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Inflammatory neuroprotection following traumatic brain injury

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Abstract

Traumatic brain injury (TBI) elicits an inflammatory response in the central nervous system (CNS) that involves both resident and peripheral immune cells. Neuroinflammation can persist for years following a single TBI and may contribute to neurodegeneration. However, administration of anti-inflammatory drugs shortly after injury was not effective in the treatment of TBI patients. Some components of the neuroinflammatory response seem to play a beneficial role in the acute phase of TBI. Indeed, following CNS injury, early inflammation can set the stage for proper tissue regeneration and recovery, which can, perhaps, explain why general immunosuppression in TBI patients is disadvantageous. Here, we discuss some positive attributes of neuroinflammation and propose that inflammation be therapeutically guided in TBI patients rather than globally suppressed.

Traumatic brain injuries (TBIs) cause many reactions; one of the most prominent is neuroinflammation. Damage to the CNS elicits inflammatory responses from resident microglia and macrophages, as well as peripheral immune cells, such as neutrophils, monocytes, and T cells. Microglia and resident macrophages immediately respond to injury after sensing damage-associated molecular patterns (DAMPs), such as the presence of adenosine triphosphate (ATP) or intracellular proteins that are released from damaged or dying cells. Signaling from DAMP receptors initiates local cytokine and chemokine production, which affects the immediate environment and provides a cue for peripheral immune infiltration (1). A major question in the field of TBI research is how the immune response influences the pathogenesis of brain injury and recovery. Although a number of studies suggest that neuroinflammation is detrimental and inhibitory to neural regeneration following TBI, the failure of anti-inflammatory drugs to achieve a therapeutic benefit in human clinical trials supports a growing need to more carefully interrogate the duality of TBI-induced immunity. Immune reactions do indeed have the means to cause damage, but they also play a critical role in promoting tissue repair and recovery following brain injury.

Pathogenic inflammation following TBI

Microglia are resident immune sentinels that respond to nearly all inflammatory events within the CNS. Their exact contribution to the pathogenesis of brain injuries is not entirely

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SUPPLEMENTARY MATERIALS

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understood, but studies have shown that microglial activation can persist for years following TBI in humans (2). For example, analysis of microglia and associated pathology in TBI patients revealed clusters of activated microglia (evidenced by CR3 and CD68 immunoreactivity) in 28% of patients that survived for more than 1 year after a single brain injury (2). These patients also showed active signs of white matter degeneration, indicative of a chronic pathological process. However, it is unclear whether microglia are active participants in this prolonged degenerative process or are simply responding to the pathology induced by other mechanisms. Investigators have attempted to interrogate microglia in animal models of TBI, although the results are not definitive. Minocycline is an antibiotic with anti-inflammatory properties that is commonly used to suppress microglia and/or macrophage activation. This compound showed some therapeutic benefit (i.e., reduced microglia activation and brain lesion size) in a weight drop model of TBI (3), but the improvement cannot be linked exclusively to the effect of minocycline on microglia. Another study similarly concluded that microglia are pathogenic by studying cortical injury in the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-2 $(NOX2)^{-/-}$ mice (4). NOX2 is a subunit of NADPH oxidase expressed by activated microglia and known to generate reactive oxygen species (ROS). Both ROS production and lesion sizes were reduced in injured $NOX2^{-/-}$ mice, which suggested that microglia-derived ROS exacerbates TBI damage (4). Because the mice in this study were globally deficient in NOX2, it will be important in future studies to link pathogenic NOX2 activity exclusively to microglia.

Peripherally derived myeloid cells have also been implicated in TBI pathogenesis. After CNS injury, blood-derived monocytes migrate to the site of damage, where they differentiate into macrophages and persist as needed until the inflammation subsides. Even though macrophages aid wound-healing responses, many studies have concluded that monocytes are inherently pathogenic after TBI. For example, a subset of circulating monocytes express C-C chemokine receptor type 2 (CCR2), and macrophage numbers were reduced by >80% following cortical injury of $CCR2^{-/-}$ mice. This resulted in improved hippocampal neuronal survival and functional recovery relative to wild-type controls (5). This study demonstrates that peripheral $CCR2^{+}$ monocytes can contribute to hippocampal damage after direct cortical injury. Additional studies are needed to address whether all injured brain regions suffer the same fate after invasion by $CCR2^{+}$ monocytes, as well as how other monocyte subsets contribute to TBI pathogenesis and recovery.

Immunosuppressive clinical trials

The association between neurodegeneration and neuroinflammation has stimulated a great deal of interest in using immunosuppressive treatments for CNS injuries. Many drugs with anti-inflammatory properties—such as corticosteroids, nonsteroidals, statins, and specific cytokine inhibitors—have been tested in different TBI animal models (6). Although many of these treatments were effective in lessening pathology and improving neurological function, the results varied according to the injury model and/or treatment window. On the basis of promising pre-clinical data, large phase III clinical trials were conducted to assess the efficacy of immuno-suppressive treatments in the acute phase of TBI. Corticosteroids had been used for decades to treat head injuries without a substantial amount of clinical data to

support their efficacy. In 2004, a definitive trial found that administration of methylprednisolone within 8 hours of injury increased the risk of death at 6 months post TBI (7). The treatment group was also less likely to have a favorable recovery (based on the Glasgow Outcome Scale) at 6 months. Another clinical trial examined the effects of treating TBI patients with progesterone, an anti-inflammatory neurosteroid, within 4 hours of head injury (8). Evaluation at 6 months post injury revealed no significant difference between progesterone and the placebo control groups in terms of mortality or favorable outcomes. These trials demonstrate that suppressing the immune response acutely after a head injury at best has no effect and at worst actually increases the risk of death 6 months later.

The benefit of CNS inflammation

Why have potent immunosuppressive drugs failed to benefit TBI patients in phase III stage clinical trials? At least some inflammation may be necessary in the acute stage of CNS injury to clear damage and set the stage for remodeling efforts. Immunity promotes regeneration in peripheral tissues, so it is counterintuitive to think that neuroinflammation is universally neurodegenerative. The immune system is usually an active participant in wound healing. Within the CNS, positive aspects of immune cells that mobilize in response to damage include clearing dead cells, supporting the barrier system, and setting the stage for wound healing. Microglia, monocytes, macrophages, neutrophils, and T cells can collectively orchestrate a response that preserves neural tissue and fosters regeneration.

Because of their ubiquitous presence and abundant DAMP sensors, microglia are usually among the first responders to brain damage. For example, microglia express several purinergic receptors that allow them to quickly respond to extracellular purines, such as ATP. Detection of extracellular ATP causes microglia to project processes toward a site of damage within minutes. In a meningeal contusion model, ATP released by surface-associated astrocytes in the damaged glial limitans (a structure that separates the meninges and brain parenchyma) caused underlying microglia to shift into honeycomband jellyfish-like morphologies that provided barrier support and debris clearance, respectively (Fig. 1 and movie S1) (1, 9). Acute inhibition of this response increased the amount of neural cell death in the parenchyma. Microglia also participate in immediate CNS barrier support following damage of cerebral capillaries (10). Capillary damage caused by focal laser injury induces a purinergic receptor–dependent extension of microglia processes toward the vascular wall in an effort to prevent leakage into the parenchyma. Inhibition of this response resulted in capillary leakage that lasted three times as long as that observed in control mice. In addition to providing barrier support, microglia and macrophages can also clear debris from the injured CNS (Fig. 2 and movie S2). After damage to the corpus callosum, impaired myelin clearance by microglia leads to a decreased recruitment of oligodendrocyte precursor cells, which results in disorganized and defective axonal remyelination (11). In general, microglia are critical participants in the acute phase of a CNS injury. Sealing barriers and clearing debris are just two benefits of having these cells involved in the response.

Despite the various protective layers separating the CNS from peripheral tissues, immune cells infiltrate the CNS in response to trauma. In a model of spinal cord injury, neutrophil recruitment was necessary for proper wound healing and repair (12). Mice treated with

neutrophil-depleting antibodies exhibited decreased astrocyte reactivity at the injury site, larger lesions, and worse neurological outcomes. Neutrophils are also recruited to the meninges in a purinergic receptor–dependent manner following acute brain injury, and interference with this response actually increased the amount of meningeal cell death (Fig. 1 and movie S3) (9). It is presently unclear how neutrophils contribute to neuroprotective responses following CNS injury, but these cells do have the ability to recruit peripheral monocytes within a day or two. Spinal cord injury elicits a marked recruitment of pro-inflammatory macrophages that is followed several days later by wound-healing macrophages (13). When this wound-healing response was blocked, mice were unable to properly repair the lesion and recover motor skills (13). Similar to peripheral tissues, innate immune cells are essential for tissue remodeling and repair in the CNS. The challenge is to develop strategies that foster wound-healing responses while impeding maladaptive neuroinflammation.

The adaptive immune system is usually associated with the containment and clearance of pathogens, but T cells can also play a positive role in CNS injury responses. CNS damage can promote the nonspecific recruitment of $CD4^+$ T cells that produce interleukin-4 (IL-4) in a major histocompatibility complex II–independent manner (14). Release of IL-4 potentiates neurotrophin signaling that helps stimulate axonal regrowth after injury. Mice lacking T cells or IL-4 demonstrated increased neuronal loss and neurological dysfunction following CNS injury. During CNS tissue repair, effector and regulatory $CD4^+$ T cells work in tandem. Regulatory T cells (T_{regs}) often keep immune responses in check by modulating inflammatory mediators to promote wound healing and remodeling. Depletion of T_{regs} before CNS injury increased the recruitment of effector $CD4^+$ T cells and improved neurological recovery (15). By contrast, T_{reg} depletion several days after injury actually interfered with the tissue repair process. Similarly to the transition from proinflammatory to wound-healing macrophages, these T_{reg} data show that the neuroinflammatory response to injury changes as time progresses.

Concluding remarks

When preclinical and clinical data are combined, a clearer picture of TBI immunity emerges. Inflammation induced by a CNS injury should not be viewed as inherently maladaptive or neurotoxic. In general, neuroinflammatory responses during the acute phase of TBI have a lot of positive attributes that include barrier maintenance, debris clearance, cytokine and/or neurotrophin production, and immune regulation, among others. Inhibition of these responses will likely enhance neural damage and impede the wound-healing response to TBI. This conclusion is supported by the failure of immune-dampening drugs (e.g., methylprednisolone and progesterone) to achieve a clinical benefit in human TBI patients when administered shortly after injury. Nevertheless, neuroinflammation can become maladaptive over time. This might occur during the chronic stages of TBI, especially when macrophages and microglia remain in an inflammatory state in the CNS for months or years and acquire aberrant functions. Genetic predispositions, environment variables, and the location and severity of the injury are also likely to shape neuroinflammatory responses. Additional research is required to explore all of these variables and to better define the temporal aspects of CNS inflammation after TBI. It is incredibly important to identify the

critical time window when inflammatory cells participate in tissue repair following TBI. Future therapies should focus on guiding CNS immunity toward a favorable outcome rather than suppressing it entirely.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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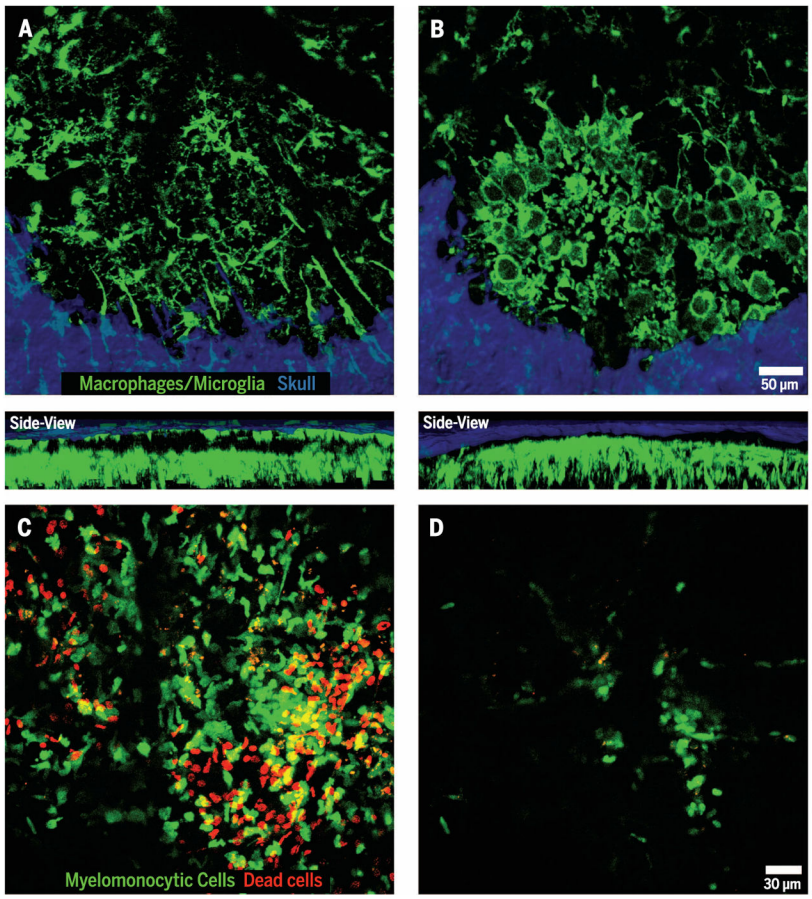

Fig. 1. Myeloid cell dynamics following brain injury

(**A** and **B**) Two-photon *z*-stacks captured through a thinned skull (blue) window of CX3CR1$^{gfp/+}$ mice show the morphology of macrophages and microglia (green) in the meninges and brain parenchyma, respectively. (A) Naïve microglia are ramified, whereas meningeal macrophages have a wormlike appearance. (B) Within minutes to hours after a meningeal contusion injury, meningeal macrophages rapidly disappear (die) and activated microglia migrate to the glia limitans and assume a "jellyfish" morphology that allows them to fill spaces previously occupied by dead astrocytes. These cells participate in phagocytosis and reduce leakage from the meninges into the underlying brain parenchyma. See corresponding movie S1. (**C** and **D**) Lysozyme-M$^{gfp/+}$ myelomonocytic cells (neutrophils/monocytes in green) are recruited to sites of meningeal cell death (red) beginning ~1 hour after TBI. A two-photon *z*-stack captured at 24 hours post injury demonstrates that nearly all the myelomonocytic cells responding to cell death are in the (C) meninges (top 30 µm of *z*-stack) as opposed to the (D) parenchyma (bottom 30 µm of the same *z*-stack). See corresponding movie S3.

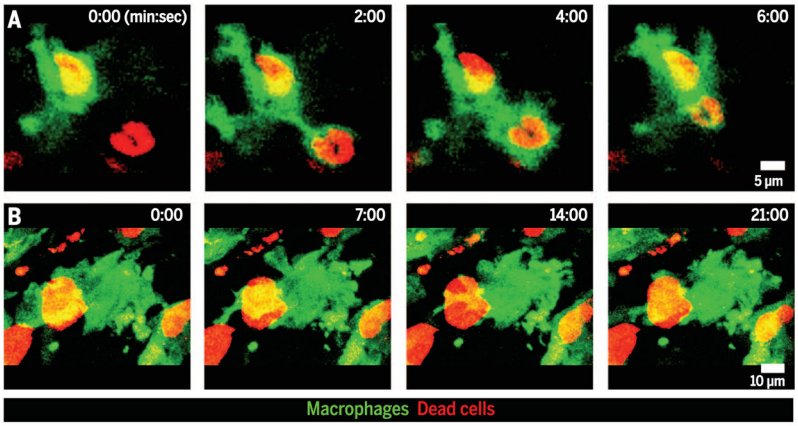

Fig. 2. Scavenging macrophages after brain injury

(**A** and **B**) Meningeal macrophages repopulate the meninges beginning ~2 days post injury, where they assume the role of scavengers. Time-lapse two-photon movies of the meningeal space show (A) a single macrophage that had previously engulfed a dead cell extending a process to phagocytose another cell at 2 days post injury and (B) a macrophage probing and sampling a dead cell at 4 days post injury. See corresponding movie S2.