TBI includes astrocytes, microglia, monocytes or macrophages, neutrophils, and T cells, which are initially activated in part by purinergic receptor signaling. In the following paragraphs, we describe the inflammatory response to TBI in more detail, focusing specifically on traditional immune cell populations. Sterile immune reactions are at least initially designed to be beneficial but can become detrimental in certain situations.

### Danger Signals

Pathogens can trigger innate immune activation via pathogen-associated molecular pattern molecules, which are conserved structures within a class of microbes recognized by Toll-like receptors or pathogen-recognition receptors. These innate signaling pathways allow plants and animals to respond quickly to invading microbes. However, it is now recognized that tissue damage in the absence of microbial infection can trigger inflammasome and innate immune activation through the release of damage-associated molecular pattern molecules (DAMPs), sometimes referred to as danger signals. Alarmins are endogenous DAMPs released by cells undergoing nonapoptotic death or by cells of the immune system. Some examples of alarmins include HMGB1, S-100 proteins, adenosine triphosphate (ATP), uric acid, DNA or RNA, and interleukin 10, among others. After TBI, alarmins are undoubtedly released, and this triggers a sterile immune reaction designed to restore tissue homeostasis. However, the severity and duration of injury can foster maladaptive immune reactions that become injurious. A previous study found that ATP release and detection via purinergic receptors elicit an acutely neuroprotective inflammatory response after mild cortical injury, but sustained immune activation may not always be beneficial. For example, therapeutic blockade of inflammasome activation reduced innate immune activation and severe TBI lesion size. Thus, additional research is required to better understand the rules that govern pathogenic vs nonpathogenic innate immune reactions after DAMP signaling in the injured brain.

### Purinergic Receptor Signaling

Purinergic receptors are an evolutionarily ancient family of transmembrane molecules that detect ATP, adenosine diphosphate (ADP), or adenosine. The receptors are divided into 2 basic classes based on whether they respond to adenosine (P1 receptors) or ATP or ADP (P2 receptors). Because ATP is a cellular source of energy, it is maintained at a high intracellular concentration during steady-state conditions. After tissue injury, ATP is released from damaged cells, which triggers an immune reaction via purinergic receptor signaling. This reaction can be amplified by pannexin and connexin hemichannels that pump ATP from healthy cells into the extracellular space. Sterile immune reactions generally subside as ATP is converted into adenosine through a 2-step reaction that involves ectonucleoside triphosphate diphosphohydrolase 1 (CD39) and ecto-5’-nucleotidase (CD73). Astrocytes and microglia each express at least one these ectoenzymes, allowing them to dampen ATP-mediated neuroinflammation.

### Microglia

Microglia are highly dynamic CNS resident innate immune sentinels that originate from primitive myeloid progenitor cells during development. Microglia participate in a variety of homeostatic CNS functions, including synaptic plasticity and learning, and are often the first responders to any inflammatory event that occurs within the parenchyma. Microglia mediate neuron removal during development via release of reactive oxygen species (ROS) and can acquire a phagocytic phenotype without an inflammatory response. Microglial expression of genes associated with neuro-
participate in diverse functions, such as phagocytosis, cytokine or chemokine release, antigen presentation, immune modulation, and tissue repair. In the naive brain, there are also populations of specialized macrophages that reside in the meninges, choroid plexus, and perivascular spaces. Their role in TBI pathogenesis is unknown. Another study also found that meningeal macrophages are among the first cells to die after focal cortical injury and may serve as an early source of alarmins and ROS (Figure 1A-C, Figure 2A and B, and Video 1). Monocyte-derived macrophages coming from the blood do not reach peak numbers in the damaged brain of animals and humans until 24 to 48 hours after injury. Monocytes are capable of crossing the blood-cerebrospinal fluid barrier with neutrophils into the injured brain as a result of CCL2 production by choroid plexus epithelium. CCL2 is significantly increased in the cerebrospinal fluid of patients with TBI. Examination of CCL2-/- mice after TBI revealed slight alterations in cytokine expression but no changes in lesion size within the first week of injury. However, when followed for a longer timeframe of 2 to 4 weeks, CCL2-/- mice had improved functional recovery, suggesting a pathogenic role for macrophages during the chronic phase of TBI. Similar results were obtained in CCR2-/- mice after TBI. CCR2 is the receptor for CCL2, and deficiency significantly reduced the number of lesion macrophages and increased hippocampal neuronal densities, spatial learning, and locomotion when measured several weeks after brain injury. Collectively, the data obtained in CCL2 and CCR2 knockout mice suggest that monocyte-derived macrophages play a pathogenic role in the chronic phase after TBI. Additional studies are required to determine whether these cells can participate in brain repair after TBI similar to what has been described in models of spinal cord injury. Whether a macrophage is pathogenic or beneficial after tissue injury likely depends on its state of differentiation.

Neutrophils

Neutrophils are an abundant population of circulating leukocytes that are usually among the first responders to tissue injuries in the periphery and CNS. Neutrophils are often viewed as a proinflammatory cell population but are known to play a vital role in wound healing through their involvement in phagocytosis, metalloproteinase release, and growth factor production. After tissue injury, neutrophils can help prepare the damaged environment for repair. Neutrophils are rapidly recruited to the CNS after TBI and enter through meningeal blood vessels and the choroid plexus. Neutrophils can also facilitate the recruitment of monocytes. A previous study focused on sterile injury of the liver found that ATP released from the damaged tissue induced inflammasome activation in a P2X7-dependent manner. This activation in turn promoted rapid recruitment of neutrophils through release of chemotaxtants (CXCL1 and CXCL2) and formyl peptides that guided these cells to the site of injury. After focal TBI, we observed that neutrophils are similarly recruited in a P2X7-dependent manner and arrive within 1 hour of injury (Figure 2C). Visualization of cellular dynamics and localization by 2-photon microscopy revealed that neutrophils localized primarily to the damaged meninges (instead of the parenchyma), where they swarmed the area and interacted with dead cells. Antagonism of this response by blocking P2X7 signaling increased the amount of cell death in the meninges, suggesting a protective role for neutrophils in the meningeal space after focal cortical injury.

Neutrophils are not always neuroprotective and have the capacity to break down the BBB by releasing metalloproteinases, proteases, tumor necrosis factor α, and ROS. Inflammatory mediators released after brain injury can facilitate this process by inducing a hyperactivated state that allows neutrophils to breach the BBB and enter the CNS. On arrival, neutrophils have the potential to induce neuronal cell death using the same soluble mediators that break down the BBB. A previous study revealed that neutrophils are...
the most abundant cell population in circulation after TBI and cause increased expression of oxidative enzymes indicative of activation. Depletion of neutrophils with anti-Gr-1 antibodies after controlled cortical impact in rodents reduced edema, microglia and macrophage activation, and TBI lesion size, but did not affect vascular leakage at 24 to 48 hours after injury.44 These data reveal that neutrophils can be pathogenic after open-skull cortical impact. However, the contribution of neutrophils to a CNS lesion may depend on their precise localization and state of activation. Open-skull controlled cortical impact is highly disruptive to meningeal architecture and likely favors neutrophil recruitment to the heavily damaged brain parenchyma. These findings contrast with mild closed-skull cortical injury, which maintains meningeal architecture and fosters a more selective pattern of neutrophil recruitment.15 To definitively establish the contribution of neutrophils to TBI pathogenesis, these cells should be evaluated in many different models of brain injury. It is conceivable that their contribution will differ based on the nature of the injury.

Figure 1. Pathogenesis of Traumatic Brain Injury (TBI)

A. Comparison of brain anatomy in the meninges and superficial neocortex before and after focal mild TBI (mTBI). The dura mater contains numerous small vessels that are lined by thin, elongated meningeal macrophages. The subarachnoid space contains vessels, fibroblastlike stromal cells, and cerebrospinal fluid (CSF). The glial limitans, composed of astrocytic foot processes, lies beneath the pia mater and forms a barrier between the CSF and underlying parenchyma. Mild focal brain injury mechanically compresses the meningeal space, compromising vascular integrity and inducing rapid necrosis of meningeal macrophages and structural cells. Leakage of fluid from meningeal blood vessels results in edema, and damaged cells within the meninges release reactive oxygen species (ROS) and adenosine triphosphate (ATP), initiating a sterile immune reaction. B and C. Maximum projections (5-μm wide) are shown in the xz plane of 2-photon z-stacks captured through the thinned skull of CX3CR1GFP/+ mice (original magnification ×20).

B. A representative image of an uninjured mouse reveals the presence of meningeal macrophages (green) in the dura and ramified microglia (green) in the brain parenchyma beneath the glial limitans (white dotted line).

C. Thirty minutes after focal mTBI, meningeal macrophages die and microglia relocate to the injured glial limitans (arrowheads). Skull bone is shown in blue. D and E. Histopathologic analysis of the superficial neocortex by confocal microscopy 8 hours after mTBI (original magnification ×20). D. An uninjured brain is shown for comparison. Dead cells were labeled transcranially with propidium iodide. Cell nuclei are blue. E. A large lesion consisting of numerous dead cells (red) (arrowhead). See Videos 1 and 2. UDP indicates uridine diphosphate.
T Cells

Although T cells play diverse roles in adaptive immune responses and the regulation of inflammation, their role (if any) in TBI pathogenesis is not clear. It has been proposed that autoreactive T cells against CNS antigens, such as myelin basic protein, can be neuroprotective after spinal cord injury. After brain injury, activated T cells are recruited to sites of damage, and ROS release may facilitate this recruitment by activating endothelial barriers. To address the role of T cells in TBI, a previous study examined the response to closed-skull head injury in RAG1 knockout mice that lack mature T and B cells. No difference in any pathologic or neurologic parameters was observed between wild-type and RAG1-deficient mice for up to 1 week. These data suggest that T cells play no role in early TBI pathogenesis. Additional studies are required to determine whether T cells actively participate in chronic TBI lesions (beyond 1 week) and/or the reparative process.

Therapeutic Modulation of TBI Pathogenesis

The pathogenesis of TBI is complex as reflected by the number of clinical trials that have failed to improve outcomes in humans. Rather than focus on the reasons for prior failures, we instead briefly discuss some successes that pertain to mechanisms of pathogenesis and inflammation covered in this review.

The concept of free radical-mediated damage of CNS tissue after injury has existed for several decades. Administration of effective antioxidants has the potential to significantly limit the spread of damage and inflammation if given soon after brain injury. In animal models, a number of previous studies have yielded promising results with antioxidants that neutralize ROS. For example, intravenous administration of the small-molecule free radical scavenger edaravone at 2 and 12 hours after weight drop--induced TBI resulted in significantly reduced inflammation, edema, BBB breakdown, lesion size, and neurologic deficits. Inhibition of NADPH oxidase complex assembly with apocynin also reduced ROS production, BBB breakdown, and neuronal cell death after weight drop--induced TBI. The only caveat of this study was that the apocynin was injected intraperitoneally 15 minutes before injury. Nevertheless, the favorable outcome implicates NADPH oxidase as a potential source of ROS after brain injury.

Using a new model of mild cortical injury, we found that transcranial administration of the antioxidant glutathione at 15 minutes...
or 3 hours after injury significantly reduced inflammation, glial limitations breakdown, and parenchymal (but not meningeal) cell death by up to approximately 70%. Pretreatment with glutathione reduced meningeal cell death by approximately 50%. These data indicate that ROS are a primary inducer of cell death and inflammation after focal brain injury and that an antioxidant can have a major effect on lesion expansion if given early. The advantage of passing a neuroprotective compound directly through the skull bone (transcranial delivery) is that a high local drug concentration can be achieved in the CNS with a limited off-target effect on the periphery.

Previous studies have supported antioxidants as neuroprotective agents in rats and humans, revealing that administration of N-acetylcysteine reduces brain damage and improves recovery after TBI. N-acetylcysteine is the cellular precursor to glutathione. A randomized, double-blind, placebo-controlled clinical trial was performed to assess efficacy in members of the military who experienced mTBI that resulted from blast exposure. Patients who received N-acetylcysteine within 24 hours had significantly improved recovery during a 7-day period when compared with a placebo control group. These findings were corroborated in 2 different rodent models of TBI (weight drop and fluid percussion), which revealed that N-acetylcysteine reversed the behavioral deficits associated with mTBI and moderate TBI. Further studies are needed to determine whether this promising neuroprotective intervention will be efficacious in patients with diverse types of brain injury.

Many clinical trials have been completed or are under way to assess the role of excitotoxic mechanisms in TBI pathogenesis. With the exception of amantadine, all drugs in this class tested to date have not been effective in promoting recovery in patients with TBI. Amantadine is thought to act as an N-methyl-D-aspartate receptor antagonist and an indirect dopamine agonist. When patients with TBI were treated during a 4-week period beginning 4 to 16 weeks after injury, amantadine improved recovery relative to the placebo control. The mechanism underlying this positive effect remains unclear. Prevention of N-methyl-D-aspartate receptor-mediated excitatory damage seems unlikely given that the drug was administered a month or more after the initial injury.

Manipulation of purinergic receptor signaling is another therapeutic approach worth considering. Use of specific purinergic receptor agonists and antagonists should allow therapeutic amelioration of different TBI lesion parameters. A previous study found that microglia responses after mTBI were dependent on P2X4, P2Y6, and P2Y12 receptors, whereas P2X7 signaling was necessary for neurobehavioral abnormalities after controlled cortical impact–induced TBI. A similar protective effect was obtained by blocking P2X7 after spinal cord injury, which was linked to receptor expression on spinal cord neurons. However, P2X7 is also expressed on inflammatory cells, and a previous study found that antagonism of this pathway increased meningeal cell death after mTBI, likely due to diminished neutrophil recruitment. Thus, purinergic receptor modulation can positively affect one CNS environment and negatively affect another. It will therefore be important in future studies to map out the exact contributions of specific purinergic receptors to different TBI lesion parameters before deciding which (if any) are best to target therapeutically in patients.

**Discussion**

The pathogenesis of TBI is initially induced by a mechanical injury that sets into motion a complex secondary reaction mediated by ROS, purines, calcium ions, excitatory amino acids, and DAMPs, among others. This pathogenesis in turn triggers a robust sterile immune reaction that consists of CNS resident and peripherally recruited inflammatory cells. The response is designed to be neuroprotective and promote wound healing but can become maladaptive over time, especially if the lesion remains active for weeks. Among the earliest soluble mediators are ROS and purines. Both are released within minutes of brain injury and initiate an inflammatory cascade. Even after mild focal cortical injury, ROS can damage the glial limitans that separate the meninges and parenchyma, which results in lesion expansion within brain tissue. Vascular damage and leakage represent another early hallmark of TBI pathogenesis that can foster edema, hypoxia, and tissue destruction. After brain injury, the innate immune system quickly mobilizes in response to purines and alarmins, and astrocytes help orchestrate this response by serving as inflammatory amplifiers. Within minutes, resident microglia are among the first to react by fortifying CNS barriers and participating in phagocytic cleanup. Neutrophils and monocytes arrive shortly thereafter and preferentially survey injured meningeal spaces if the CNS architecture remains intact. Focal brain injury elicits an anatomically partitioned immune reaction (at least acutely) with myelomonocytic cells tending to the damaged meninges and microglia responding within the parenchyma. Eventually, myelomonocytic cells can enter the damaged brain, and studies have found that their presence there is sometimes neurotoxic. However, sterile immune reactions are not inherently neurotoxic and are usually elicited to prepare a damaged tissue for wound healing. Thus, the entire contribution of immune cell subsets to TBI lesions needs to be considered before targeted therapeutic interventions can be intelligently designed. Another important variable is time. The exact contribution of immune cells to a TBI lesion may shift over time. For example, an initially neuroprotective immune response may become maladaptive as secondary inducers of tissue destruction diversify.

Although TBI has proven difficult to treat, promising interventions lie on the horizon. Given the importance of ROS in TBI pathogenesis and the success with N-acetylcysteine in patients with mTBI, clinical pursuit of antioxidant therapy seems warranted. The likely key to success is early treatment with antioxidants so that TBI lesion expansion and subsequent inflammation can be stopped as soon as they are initiated. Because TBI lesions begin to expand within hours of injury, development of strategies to rapidly preserve brain tissue is paramount. The kinetics of lesion expansion must be carefully considered when attempting to manipulate purinergic and excitatory neurotransmitter pathways, which engage rapidly after in-
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Conclusions

Traumatic brain injury encompasses a complex spectrum of injuries that tax the neural-immune interface and can result in permanent neurologic dysfunction. Detailed knowledge of this interface during the acute and chronic phases of TBI will help us design the most efficacious interventions.

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