Role of Microglia in Neurotrauma

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Summary: Microglia are the primary mediators of the immune defense system of the CNS and are integral to the subsequent inflammatory response. The role of microglia in the injured CNS is under scrutiny, as research has begun to fully explore how postinjury inflammation contributes to secondary damage and recovery of function. Whether microglia are good or bad is under debate, with strong support for a dual role or differential activation of microglia. Microglia release a number of factors that modulate secondary injury and recovery after injury, including pro- and anti-inflammatory cytokines, chemokines, nitric oxide, prostaglandins, growth factors, and superoxide species. Here we review experimental work on the complex and varied responses of microglia in terms of both detrimental and beneficial effects. Addressed in addition are the effects of microglial activation in two examples of CNS injury: spinal cord and traumatic brain injury. Microglial activation is integral to the response of CNS tissue to injury. In that light, future research is needed to focus on clarifying the signals and mechanisms by which microglia can be guided to promote optimal functional recovery. **Key Words:** Microglia, spinal cord injury, traumatic brain injury, inflammation.

INTRODUCTION

The role of microglia in the injured CNS is under scrutiny, as research has begun to determine how microglia-mediated inflammation contributes to secondary injury and recovery of function following trauma. Whether microglial response to injury is good or bad is under debate, with strong support for a dual role and differential activation.

Microglia, which were first described by del Rio-Hortega[1] in the early part of the 20th century, represent 10–20% of the total cell population in the adult CNS.[2] These cells are the resident immune cells of the CNS, belonging to the mononuclear phagocyte lineage, and are the primary mediators of the brain's innate immune response to infection, injury, and disease. It is commonly believed that these cells migrate into the CNS during development, and may continue to invade over the course of life, particularly after injury or insult.[3]

This review will explore the current research on microglial responses to CNS injury. The argument for a positive *versus* negative role of microglia will also be discussed. Two models of CNS injury, brain and spinal cord trauma, will be used to exemplify these responses *in vivo*. A large variety of CNS injury models have been used in investigating microglial and inflammatory responses to CNS injury, and microglial responses vary depending on the injury model used and the severity of the injury inflicted. Data from brain and spinal cord trauma models, in which direct mechanical forces are applied to the CNS, were chosen in this review to reflect both the similarities and differences in responses, and to show the generality that one can expect from microglia *in vivo* (FIG. 1). Reviews of microglial responses in other models of CNS injury and disease, including stroke and ischemia,[4] infection,[5] multiple sclerosis,[6] and neurodegenerative diseases,[7,8] should also be consulted for a fuller understanding of microglial activity.

GENERAL RESPONSE OF MICROGLIA TO INJURY

Microglia are dynamic cells, constantly surveying their microenvironment for noxious agents and injurious processes.[9] They respond to extracellular signals and are responsible for clearing cellular debris and toxic substances by phagocytosis, thereby maintaining normal cellular homeostasis in the CNS. To detect potential harmful insults, microglia express a set of pattern recognition receptors that recognize small molecular motifs found on pathogens or factors associated with tissue damage. These highly conserved pattern recognition receptors detect exogenous

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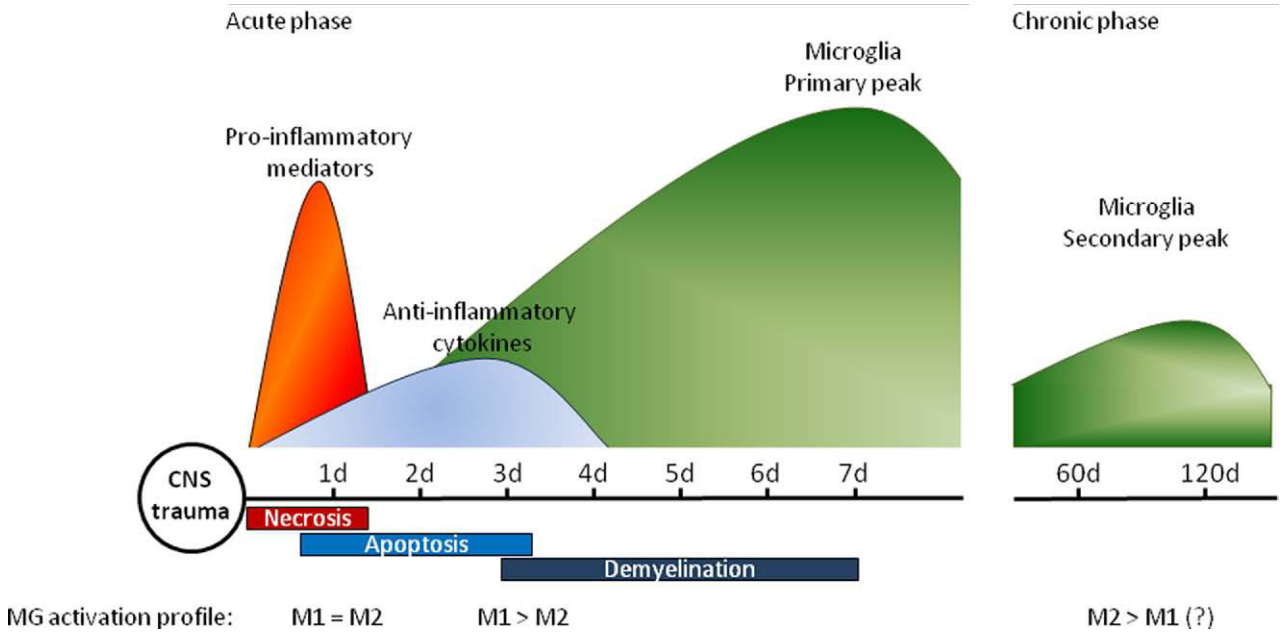

FIG. 1. Schematic of the microglial (MG) activation timeline after CNS injury, including inflammatory events and neurotoxic profiles in the acute and chronic phases. Also indicated are the proposed M1 and M2 microglial ratios over time.

pathogen-associated molecular patterns and endogenous danger-associated molecular patterns and enable microglia to identify and react to noxious stimuli and harmful events.[10] Microglia also express receptors for a number of factors that are released by injured neurons, including ATP, glutamate, growth factors, and cytokines.

In the healthy adult brain, microglia are in a resting state and have a dendritic morphology with many processes.[11] In response to injury, however, microglia undergo dramatic changes in cell morphology and behavior. For example, upon activation microglia contract their processes and transform from a ramified to an ameboid morphology resembling that of blood-borne macrophages, followed by proliferation and migration toward the site of injury.[11] This convergence upon the site of injury is in response to ATP and other signals released by injured cells[12,13]; once there, microglia act as a barrier between the injured and healthy tissue.[12]

No markers are currently available that can distinguish cells in the CNS as resident activated microglia *versus* infiltrating macrophages. The two cell types have similar morphology, gene expression, and antigen presentation capabilities (for a review, see Streit et al.[14]). Microglia do, however, have significant differences from their blood-borne relatives, differences that are most likely related to their local microenvironment. For example, recent studies have suggested differences in protein expression profiles between macrophages and microglia, including differences in the level of CD45 expression[15,16] and galectin-3/MAC-2 expression.[17] Furthermore, macrophages have twice as much proteolytic activity as microglia, and microglia have a more robust response to cytokine stimulation, *in vitro*.[18,19] Preliminary proteomic analysis shows differences in 19 proteins, including superoxide dismutase, among microglia, bone marrow macrophages, and spleen macrophages[18]; in that study, however, the microglia and macrophages were isolated from different aged mice. Because age can affect macrophage activity,[14,20] the reported differences in protein expression must be considered with caution.

DETRIMENTAL RESPONSES OF ACTIVATED MICROGLIA

The production of vast numbers of cytotoxic chemicals and their association with neuronal cell death has long contributed to the view that microglia play a detrimental role in the CNS (FIG. 2). A large number of studies have shown that microglia release proinflammatory mediators that contribute to neuronal dysfunction and cell death in response to injury and various immunological stimuli.[21–23] These neurotoxic substances include proinflammatory cytokines, chemokines, nitric oxide (NO), and superoxide free radicals that generate reactive oxygen species (ROS) and reactive nitrogen species (RNS).

Using the lipopolysaccharide (LPS) model of microglial activation, much research has shown that microglia can develop a neurotoxic phenotype. Lipopolysaccharide acts through either the Toll-like receptor 4 (TLR4) or CD11b/CD18 (MAC1) receptor; both receptors induce a signal transduction cascade that modulates NFκB-mediated proinflammatory gene expression, potentially resulting in neuronal cell death. Knockout of the MAC1 receptor or MyD88 within the TLR4 pathway blocks microglia-mediated neuronal cell death.[24,25] Activation

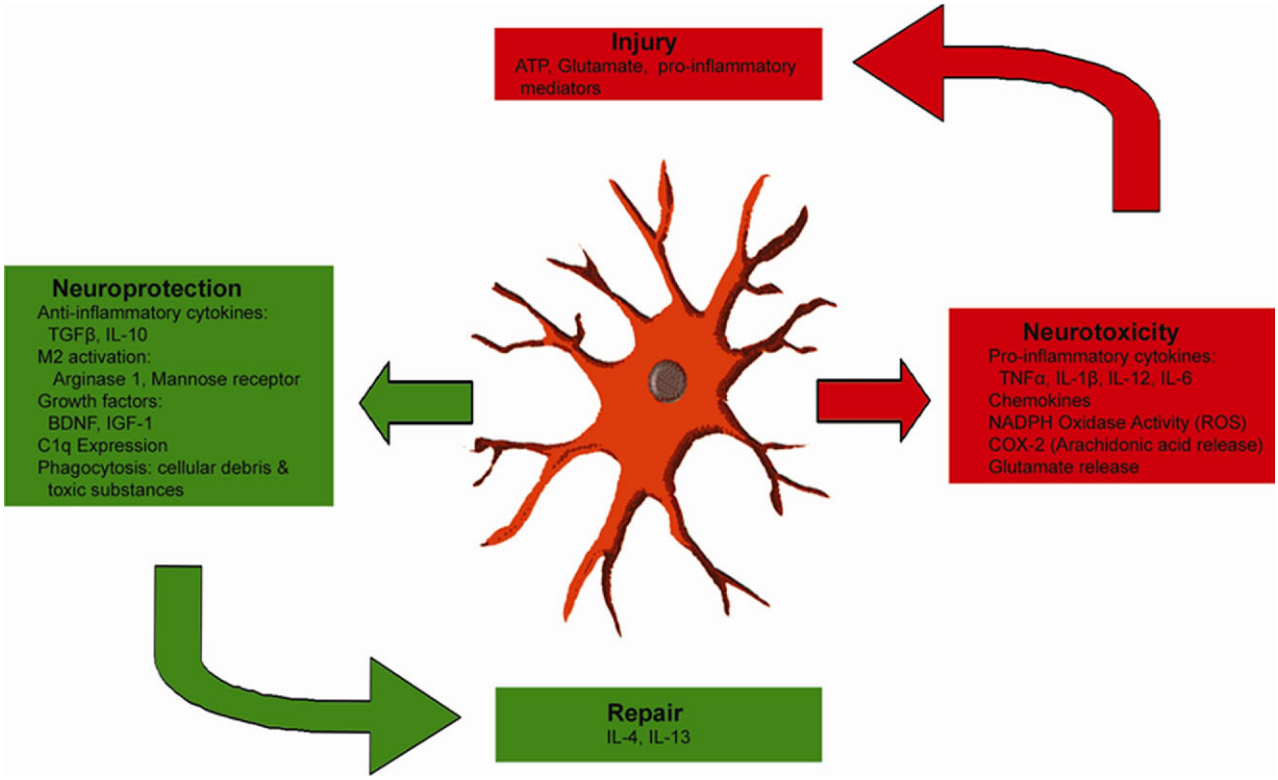

FIG. 2. Schematic of detrimental and beneficial effects of activated microglia. Red arrows indicate potential cytotoxic activities; green arrows indicate the pathways that may be protective.

of the MAC1 receptor also results in a phagocytic and toxic microglia phenotype, which is characterized by activation of NADPH oxidase and ROS production,[10] as well as tumor necrosis factor-α (TNF-α) release.[25]

Numerous other factors can induce a neurotoxic phenotype in microglia. For example, exposure of microglia to myelin in a cell culture model to mimic myelin debris produced after injury resulted in the release of NO, TNF-α, and glutamate, with subsequent neuronal death.[26] The ATP and glutamate released by damaged neurons can also induce microglial activation,[27] and abnormal protein aggregates, such as amyloid-β (Aβ) and α-synuclein, activate microglia to secrete proinflammatory mediators such as TNF-α, NO, and superoxide species.[28–30] Exposure of cultured microglia to dying neurons results in the release of cytokines such as TNF-α, interleukins IL-12 and IL-6, and RNS, as well as an increase in the expression of cell-surface antigens, CD40, major histocompatibility complex II (MHC-II), CD11b, and enzymes, such as inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX-2).[31] Notably, media from microglia exposed to dying neurons induced subsequent neuronal death.[31]

The mechanisms by which microglia induce neuronal cell death are not fully understood, but several pathways have been shown to be involved. For example, microglial NADPH oxidase-related ROS release leads to increased internal zinc and potassium concentrations, resulting in neuronal apoptosis.[32] The NADPH oxidase enzyme is activated by exposure of microglia to damaged neurons,[33] neurotoxins such as rotenone[34] and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP),[33,35] LPS,[36,37] Aβ,[29] and α-synuclein.[28] This multicomponent enzyme has two membrane components ($p22^{phox}$ and $gp91^{phox}$), as well as cytosolic components ($p47^{phox}$, $p67^{phox}$, and $p40^{phox}$).[38] Stimulation results in the transport of the cytosolic components to the membrane to form the active enzyme complex and enables the production of extracellular ROS. NADPH oxidase is also thought to be a crucial component of microglial signaling, one that regulates the NFκB pathway and proinflammatory gene expression in these cells.[37,39] NADPH oxidase knockout results in attenuated proinflammatory production and reduced microglia-mediated neurotoxicity.[37]

With age, resting microglia have been shown to take on a more activated phenotype, increasing expression of several receptors and markers, such as ionized calcium-binding adapter molecule 1 (Iba-1)[40] and MHC-II.[41,42] Aged microglia also exhibit hypertrophic and shortened processes that resemble an activated cellular morphology.[40] Consistent with the view that activated microglia are the primary source of proinflammatory cytokines in the brain, several studies have demonstrated age-related increases in IL-1β, IL-6, and TNF-α expression.[43–45] Further, IL-1β-positive microglia have been reported in the brains of aged individuals,[46] and increased expression of

IL-6 and TNF-α has been associated with age-related cortical atrophy in humans.[47]

Microglia are not only toxic to neurons but to other glial cells as well. Recently, it was shown that peroxynitrite, a short-lived potent oxidant and the reaction product of NO and superoxide, was the toxic microglial factor responsible for LPS-induced death of developing oligodendrocytes .[48] The presence of astrocytes alters the LPS-induced cell death mechanisms and shifts LPS-induced microglia-dependent toxicity of oligodendrocytes from a peroxynitrite-mediated mechanism to a TNF-α-mediated mechanism.[49]

Short-term microglial activation is not considered to be detrimental (a concept that will be addressed in greater detail in subsequent sections). Chronic microglial activation, however, is considered to be the most damaging response of microglia to injury.[50] For example, in the injured or diseased CNS, interactions between damaged neurons and dysregulated, hyperactivated microglia create a vicious self-propagating positive feedback loop that leads to uncontrolled, prolonged microglial activation and neuronal cell death that drives the chronic progression of neurodegeneration and disease.[22]

BENEFICIAL RESPONSES OF MICROGLIAL ACTIVATION

Short-term microglial activation may have beneficial effects (FIG. 2). As mentioned earlier, microglia phagocytose cellular debris and maintain normal cellular homeostasis, thereby preserving and protecting healthy tissue.[51] Microglial phagocytosis is dependent upon C1q expression, among other things.[52] Notably, C1q exposure has been shown to reduce LPS-induced cytokine expression, suggesting differences in level of activation between phagocytic and cytokine-expressing microglia.[52] It has been suggested that the level of the inflammatory stimulus regulates the level of the microglial response. For example, Li et al.[53] demonstrated that, although high levels of LPS (>1 μg/mL) induced proinflammatory cytokine expression and neurotoxicity, lower concentrations ($\leq$500 ng/mL) increased neuronal cell viability and promoted neurite extension.

In recent years, a new classification of microglia has entered the literature. Described as an alternatively activated subset of microglia (M2), these cells have markers that can differentiate them from classically activated microglia (M1).[54,55] The M2 microglia are typically considered to be less inflammatory than M1 microglia; they are characterized by reduced NO production and increased anti-inflammatory cytokine production. The M2 microglia express specific antigens such those to arginase 1, mannose receptor, found in inflammatory zone 1 (FIZZ1), and chitinase 3-like 3 (YM1)[56] and are involved in tissue repair, wound healing, and extracellular matrix remodeling.[55] After injury or *in vitro* following exposure to IL-4 or IL-13, microglia develop this nontoxic M2 phenotype, resulting in extensive neurite elongation and outgrowth across inhibitory surfaces.[54,57] Furthermore, microglia exposed to IL-4 also demonstrate reduced proinflammatory cytokine production (e.g., TNF-α and IL-1β) and increased anti-inflammatory or growth promoting factor production.[56,58]

Microglia produce a number of neuroprotective substances in response to injury, such as anti-inflammatory cytokines and neurotrophic factors, including nerve growth factor, transforming growth factor β (TGF-β), IL-10, and IL-1 receptor antagonist (IL-1ra).[59–62] Because of its ability to bind to IL-1 receptor (IL-1RI) without initiating signal transduction,[63] IL-1ra plays a major role in counteracting the biological effects of IL-1β. Furthermore, both TGF-β and IL-10 inhibit macrophage and microglia activation by downregulating the expression of molecules associated with antigen presentation and production of proinflammatory cytokines, chemokines, and nitric and oxygen free radicals.[59,64,65] After exposure to 6-hydroxydopamine (6-OHDA), increase was observed in microglial production of brain-derived growth factor (BDNF), insulin-like growth factor 1 (IGF1), and TGF-β2,[66] which led to improved neuronal cell viability in this model. Exposure to hypoxic neurons induced the production of BDNF and glial-derived neurotrophic factor (GDNF) in microglia.[67] Similarly, exposure of microglial cultures to conditioned media from NMDA- or AMPA-treated neurons resulted in increased release of IL-1ra and was neuroprotective in this excitotoxicity model.[68]

Microglia can interact with other cells of the CNS and have significant beneficial effects. Recent data reported by Roy et al.[69] suggest that cell-to-cell contact between T lymphocytes (Th2 cells) previously exposed to CNS antigens and microglia promotes the expression of neurotrophins (e.g., BDNF) without inducing release of proinflammatory cytokines. In contrast, the Th1 lymphocyte subtype cells stimulate microglia to produce proinflammatory cytokines rather than neurotrophins, demonstrating that T-cell subsets are associated with differential effects on microglial activity and gene expression. Note, however, that microglia themselves inhibit the proliferation of $CD4^+$ T cells.[70] Furthermore, activated microglia recognize and phagocytose infiltrating neutrophils, thereby limiting neutrophilic damage to healthy tissue.[71]

ROLE OF MICROGLIA IN SPINAL CORD INJURY

Spinal cord injury (SCI) results from an initial, mechanical insult on spinal cord tissue and is followed by secondary biochemical changes that produce long-term dysfunction. This secondary injury includes delayed

events, such as ischemia, lipid degradation, free radical formation, excitotoxicity, and protease release,[72,73] leading to demyelination, axonal degeneration, neuronal death, cavitation, and glial scarring surrounding the area of the initial damage.[74–77] Inflammation, including the activation of resident microglial cells, plays an important role in these secondary changes.[73,78,79]

Direct damage to neurons and surrounding cells results in the release of a number of intracellular components and changes in extracellular ion content, such as glutamate and ATP, to which microglia are particularly sensitive.[2,80] For example, expression of microglial cytokine, ATP, CD4, and glutamate receptors are upregulated, as well as MHC class I and II antigens, leading to an improved ability of microglia to respond to signals at the site of injury.[77,81–83] Proinflammatory cytokines and chemokines are also rapidly upregulated and likely contribute to microglial activation. TNF-α, for example, is produced by neurons, astrocytes and microglia[84–86] and is upregulated rapidly after SCI.[87–89] TNF-α acts to initiate a number of downstream signal transduction pathways, such as the NFκB and MAPK systems,[90] and may promote glutamate-induced neurotoxicity by impairing microglial uptake of extracellular glutamate.[91]

Advances in molecular biology, including improvements in microarray technology, have enabled more detailed analyses of injury and cellular responses. Gene expression analyses of SCI have demonstrated a strong inflammatory component, with nearly 200 inflammatory-related genes upregulated after injury, including genes for COX2, iNOS, MnSOD, HSP70, IL-1 receptor, and IL-1β.[92–95] Microglia-related genes, specifically, have also been profiled after SCI, demonstrating a strong elevation of gene expression both acutely and chronically after insult.[96]

Macrophages and microglia together comprise the monocytic reaction in the injured spinal cord, and there is significant upregulation of bromodeoxyuridine incorporation in microglial cells within residual spinal cord tissue after SCI.[97] Macrophages from the periphery and activated microglia appear in the spinal cord between 12 and 24 hours after injury, with maximal infiltration at 4–8 days after injury (FIG. 1).[98,99] Recent data have revealed that there is also a secondary peak of microglia and macrophage presence in the spinal cord at 60 days, with continued elevation through 180 days after SCI.[100] Activated microglia and macrophages demonstrate gene expression profiles that reflect an increase in phagocytosis, upregulation of antigen-presenting capabilities, and secretion of proinflammatory molecules, ROS, and RNS.[101]

A study using bone marrow chimeric rats has shown that microglia are responsible for much of the immune response at the lesion site within the first days after SCI, as well as in rostral and caudal regions.[102] Yang et al.[103] also demonstrated that resident microglia, rather than infiltrating macrophages, are the primary source of the proinflammatory cytokines IL-1β, IL-6, and TNF-α acutely after SCI. Moreover, at 14 and 28 days after injury there are areas of increased blood-spinal cord barrier permeability rostral and caudal to the lesion site that are associated with OX-42$^+$ microglia expression.[104] A recent study by Shechter et al.[105] demonstrated that ablation of circulating monocytes and macrophages while maintaining microglial viability impaired functional recovery, suggesting that microglia strongly contribute to the loss of function in the injured spinal cord.

Studies have shown that ROS, components of NADPH oxidase, and peroxynitrite are chronically upregulated after SCI.[106] Many of these factors are cytotoxic,[107–109] as described earlier, or can inhibit cellular proliferation or progenitor replenishment.[110] Further, there is evidence that microglia can cause axonal retraction through direct interactions.[111]

Nonetheless, a variety of microglia-related factors may also play a role in neuroprotection and axonal survival after SCI. For example, macrophages and activated microglia expressing the M2 phenotype (arginase 1 and mannose receptor positive) are expressed immediately after SCI to 14 days after injury, along with the IL-4 receptor, which is associated with the M2 phenotype.[54] Expression of these M2 microglial factors may promote CNS repair while limiting secondary inflammatory-mediated injury. Further, research has shown that chondroitin sulfate proteoglycans, proteins expressed by astrocytes, can induce microglial expression of IGF-1.[112]

Microglia also increase phagocytic activity after SCI in an effort to eliminate cellular and myelin debris in the injured spinal cord. Upregulation of ED1, a lysosomal protein, is indicative of increased lysosomal bodies within a cell and is a marker for increased cellular phagocytosis, whereas increased ED1 expression is highly correlated with the ability of microglia to phagocytose targets.[113] Microglia response factor (MRF) and galectin-3 are also upregulated and correlated with phagocytosis by microglia, as demonstrated by immunostaining for phagocytosis of myelin in response to axonal transection or SCI.[96,114,115]

ROLE OF MICROGLIA IN TRAUMATIC BRAIN INJURY

Mechanical forces at the moment of traumatic brain injury (TBI) cause rapid tissue deformation, resulting in primary physical damage.[116] The mechanisms involved in cell death and tissue loss following TBI are complex interactions between acute and delayed biochemical, molecular, and physiological events that collectively mediate widespread neurodegeneration and loss of neurolog-

ical function. These secondary injury mechanisms include glutamate excitotoxicity, blood–brain barrier disruption, secondary hemorrhage and ischemia, mitochondrial dysfunction, apoptotic and necrotic cell death, and inflammation.[117] Such secondary injury events begin within seconds to minutes after the primary insult and may continue for days, weeks, and months, progressively contributing to worsening neurological function.

One of the central inflammatory responses to brain injury is activation of microglia.[118,119] After acute injury, microglia have been shown to react within a few hours with a migratory response toward the lesion site. In fact, elegant *in vivo* two-photon microscopy imaging studies of fluorescently labeled microglia in transgenic mice following laser-induced injury demonstrated rapid proliferation and movement of ramified microglial cells to the site of injury in response to extracellular ATP released by the injured tissue.[12,120] The microglial processes then fused to form an area of containment between healthy and injured tissues, suggesting that microglia may represent the first line of defense following injury.[12] In humans with TBI, microglial activation has been reported as early as 72 hours after injury,[121] and it can remain elevated for months after injury.[122,123] This activation profile is mirrored in rodent models of TBI (FIG. 3), and chronic microglial activation surrounding the lesion is evident weeks and months after the initial brain injury.[124–126]

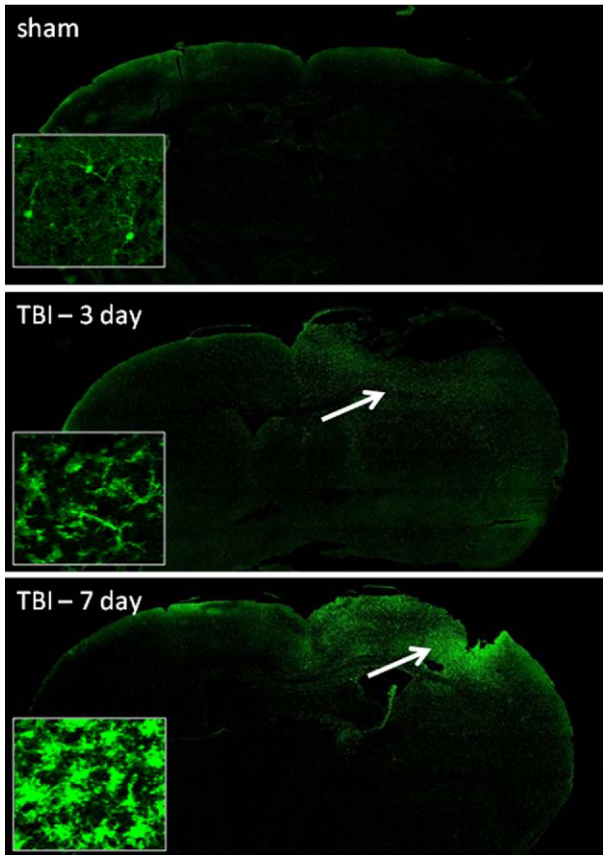

FIG. 3. Timecourse of microglial activation following experimental traumatic brain injury (TBI). Sham, 3 day TBI, and 7 day TBI brain samples were immunostained with anti-Iba-1 to label activated microglia. Upon activation, microglia contract their processes and change from a ramified to an ameboid shape (inset), proliferate and migrate to the site of injury.

Numerous gene profiling studies of TBI have been conducted using microarray technology, and genes related to inflammation are strongly upregulated in the acute phase after injury in both rats and mice.[127–129] Follow-up studies on microglia-related genes and their temporospatial localization after TBI demonstrated that markers of activation (e.g., CD68, MHC-II), stress responses (e.g., $p22^{phox}$, heme oxygenase 1), and chemokine expression (e.g., CXCL10, CXCL6) were markedly increased after injury.[130] Consistent with the early activation profile of microglia following injury, there is rapid upregulation of proinflammatory IL-1β mRNA within hours of experimental TBI.[131–133] The damaging effects of IL-1β are mediated through IL-1RI, which is strongly expressed on microglia and neurons.[131,134] This damage is not due to the cytokine itself, but rather to its effect on activating other proinflammatory pathways, such as TNF-α.[135] Inhibiting IL-1β in experimental models of TBI has been shown to be neuroprotective, improving functional recovery.[131,132,136–138] In addition, TNF-α levels are elevated in both the serum and CSF of patients with severe TBI.[139,140] TNF-α expression after experimental TBI is detectable after 1 hour, peaks between 3 to 8 hours, and returns to normal levels at 24 hours after injury.[141–143] However, the role of TNF-α in the pathogenesis of TBI is somewhat controversial and complex in nature, with different functional outcomes in the acute and delayed phases after TBI.[144,145]

Anti-inflammatory cytokine levels are also modulated by TBI. In humans, IL-10 and TGF-β levels are elevated acutely after injury,[146,147] and experimental studies have shown that IL-10 has beneficial effects following trauma.[148] For example, intravenous administration of IL-10 after experimental TBI in rats improved neurological recovery and significantly reduced TNF-α and IL-1β expression in the traumatized cortex and hippocampus. These neuroprotective effects may be a result of suppressed microglial activation, in that IL-10 treatment has been shown to decrease production of proinflammatory cytokines.[149] Furthermore, injection of the anti-inflammatory cytokine TGF-β1 after injury in rodents reduces lesion size,[150] improves function, and reduces iNOS production.[151]

It is well accepted that age influences microglial activation.[46,152] Exacerbated microglial and astrocytic responses to injury are thus likely to be involved in enhanced susceptibility to and poor recovery from TBI in elderly patients.[153,154] Recent studies have demonstrated that the microglial response to experimental TBI was exaggerated and prolonged in aged mice, relative to adult mice.[155] These differences included increased microglial activation

(CD11b and Iba-1 immunoreactivity) in the hippocampus of aged (21–24 months) *versus* adult (5–6 months) mice. This observation is consistent with reports of elevated microglial activation in the aged brain following injury such as facial nerve axotomy[156] and cerebral ischemia.[157] It has been proposed that, after injury, microglia in the aged brain are primed to respond more rapidly, produce more pronounced inflammatory responses,[41] and proliferate more vigorously than microglia in the younger brain.[156] Thus, hyperactivated and dysfunctional microglia in the aged hippocampus following TBI may contribute to enhanced neuronal loss in the hippocampus and worse neurological outcome in the elderly.

Compelling data from several epidemiological studies demonstrate that a history of TBI is one of the strongest risk factors for the development of Alzheimer's disease (AD) later in life.[158–160] Further, it has been shown that Aβ plaques, a hallmark of AD, may be found in patients within hours of TBI.[161–163] Several possible pathophysiological mechanisms linking brain injury and AD, such as the accumulation and clearance of Aβ peptides following TBI, have received much attention recently (for a review, see Johnson et al.[164]). However, chronic neuroinflammation is a common neuropathological feature of TBI and AD, and chronic microglial activation may be a key causative factor. In AD, Aβ is implicated in the pathology, both through direct toxicity to neurons[165] and by potentiating neuronal damage by microglial activation.[30] In patients with AD, activated microglia cluster at sites of aggregated Aβ and penetrate the neuritic plaques.[166,167] Furthermore, Aβ is proinflammatory and activates microglia to release neurotoxic factors such as NO, TNF-α, and superoxide.[29,168] Following TBI, activated microglia surround the lesion and remain chronically activated for weeks and months after the initial brain trauma.[125] Persistent long-term microglial activation was observed in the traumatized cortex 3 months after experimental brain injury and was associated with increased expression of proinflammatory cytokines, IL-1β and TNF-α.[169] In humans, long-term microglial activation and chronic inflammation may persist for many years in head injury survivors,[123] with increased microglial activation detected in both parasagittal and hippocampal white matter in head-injured cases up to 16 years after injury. These long-term persistent inflammatory changes may cause post-traumatic neurodegeneration, which could form the basis of the cognitive decline that is often observed in long-term survivors of TBI.

THERAPEUTIC IMPLICATIONS

A number of therapeutic interventions have been developed that target microglia or microglia-related inflammation after traumatic CNS injury, such as minocycline, peroxisome proliferator-activated receptor γ (PPARγ) agonists, and anti-inflammatory cytokines, among others. Minocycline is a second-generation tetracycline that is known to have anti-inflammatory properties independent of its antimicrobial activity.[170] Studies have shown that minocycline inhibits microglia-mediated neurotoxicity[171] and suppresses the production of several proinflammatory cytokines.[172,173,174] In experimental models of TBI and SCI, minocycline treatment reduced tissue loss and improved functional recovery after injury.[175,176–181] PPARs are ligand-activated transcription factors of the nuclear hormone receptor family[182]; activation of the PPARγ isoform has demonstrated significant anti-inflammatory effects, including attenuation of proinflammatory cytokine, iNOS, and COX2 expression.[183] PPARγ agonists, such as rosiglitazone and pioglitazone, confer neuroprotection in models of acute CNS injury and neurodegeneration.[184–188] In SCI and TBI models, anti-inflammatory cytokine administration, such as IL-10 and TGF-β1, has been shown to improve functional outcome.[148,189,150] The proinflammatory cytokine receptor antagonist IL-1 receptor antagonist (IL-1ra) has also been found to have anti-inflammatory actions. In experimental models of TBI, neutralization of IL-1ra or IL-1β resulted in attenuated proinflammatory cytokine and chemokine production, reduced hippocampal damage, and improved neurological behavior after injury.[131,132,136–138]

Nonetheless, the dual role of microglia must be kept in mind.[190,191] Although treatment to suppress microglial activity may reduce inflammation and improve neuronal survival or plasticity,[192,193] it is possible that the beneficial effects of microglia may also be lost. For example, proinflammatory cytokines released by microglia are associated with increased nerve growth factor production by astrocytes.[194] In addition, studies have shown that upregulation of microglial activity, by granulocyte-macrophage colony-stimulating factor (GM-CSF) injection or addition of activated microglia, can improve recovery after CNS injury.[195,196] These studies indicate that much research remains to be done to fully understand the contribution of microglia to CNS injury recovery.

SUMMARY

Microglia are the primary mediators of the innate immune response to injury and disease in the CNS. These cells respond rapidly and specifically to the signals presented to them, and have the potential to play either a neuroprotective or a neurotoxic role after injury. Further understanding of the microglial cellular responses to traumatic injuries, including the initiating events, the signal transduction pathways involved, and the mediators produced, may facilitate identification of future therapies that promote the beneficial effects while preventing the detrimental and neurotoxic effects.

To date, a number of therapeutic interventions have

been developed that target microglia or microglia-related inflammation after traumatic CNS injury. Although it is not necessary that these interventions act exclusively on microglia to elicit their beneficial effects, the evidence suggests that attenuating microglial activation and associated inflammation is a major mechanism of action of these therapies. In fact, several lines of research indicate that multipotential therapies that target microglial activation in addition to other secondary injury mechanisms, such as apoptotic cell death, are most likely to succeed in treating acute CNS injury and improving long-term functional outcome.[197,198]

In conclusion, it is clear that the microglial response to injury is complex and multifaceted. The influence of microglia on both propagation of secondary injury and continuing neuronal damage, as well as their influence on the beneficial, reparative, and wound-healing effects following injury, is beginning to be understood. These complex microglial responses need to be considered when investigating traumatic CNS injuries, and research must now focus on identifying the signals and mechanisms by which microglia can be guided to promote optimal recovery. In addition, a clear understanding of the influence of the various CNS injury models, severity of injury, and time on the microglial phenotypic responses would greatly enhance therapeutic approaches that target microglia. Finally, while the discovery of the M1 *versus* M2 microglial phenotypes is a considerable advance within the field, a detailed understanding of the role of the classic and alternatively activated microglial phenotypes, particularly with relation to chronic microglial responses to injury, is essential.

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