Review

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Inflammatory reaction after traumatic brain injury: therapeutic potential of targeting cell–cell communication by chemokines

Stefka Gyoneva and Richard M. Ransohoff

Neuro/Immuno Discovery Biology, Biogen, Cambridge, MA, USA

Traumatic brain injury (TBI) affects millions of people worldwide every year. The primary impact initiates the secretion of pro- and anti-inflammatory factors, subsequent recruitment of peripheral immune cells, and activation of brain-resident microglia and astrocytes. Chemokines are major mediators of peripheral blood cell recruitment to damaged tissue, including the TBI brain. Here we review the involvement of specific chemokine pathways in TBI pathology and attempts to modulate these pathways for therapeutic purposes. We focus on chemokine (C-C motif) ligand 2/chemokine (C-C motif) receptor 2 (CCL2/CCR2) and chemokine (C-X-C motif) ligand 12/chemokine (C-X-C motif) receptor 4 (CXCL12/CXCR4). Recent microarray and multiplex expression profiling have also implicated CXCL10 and CCL5 in TBI pathology. Chemokine (C-X3-C motif) ligand 1/chemokine (C-X3-C motif) receptor 1 (CX3CL1/CX3CR1) signaling in the context of TBI is also discussed. Current literature suggests that modulating chemokine signaling, especially CCL2/CCR2, may be beneficial in TBI treatment.

Traumatic brain injury (TBI)

TBI is defined as an open or closed head injury (CHI) that disrupts brain function. Millions of people worldwide seek medical attention for TBI as a result of, for example, falls, motor vehicle accidents, or sports- and war-related activities. Depending on the etiology, TBIs can be closed head or penetrating and occur in a single event or in a repetitive fashion [1]. Any of these types of injury can result in various severities that are clinically classified as mild, moderate or severe based on a series of neurological tests [2].

Regardless of origin, TBI sufferers experience a relatively stereotyped array of symptoms associated with the injury: dizziness, confusion, and sometimes loss of consciousness (especially in severe injury). Even after the initial injury is managed and resolved, approximately 70–80% of TBI patients develop long-lasting effects such as changes in personality and cognition, anxiety and depressive-like behaviors [3–6]. TBI also increases the risk for certain neurodegenerative conditions. For instance, repeated concussive TBI has been associated with the development of chronic traumatic encephalopathy (CTE) in athletes [7–9]. Furthermore, both repeated and single TBI show a strong association with increased Alzheimer's disease (AD) risk or earlier AD onset [10–12]. Correlations with Parkinson's disease and amyotrophic lateral sclerosis (ALS) have also been reported, but the supporting evidence is not as strong as for CTE and AD [13].

An important determinant of brain pathology and functional recovery after TBI is whether the injury was focal or diffuse: whether it affected only the site of impact or led to the involvement of functionally and anatomically connected brain regions [14]. The minutes and hours after any TBI are characterized by acute pathology that includes – depending on the nature and extent of injury – tissue and blood–brain barrier (BBB) disruption, release of excitotoxic compounds, axonal injury, and neuronal death [15]. This primary pathology sets in motion events that perpetuate dysfunction over time, often associated with spreading of pathological changes to surrounding brain regions (diffuse injury) [14]. One of the main driving forces of this secondary pathology appears to be the inflammatory reaction after TBI [12].

TBI initiates an inflammatory reaction encompassing several interrelated components: release of intracellular components to the parenchyma from damaged cells; activation of brain-resident microglia and astrocytes; production of cytokines and chemokines; and recruitment of peripheral immune cells into the brain. Many of these processes influence each other, leading to complex interactions. For example, brain-resident cells secrete chemokines that attract peripheral cells, which in turn release signaling factors. These serve to recruit additional cells from the periphery, perpetuate activation of microglia and astrocytes, and damage neurons.

Considering the high prevalence of TBI and its association with serious neurological problems and risk for neurodegenerative diseases, there is a strong impetus to develop new TBI therapies that not only promote cell survival

Corresponding author: Ransohoff, R.M. (richard.ransohoff@biogen.com).
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immediately after the injury but also address the development of secondary pathology. Because of the close association between neuropathology and inflammation in space and time, the latter has emerged as an important target for the amelioration of TBI [12,16,17]. Moreover, the persistent nature of inflammation suggests that modulating inflammatory pathways may provide an extended therapeutic window to prevent the development of secondary pathology and in this way promote subsequent neurological recovery.

Inflammatory reaction following TBI

Molecular mediators

The use of animal models (Box 1), human surgical and postmortem tissue samples, and analysis of cerebrospinal fluid (CSF) and plasma from TBI patients has elucidated the temporal profile of inflammatory events following TBI (Figure 1) [18]. Within minutes and hours of the injury, damaged cells release certain intracellular components that act as danger-associated molecular pattern (DAMPs) and signal to other cells via pattern recognition receptors (Figure 1A). DAMPs include heat shock protein (HSP) 60 and 70, nucleic acids, and high mobility group protein B1 (HMGB1), which signal principally to Toll-like receptor (TLR) 2 and 4 [19]. In response to these and other stimuli, astrocytes, microglia and damaged neurons at the injury site start secreting cytokines and chemokines. This initial wave of inflammatory signals serves to activate microglia and astrocytes, possibly increase their migration to the site of damage, and recruit peripheral immune cells. After they enter the brain, leukocytes initiate a second wave of inflammatory mediators that contributes to the tissue damage.

The number of inflammatory mediators recognized to participate in the response to TBI has expanded over the past few years through the application of multiplex assays that simultaneously measure multiple unique mRNA or protein analytes. These approaches enable kinetic studies of mediator production in TBI animal models [20–23]. For example, more than one study reported expression of interleukin (IL)-1β, tumor necrosis factor alpha (TNF-α), IL-6, CCL2, CCL3, CXCL1, CXCL2, CXCL8/IL-8, CXCL10, CCR2, CCR5, CXCR4, and CX3CR1 within 6 h of TBI. Importantly, the same mediators have been detected in the early stages after injury in TBI patients, at the mRNA level by ribonuclease protection assay or the protein level by multiplex analysis of microdialysis samples. As in animal models, the levels of many cytokines and chemokines peak within 4–12 h after TBI [24,25]. Moreover, most chemokines are present at higher levels in the CSF than plasma, indicating local production by brain-resident cells [24,25].

In the past few years, the central role of inflammatory signaling in TBI response has been even further supported by large-scale microarray analyses covering much or all of the genome [26–30]. In bioinformatic analysis of all studies so far, functional pathways related to inflammation, stress, (inflammatory) cell movement, and cell signaling were among the top pathways differentially affected by TBI. These data were obtained by several groups using different animal models, showing that the importance of inflammation is model- and platform-independent. Examination of specific genes differentially affected by TBI in microarray experiments converged on cytokine and chemokine pathways similar to the ones identified by the narrowly focused multiplex assays [24–30]. Together, these studies confirm that early upregulation of inflammatory mediators is a robust response that is likely to contribute to the subsequent neuropathological sequelae of TBI.

Cellular response

The tissue damage and subsequent release of inflammatory mediators after TBI lead to changes in the function of brain-resident cells and recruitment of peripheral cells (Box 2). If one imagines a prototypical cortical impact, among the first cell types to respond to injury are neutrophils, whose accumulation starts in the subarachnoid space and vascular elements near the injury. Neutrophils subsequently enter the brain parenchyma around 1 day post injury (dpi) (Figure 1A), mediated at least in part by upregulation of adhesion molecules on the endothelium [18,31–35]. While neutrophil recruitment to tissues is essential in responses to peripheral infections and damage, they release reactive oxygen and nitrogen species that damage the brain parenchyma. Neutrophil presence in the brain is greatly reduced

Box 1. Animal models of TBI

There are several established animal models of TBI that simulate human injury to varying extents. Below are brief descriptions of models used in the studies that we reference. For more information, see Xiong *et al.* [86] for a review of the most commonly used models.

Stab injury. Stab injury is a type of penetrating injury that is delivered through a small craniotomy. Fine scissors or a thin membrane are inserted a defined distance (1–5 mm) into the brain parenchyma [87]. This type of injury was used for earlier studies of TBI in mice.

Weight drop/CHI. There are several weight-drop models developed by different groups that simulate CHI. Although generally applied to the closed skull, some of these injuries are delivered through a craniotomy, with the dura mater intact. Injury intensity is controlled by adjusting the weight of the dropped object and the height from which it is dropped. Depending on the exact model and injury intensity, CHI models induce concussions, brain contusion, diffuse axonal injury, hemorrhage, and other features of human TBI. However, different weight-drop models can have significant inter-animal variability.

Controlled cortical impact (*CCI*). A piston driven by air pressure or electromagnetism impacts the head at a controlled angle, velocity, and depth. CCI is most often delivered through a craniotomy, but newer variations of this model deliver an injury to the intact skull. Similar to CHI, CCI simulates aspects of concussions, brain contusion, and hemorrhage seen in human TBI. Unlike CHI, the injury is highly reproducible between animals.

Fluid percussion injury (*FPI*). For FPI, rodents are connected through a craniotomy to a fluid-filled chamber with a small opening; a swinging pendulum hits one end of the chamber to generate a water pulse that impacts the exposed brain at the other end of the chamber. Depending on the location of the craniotomy, the injury can be delivered to the side of the brain (lateral FPI) or the midline (central FPI). Injury intensity is controlled by adjusting the height from which the pendulum is dropped. Like CCI, FPI delivers reproducible injury that mimics aspects of concussions, brain contusion, diffuse axonal injury, and hemorrhage seen in humans.

Utility of models. CCI and lateral FPI are commonly used to generate focal injuries. Central FPI or Marmarou's weight-drop models are most appropriate to simulate diffuse injuries.