Title: Cellular Players that Shape Evolving Pathology and Neurodegeneration Following Traumatic Brain Injury.

Authors: Shweta S. Puntambekar[1*] (PhD), Maha Saber[2*] (PhD), Bruce T. Lamb[1] (PhD), Olga N. Kokiko-Cochran[3*] (PhD)

*These authors contributed equally to this work.

Author information:

Shweta S. Puntambekar, Ph.D.

Stark Neuroscience Research Institute,

Indiana University-Purdue University,

Indianapolis, IN 46202, USA

Email: sspuntam@iu.edu

Maha Saber, Ph.D.

Barrows neurological institute,

Phoenix Children's Hospital

1919E Thomas Rd Ambulatory bldg, 3rd floor

Phoenix, AZ 85016, USA

Email: saberm@email.arizona.edu

Bruce T. Lamb, Ph.D.

Stark Neuroscience Research Institute,

Indiana University-Purdue University,

Indianapolis, IN 46202, USA

Email : btlamb@iu.edu

Corresponding Author:

Olga N. Kokiko-Cochran, Ph.D.

Department of Neuroscience

Institute for Behavioral Medicine Research Building

460 Medical Center Drive

Columbus, OH 43210, USA

Email: olga.kokiko-cochran@osumc.edu

Affiliations:

1. Stark Neuroscience Research Institute, Indiana University-Purdue University, Indianapolis, IN 46202, USA
2. Barrows Neurological Institute at Phoenix Children's Hospital, Phoenix, AZ 85016, USA
3. Department of Neuroscience, Institute of Behavioral Medicine Research, Ohio State University, Columbus, OH 43210

Abstract (292 words)

Traumatic brain injury (TBI) is one of the leading causes of death and disability worldwide, and has emerged as a critical risk factor for multiple neurodegenerative diseases, particularly Alzheimer's disease (AD). How the inflammatory cascade resulting from mechanical stress, axonal shearing and the loss of neurons and glia following initial impact in TBI, contributes to the development of AD-like disease is unclear. Neuroinflammation, characterized by blood-brain barrier (BBB) dysfunction and activation of brain-resident microglia and astrocytes, resulting in secretion of inflammatory mediators and subsequent recruitment of peripheral immune cells has been the focus of extensive research in attempts to identify drug-targets towards improving functional outcomes post TBI. While knowledge of intricate cellular interactions that shape lesion pathophysiology is incomplete, a major limitation in the field is the lack of understanding of how distinct cell types differentially alter TBI pathology. The aim of this review is to highlight functional differences between populations of bone marrow derived, infiltrating monocytes/macrophages and brain-resident microglia based on differential expression of the chemokine receptors CCR2 and CX_3CR1. This review will focus on how unique subsets of mononuclear phagocytes shape TBI pathophysiology, neurotoxicity and BBB function, in a disease-stage dependent manner. Additionally, this review summarizes the role of multiple microglia and macrophage receptors, namely CCR2, CX_3CR1 and Triggering Receptor Expressed on Myeloid Cells-2 (TREM2) in pathological neuroinflammation and neurodegeneration vs. recovery following TBI. TREM2 has been implicated in mediating AD-related pathology, and variants in TREM2 are particularly important due to their correlation with exacerbated neurodegeneration. Finally, this review highlights behavioral outcomes associated with microglial vs. macrophage variances, the need for novel treatment strategies that target

unique subpopulations of peripheral macrophages, and the importance of development of therapeutics to modulate inflammatory functions of brain-resident microglia at specific stages of TBI.

1. **Introduction.**

Traumatic Brain Injury (TBI) is defined by The Centers for Disease Control and Prevention (CDC) as the functional disruption of the brain from an impact or penetrating injury [1], resulting in apoptotic and necrotic cell death of neurons and glia, disruption of the blood-brain barrier (BBB) with subsequent infiltration of the injured brain by peripherally-derived, immune cell populations such as neutrophils and macrophages. This is accompanied by phenotypic activation of brain-resident glia, namely astrocytes and microglia [2-4]. Approximately 10 million people experience some form of TBI every year [5]. In the United States, 1.4 million people suffer from TBI each year, with approximately 235,000 patients requiring hospitalization and specialized healthcare. Strikingly, TBI accounts for approximately 50,000 mortalities each year, along with over 90,000 individuals facing a lifetime of permanent post-injury impairment [1]. Post-injury disabilities can encompass a myriad of cognitive, motor, and emotional deficits and result in healthcare costs that range into the upper millions to billions of dollars. Of particular importance is the fact that TBI has been identified as a significant risk factor for the development of Alzheimer's disease (AD) related pathological changes including intra-neuronal neurofibrillary tangles (NFTs) of hyperphosphorylated tau (pTau) and deposits of extracellular amyloid-β (Aβ) [6]. It is important to note that single occurrence brain injuries are predominantly associated with AD, Parkinson's disease, and Amyotrophic Lateral Sclerosis (ALS), while Chronic Traumatic Encephalopathy (CTE) is the most prevalent disease associated with repetitive TBI. Given the increasing prevalence of AD worldwide, and that it is currently classified as the most common form of dementia, this review will focus on the development of AD/AD-like pathology following single occurrence TBI. Although there is a growing need to understand disease mechanisms resulting in CTE, these remain beyond the scope of this review.

5

While mechanisms that govern the pathophysiology of TBI lesions and result in AD-like neurodegenerative changes are poorly understood, increasing experimental and clinical data implicate chronic post-injury neuroinflammation as a potential catalyst for TBI-related neurodegeneration and cognitive decline. The lack of a clear understanding of the complexity of the inflammatory response following TBI has been a significant hindrance to the development of successful therapeutics to minimize and/or ameliorate post-injury deficits. The unique pathological and inflammatory features of TBI vary based on the severity (mild vs. moderate vs. severe) and duration (single vs. repetitive) of the injury [2-4]. This, in combination with the evolving nature of TBI lesions as injury becomes chronic, has added to the dilemma of failed treatment paradigms. Lastly, extensive research has been dedicated towards development of anti-inflammatory drugs targeting functional responses of immune cell populations, namely the blood derived macrophages and the brain-resident microglia. However, an incomplete understanding of their unique contributions to TBI pathogenesis in a disease-stage dependent manner has led to several failed clinical trials and ineffective treatment strategies [7]. This review seeks to outline what is currently known about cellular mechanisms that shape inflammatory responses post moderate to severe single occurrence TBI, with a particular focus on defining functional differences between activated peripheral macrophage populations and brain-resident microglia, and how they relate to development of AD-like, neurodegenerative pathology post TBI.

1.1. The problem with 'immune privilege' in TBI.

The central nervous system (CNS), composed of terminally differentiated neurons and highly specialized glial cells, the astrocytes, microglia and oligodendrocytes, comprises a unique neuro-immunologic niche. Complex interactions between various populations of glia and neurons are critical for the functional integrity of the CNS. Based on homeostatic mechanisms

6

involving critical immune interactions evolved to limit immune-mediated damage and loss of CNS function, the CNS is considered 'immunologically privileged' where responses with a potential to cause bystander injury to healthy tissue are selectively restricted and those that promote repair and homeostasis are preserved and/or enhanced [8,9].

Specialized draining lymphatics and the recently discovered, glial-dependent glymphatic system that allow for controlled cellular and molecular egress into the deep cervical lymph nodes [9,10], and limited cellular expression of major histocompatibility complex (MHC) molecules limit endogenous antigen presentation [8,9]. Constitutive expression of soluble immune-suppressive factors like TGFβ, macrophage migratory inhibition factor (MIF) and neurotrophins results in tolerance-inducing antigen presenting cells (APCs) leading to overall T-cell suppression and inhibition of Natural Killer (NK) cell-mediated cytolysis. Furthermore, bi-directional signaling between neurons and microglia via fractalkine (CX_3CL1), CD200 and neurotransmitter signaling actively maintains microglia in their homeostatic activation state [11]. Finally, blood vessels lining the CNS are composed of specialized endothelial cells with restricted expression of adhesion molecules and presence of tight junction proteins that act to restrict the access of large molecules and peripheral immune cells [3,12]. The basement membrane separates the endothelium from the brain parenchyma, and is juxtaposed with pericytes, astrocytic endfeet and microglia. This neurovascular unit is collectively called the 'blood-brain barrier (BBB)' and is the final, important factor in CNS immune privilege [12].

This neurological milieu presents a unique challenge following trauma or injury. Pro-inflammatory immune signaling is critical for the resolution and containment of pathogens, but also causes bystander, immune-mediated cell death. Although heightened anti-inflammatory responses can minimize bystander damage, early inhibition of inflammation can lead to chronic

pathology and lesion spread. Thus, the CNS immune response has to be finely tailored to a) allow for a controlled pro-inflammatory response that effectively resolves initial pathology and limits the expansion of injury lesions into surrounding, uninjured tissue and b) mount a well-timed anti-inflammatory response that facilitates repair mechanisms without resulting in the persistence of the initial injury.

1.2. Injury Mechanisms in TBI: The 'double edged sword' of neuroinflammation.

Pathology following brain trauma is an evolving continuum of two phases of injury. Primary pathology that manifests as neuronal and glial cell death, axonal shearing/injury, edema and disruption of the BBB is a direct consequence of physical and mechanical impact on the brain. Cellular death results in the release of DNA, RNA, ATP, reactive oxygen species (ROS) and necrotic cellular debris [2-4]. These 'damage associated molecular patterns' (DAMPs), via signaling through pattern recognition receptors (PRRs) and Toll like receptors (TLRs) trigger activation of the resident immune cells of the brain, namely the microglia and astrocytes, and initiate the first wave of neuroinflammation (Fig 1). This involves inflammasome-activation and release of cytokines (TNF, IFNγ, IL-1β, IL-10), chemokines (CCL2, CCL5, CXCL9) and inflammatory mediators (GM-CSF, Type-I Interferon, complement proteins, Nitric Oxide), that further coordinate the recruitment, expansion and survival of peripheral immune cells such as the macrophages, dendritic cells, T cells and B cells within the injured tissue [2-4,13]. Gliosis associated with the injury serves to segregate the lesioned region from the surrounding healthy tissue and thereby prevents pro-inflammatory cytokine and ROS mediated bystander cell death [2,3]. Additionally, studies of traumatic spinal cord injury (SCI) have demonstrated that lesion-associated reactive astrocytes and microglia secrete BDNF, TGFβ, and FGF2 that promote neuronal survival and recruitment and differentiation of oligodendrocyte progenitor cells (OPCs)

[14]. Thus, the acute inflammatory response creates an environment conducive for tissue repair by promoting efficient clearance of cellular debris and effective regenerative/repair promoting cell activation (Fig 1).

Although necessary for initiation of active repair, the dysregulation of the acute immune response results in enhanced and sustained inflammation and subsequent, secondary, immune-mediated tissue damage. Chronic microglial activation and persistent production of pro-inflammatory mediators like ROS, IFNγ, IL-1β, and complement protein C1qa result in neuronal cell death. Microglial derived pro-inflammatory mediators such as IL1-α, TNF and C1qa can alter astroglial activation and skew them towards a highly neurotoxic A1 phenotype (Fig.1) which is characterized by enhanced expression of complement component 3 (C3) [15]. Additionally, these astrocytes are impaired in their ability to engulf synapses, leading to defects in synaptic pruning and synapse elimination [15]. Lastly, the 'glial scar', composed of glial cells such as astrocytes and NG2 glia, along with pericytes and meningeal cells, surrounds the primary injury lesion and represents a molecular and physical barrier that inhibits transected axons from regenerating beyond the fibrotic tissue [2,3]. The cognitive and neurological deficits that persist for years following initial injury have largely been attributed to this chronic pro-inflammatory response impeding axonal growth/regeneration.

2. BBB dysfunction following TBI.

The BBB is a selective barrier formed by cerebral microvascular endothelial cells and offers physical, transport, and metabolic protection to the brain. Tight junctions between endothelial cells form a physical barrier that selectively limits the transport of molecules and prevents entry of plasma proteins and peripheral antigens into the brain [3,12]. However, various small molecules (e.g., O_2 and CO_2) and lipophilic agents can diffuse through the lipid membrane.

In addition, a transport barrier is formed by specific systems on the luminal and abluminal membranes, which regulate transcellular movement and endocytosis [16]. As a result, large hydrophilic peptides and proteins, including cells of the peripheral immune system, are unable to cross the BBB unless they engage in a specific receptor-mediated transcytosis. Finally, various intra- and extra-cellular enzymes such as peptidases, nucleotidases, and monoamine oxidases that have the ability to inactivate neurotoxic compounds form a metabolic barrier. Thus, the BBB provides both passive and active protection to the brain, which can be significantly compromised following TBI [3,12].

Ultimately, the BBB serves as a mechanism by which essential nutrients are allowed access to the brain while simultaneously mediating the clearance of waste or cellular debris from the CNS, thereby actively maintaining a homeostatic equilibrium. By restricting ionic and fluid movement between the blood and brain, the BBB assists in creating an optimal environment for neuronal functioning. The BBB also acts as a barrier between neurotransmitters and other lipophilic agents that are active in the CNS, and peripheral tissue, thereby limiting crosstalk between the two systems in a healthy state [3,12]. Consequently, disruption or damage to the BBB can hamper barrier protection and jeopardize brain homeostasis. In the case of TBI, post-injury BBB dysfunction can be biphasic [17] and occur as a direct result of the primary injury or as a delayed consequence of sustained inflammatory and cellular responses associated with the primary injury [2,3]. Influx of plasma proteins into the CNS through a damaged BBB following TBI is sufficient to induce a neuroinflammatory response (Fig.1). Subsequent post-injury edema and increased intracranial pressure facilitate secondary injury cascades that affect all elements of the neurovascular unit, including neurons, astrocytes, pericytes, and microglia [2-4]. Increased production of free radicals and cytokines as well as matrix metalloproteinases (MMPs) such as

MMP2, MMP3 and MMP9 increase BBB permeability and promote activation of local astrocytes and microglia (Fig.1) [3,12]. The long-term consequence of this acute inflammatory response is largely dependent on the brain's ability to reorganize and reestablish homeostasis. As a result, mild to moderate TBI may result in BBB dysfunction characterized by a transient opening of endothelial tight junctions resulting in a temporary influx of inflammatory molecules and immune cells from the periphery. Conversely, severe or repetitive TBI can result in a chronic breakdown of BBB function with subsequent long-term inflammatory consequences in the brain [2-4].

Increasing evidence supports the notion that microglial activation impairs BBB function. First, microglial production of MMP2, MMP3, and MMP9 disrupts proteins in the basal lamina and degrades tight junctions following TBI [17]. Second, experimental models have shown that reactive microglia produce oxygen free radicals and their reaction products, namely hydrogen peroxide and NO, facilitate oxidative damage and neurodegeneration [2,3,12]. Consequently, this oxidative stress increases the permeability of endothelial cells, heightens expression of cell-adhesion molecules and enhances immune cell infiltration [18]. By contrast, recent experimental studies revealed that juxtavascular microglial cells are critical in mediating rapid closure/repair of small leaks in the BBB [19]. Specifically, movement of microglial processes in response to small openings in the BBB is essential in determining how quickly the leaks are resolved. This physical barrier, in addition to potential trophic factors released from microglia, lend support to the notion that microglia are integral to the neurovascular unit and facilitate structural integrity of the BBB [12,19]. Thus in the context of TBI, brain injury induced microglial reactivity or microglial damage may significantly impair the cell's ability to appropriately respond to BBB disruption and support structural recovery from the primary insult. Signaling at the BBB can

potentially actively shape pathological changes occurring at the lesion-site following TBI. How these various cells and inflammatory mediators shape the function of the BBB post TBI, and whether the severity of TBI can skew astroglial and/or microglial responses towards exacerbating or repairing BBB dysfunction is still not completely understood (Fig.1).

3. <u>Cellular Responses in TBI: Microglia vs. Macrophages.</u>

Although elaborate cellular interactions are implicated in TBI pathology, activation of CNS resident microglia and subsequent recruitment of inflammatory macrophages constitute the majority of the immune response within 48-72 hours of TBI (Fig.1). Additionally, while the peripheral immune response subsides by 2-3 weeks, the lesion-associated activation of microglia/macrophages persists for months to years following initial injury [2,3]. Thus, dysregulated acute activation of microglia and macrophages can not only exacerbate lesion pathology, but can also result in chronic bystander tissue damage. The experimental ablation of CD11b-expressing macrophage populations has been employed to understand their functional contributions to pathophysiology of TBI lesions, but have proven to be inconclusive. This is likely to be due to the depletion of both CD11b-expressing microglia along with infiltrating peripheral macrophages, thereby making it difficult to attribute the unaltered axonal injury and lesion volume to their differential neurotoxic and/or neuroprotective functions [2]. Additionally, the ablation requires the systemic administration of drugs, which act to deplete all CD11b$^+$ populations including monocytes, macrophages and dendritic-cell subsets. This creates a heightened peripheral inflammatory milieu that may further influence the acute immune response post TBI. Moreover, while acute depletion of CD11b cells before TBI skews the CNS towards a

pro-inflammatory state, these studies showed that the drugs used to deplete the CD11b cells in the murine models themselves exerted neurotoxic effects that were independent of the injury [2].

Several gene expression studies have attributed distinct genetic signatures to CNS resident microglia and peripherally derived macrophages under homoeostatic and inflammatory/neurodegenerative conditions. There has been an increasing interest in understanding how resident microglia and infiltrating monocytes/macrophages may differentially modulate TBI lesions. Based on their surface expression of the chemokine receptors CCR2 and CX₃CR1, various subsets of bone marrow and yolk sac derived myeloid populations have been identified in rodent models of disease [20]. In peripheral blood, two distinct monocyte subsets express distinct profiles of CX₃CR1 and CCR2. CCR2^{high} CX3CR1$^{low/int}$ Ly6c^{high} monocytes are the inflammatory subset that enters target tissues in response to injury and differentiates into inflammatory macrophages. In contrast, the CCR2^{+} CX₃CR1^{hi} Ly6c^{-} subset represents the patrolling monocyte population that is involved in active surveillance of the vasculature, where they are known to scavenge and eliminate cellular debris and resolve inflammation [20].

3.1. Roles of peripheral, 'inflammatory' macrophages in TBI – not all macrophages are equal.

Predominant expression of CCR2 on populations of inflammatory and patrolling monocytes has been used as a basis to distinguish them from CCR2^{-} resident microglia [14]. Using genetic ablation of elements of the CCR2-CCL2 axis or treatment with CCR2 antagonists to restrict the peripheral macrophage response to TBI, various groups have identified unique roles for infiltrating macrophages [21,22]. While loss of CCR2 signaling does not alter chronic cortical or hippocampal tissue loss, CCR2$^{-/-}$ mice display improved long-term spatial memory and

13

learning 9 weeks post TBI. Interestingly, although chronic volume loss in the CA1-CA3 region was similar in the WT and CCR2$^{-/-}$ cohorts, CCR2 deficiency protected CA1-CA3 neurons from TBI-induced death [21]. Apart from being crucial for monocyte recruitment to the site of injury, CCR2-CCL2 signaling is critical for the egress of monocytes from the bone marrow. A similar improvement in hippocampal-dependent learning and memory has been reported using the CCR2 antagonist, CCX892 [22]. It is important to note that improved cognition and neuronal protection associated with CCR2 deficiency manifests almost 9 weeks post TBI. However, accumulation of CCR2^{+} macrophages is an acute inflammatory response that peaks within hours-days of the injury and contracts to baseline levels by 2 weeks post TBI [2,3]. In contrast to the chronic effects of CCR2 deficiency, disrupted CCR2 signaling in *CCR2$^{RFP/RFP}$* reporter mice results in smaller lesion cavities with reduced axonal pathology 3 days post TBI [23]. However, while TBI increases overall tau phosphorylation, the absence of CCR2 causes translocation of axonal pTau to neuronal soma. These data suggest that an acute loss of a CCR2^{+} peripheral macrophage response limits tissue loss and axonal damage but may aggravate development of neuro-fibrillary tangle pathology [23]. These observations suggest two functionally distinct macrophage subsets – one associated with exacerbation of pathology, while another involved with prevention of intra-neuronal aggregates of pTau. An approximate 25-50% preservation of the macrophage response in the CCR2$^{-/-}$ mice or following treatment with CCX892 [22], further suggests CCR2-CCL2 independent recruitment of macrophage subpopulations following TBI.

Lastly, studies using reporter mice to track arginase-1 (Arg1) expressing cells have identified Arg1^{+} and Arg1^{-} populations of infiltrating macrophages [24]. Although both subpopulations express a mixed phenotype of pro- and anti-inflammatory markers, Arg1^{+} macrophages preferentially upregulate genes associated with wound healing responses (*CD9,*

Spry2), regulation of tissue injury (*CD36, Hmox1*) and neuroprotection (*Mt2, CD38*), while Arg1⁻ macrophages show a biased upregulation of *IL1β, MHC class II* and *Ciita* indicative of a pro-inflammatory phenotype [18]. These data raise some pertinent questions/observations that remain unanswered:

1. How do various subsets of infiltrating macrophages work in concert to shape the evolution of TBI lesion pathology? Activation phenotypes of macrophage subsets are highly fluid and dynamic, thus creating a lesion microenvironment of a mixed monocyte/macrophage response. The heterogeneity of chemokines that recruit peripheral macrophages to the CNS, gives rise to the possibility of distinct signaling mechanisms driving infiltration of these individual macrophage subsets (Fig.1).

2. Can activated, infiltrative macrophages drive protective responses post TBI? TBI has been associated with an increased risk of developing AD-like pathology, including aggregated tangles of pTau and subsequent dementia[5]. It is therefore an interesting speculation that there may be a distinct macrophage subpopulation that confers protection from development of tauopathies post TBI (Fig.1).

3. How does an acute and/or partial ablation of the macrophage response ameliorate chronic cognition and memory? Data suggests that either acute ablation of a pro-inflammatory macrophage population and / or selective, chronic retention of a repair promoting subset may be critical for neuroprotection and subsequent improved cognition.

Using the *CX3CR1^{GFP/+}CCR2^{RFP/+}* reporter mice, Morganti et al have characterized the kinetics of the macrophage response following TBI [22]. This study shows that while CX_3CR1 expression is limited to the CNS resident microglia following acute injury, chronic injury results in upregulation of CX_3CR1 expression on infiltrating CCR2⁺ inflammatory macrophages. These

15

$CX_3CR1^+CCR2^+$ macrophages are distinct from the $CX_3CR1^+CCR2^-$ resident microglia and persist for up to 3 weeks post TBI. However, $CX_3CR1^+CCR2^+$ macrophages fail to persist in CNS as the injury becomes chronic, and the chronic TBI lesion remains associated with $CX_3CR1^+CCR2^-$ cells [22]. The up regulation of CX_3CR1 on activated peripheral macrophages may allow for in-situ control of their inflammatory response via neuronal and endothelial CX_3CL1 signaling, thereby tailoring them towards a protective phenotype. Whether myeloid cells associated with chronic injury lesions represent a purely chronic microglial response or are composed of a combination of resident microglia and CX_3CR1^+ peripheral macrophages that have down-regulated their CCR2 expression is currently unknown. Thus, while TBI therapies have targeted a pan-CCR2 response, these studies suggest that a more tailored approach that ablates or enhances specific macrophage subsets may be the way forward.

3.2. Targeting brain resident microglia in TBI

Persistent and progressive physical and/or cognitive decline as a consequence of brain injury has been largely attributed to sustained pro-inflammatory microglial activation [25]. However, the precise contribution of these cells to chronic TBI pathology has remained controversial. Neuroimaging with the positron emission tomography (PET) ligand [11C](R)PK11195 (PK) has been widely used to study chronic microglial responses in TBI patients. PK 11195 binds to the 18kDa translocator protein (TSPO) expressed on the outer mitochondrial membrane and is upregulated on activated astrocytes, microglia and peripheral macrophages [4]. TSPO ligands thus serve as a good measure of chronic gliosis. Elucidating microglial responses in chronic pathophysiology is further complicated by the paucity of differential markers to distinguish resident microglia from peripheral macrophages.

Given that microglia are the resident macrophage population of the CNS, their roles under physiological conditions and in the post injury brain are crucial for the maintenance and/or return to homeostasis. Two-photon imaging of microglia has revealed that these cells constantly survey the CNS microenvironment with highly motile processes. Through *de novo* process formation and retraction, microglia are responsible for organized scanning of CNS micro-regions, ensuring exhaustive and repetitive surveillance of the healthy brain tissue. Unlike homeostatic immune surveillance, microglial response to CNS trauma results in the rapidly polarized extension of processes towards the injury [26]. Hence, microglia are the primary innate immune response to injury, and also constitute the predominant macrophage population associated with chronic lesions. Furthermore, owing to their continuous and active inspection of the CNS, microglia are uniquely situated to detect and/or contribute to any pathological changes that are temporally and anatomically distal to the initial injury.

In the healthy brain, microglia are maintained in a homeostatic state through a network of paired cell surface receptor-ligand interactions between microglia and neurons such CD200-CD200R1 and CD47-CD127a [11]. One such mechanism is through the chemokine, fractalkine (CX_3CL1) which signals via the dedicated receptor CX_3CR1. Survivors of severe TBI show long-term elevation in CSF levels of CX_3CL1 indicating a role for fractalkine signaling in TBI pathophysiology [27]. A microglial phenotype as a result of chronic activation/inflammation is associated with heightened neurotoxicity in several models of neurodegeneration (AD, PD and ALS) and injury. The primary disease process results in 'microglial priming' that is associated with downregulation/loss of CX_3CR1 expression, and is associated with an enhanced secretion of inflammatory cytokines, chemokines and ROS upon lesion induced pro-inflammatory stimulation (Fig.1) [28]. Exclusive expression of CX_3CR1 by the microglia within the CNS has

17

thus made the CX_3CL1-CX_3CR1 axis an attractive therapeutic target for the modulation of microglial responses post TBI.

3.3. CX_3CR1 and its role in microglial protection of the brain

Studies using mice deficient in CX_3CR1 have revealed an evolving role of CX_3CR1 signaling as pathology transitions from the acute to chronic stages post mild TBI. Lesions in the $CX_3CR1^{-/-}$ mice show increased association with cells expressing YM1 and CD206/mannose receptor at 7 days post injury (dpi)[29]. This activation phenotype, which is induced by IL-4/IL-13 mediates clearance of cellular debris via endocytosis and phagocytosis and is involved in tissue repair/wound healing responses. A concomitant reduction in the numbers of CD68$^+$ cells along with reduced iNos and IL-1β gene expression in the cortices of injured $CX_3CR1^{-/-}$ mice indicate a skewing of the acute lesion microenvironment towards a protective microglial phenotype [29,30]. As the injury becomes chronic, the $CX_3CR1^{-/-}$ lesions display an increased density of iNos$^+$ cells relative to injured WT controls. Interestingly, the chronic loss of CX_3CR1 leads to increased abundance in the hippocampus of cells expressing the scavenger receptor 'Macrophage Receptor with Collagenous Structure' (MARCO), which is implicated in enhanced pro-inflammatory cytokine secretion. Although injured $CX_3CR1^{-/-}$ and WT controls showed similar absolute numbers of CD68$^+$ cells in the hippocampus at chronic time-points, these inflammatory phagocytes displayed a hypertrophic morphology [29] with elevated CD11b expression, consistent with sustained activation which persists for up to 5 weeks post injury [30]. This shift from a protective to an inflammatory activation status in the absence of CX_3CR1 signaling correlates with reduced numbers of neurons in the cortex of $CX_3CR1^{-/-}$ mice with no differences in chronic lesion volumes. These data suggest a duality in CX_3CR1 signaling where an acute, pro-inflammatory lesion driven by CX_3CR1^+ microglia results in enhanced neurotoxicty. However,

18

chronic pro-inflammatory microglial activation is attenuated by CX_3CR1, and is critical in limiting bystander hippocampal pathology (Fig.1). Interestingly, unlike the worsening of chronic pathology in the $CX_3CR1^{-/-}$ mice, a heterozygous deletion in CX_3CR1 ($CX_3CR1^{+/-}$) results in reduced neurodegeneration, smaller lesions and better functional recovery in comparison with WT controls at 80 days following moderate TBI [31]. *Erturk et al* report that while moderate TBI results in a chronic lesion cavity (1 yr post TBI) that extends into the deeper regions of the hippocampus and thalamus, lesions in $CX_3CR1^{+/-}$ mice are restricted to the superficial cortical layers with a complete preservation of hippocampal structures. These data suggest that a complete loss of CX_3CR1 ($CX_3CR1^{-/-}$) mediated control of microglial activation can drive chronic bystander neurotoxicity via exaggerated chronic pro-inflammatory microglial responses. However, a dampened CX_3CR1 response ($CX_3CR1^{+/-}$) may allow for controlled microglial activation that not only restricts the chronic access of potentially neurotoxic peripheral immune lymphocytes and macrophages to the injured CNS but also protects deeper brain regions from neurodegeneration [31].

How do microglia mediate long-term neuroprotection? The paradigm of CNS preconditioning has been studied extensively as a neuroprotective therapeutic approach following TBI and stroke. A broad range of stimuli, including the bacterial endotoxin lipopolysaccharide (LPS), is currently being tested to this end. Using a bone-marrow chimera approach, *Chen et al* generated mice with LPS-mediated TLR4 signaling restricted to the periphery or the CNS [32]. These authors demonstrate that systemic treatment with low-dose LPS skews microglia towards a neuroprotective phenotype, as evidenced by enhanced expression of genes associated with 'alternative activation' that are responsible for an anti-inflammatory/repair-promoting phenotype, characterized by the expression of *Ym1, Arg1, Socs3,*

IL4-Rα and IL1Ra with minimal expression of pro-inflammatory genes such as *CCR2, iNos,* and *TNF*. Furthermore, this pre-conditioning response depends upon TLR4 expression within the brain and remains unaltered by the absence of TLR4 on peripheral immune cells. Interestingly, LPS pre-conditioning resulted in increased neuronal ensheathement by activated microglia. Immuno-electron microscopy revealed a displacement of synaptophysin[+] afferent synapses at the neuronal surface directly apposed by primed-microglia, and this correlated with significantly reduced lesion volumes and neuronal death following experimental cryogenic brain injury [23]. As increased TLR-signaling downstream of TBI is a predominant acute immune response, it is tempting to speculate that controlled TLR-dependent microglial activation can not only alter the acute lesion microenvironment but may also drive chronic synaptic remodeling/modification post injury.

Another possible mechanism of chronic neuroprotection is the active repair of the disrupted BBB[19,33]. Indeed, significant reduction in the frequencies of peripheral immune cells in $CX_3CR1^{+/-}$ injured brains relative to WT controls at 1 year post injury indicate a microglial contribution to chronic retention of macrophages and the re-establishment of an intact BBB [31]. An elegant study using two-photon laser scanning microscopy has demonstrated microglial subsets that undergo dramatic morphological alterations forming 'honeycomb' or 'jellyfish' like structures following a closed skull, mild, compression induced TBI [33]. An extensive network of honeycomb microglia lines the compromised glia limitans within an hour post injury. These microglia surround, and presumably protect the surviving astrocytes in the acute lesion. Astrocytic death associated with lesion progression corresponds with extension of non-branching microglial processes, also called 'jellyfish projections' towards the glia limitans. The formation of an uninterrupted layer of phagocytic jellyfish projections at the glia limitans denotes an effort by

CNS resident microglia to protect a disrupted BBB in the event of astrocytic death or dysfunction. The eventual death of these jellyfish microglia causes the lesion to spread and results in neutrophil infiltration and neuronal loss. Interestingly, while inhibition of microglial-specific purinergic receptors P2RY12 and P2RX4 blocked formation of honeycomb and jellyfish structures, inhibition of P2RX7 completely blocked the neutrophil response to injury. Lastly, blocking connexin hemichannels to dampen astrocytic ATP production compromised purinergic receptor dependent formation and these microglial networks, resulting in enhanced BBB permeability following injury [33]. Indeed, ATP mediated polarization of stable microglial processes towards injury lesions has been previously described as a protective mechanism that limits diffuse neurotoxicity [26]. These data shed light on the intricate crosstalk between the astrocytes and microglia involved in the maintenance of BBB integrity (Fig.1). Thus astrocytic ATP mediated enhancement of purinergic receptor signaling by CNS resident microglia may be an important factor in the formation of stable microglial networks that facilitate the re-establishment of an intact BBB in chronic TBI.

4. TREM2 as a modulator of pathological neuroinflammatory responses: a link between TBI and AD pathology.

Post mortem analysis of brain tissue has demonstrated increased accumulation of extracellular deposits of Aβ and intra-neuronal NFTs, hallmarks of AD-pathology, in patients with TBI. While the degree of AD-like pathology varies depending on the severity and duration of the initial injury, the incidence of these pathological features in a significant cohort of TBI cases, underscores the possibility of an immunological overlap in disease mechanisms in TBI and AD [6]. Recent Genome Wide Association Studies (GWAS) have identified crucial genetic variants related to innate immune signaling, including TREM2, that confer increased

susceptibility to development of AD and other neurodegenerative diseases and can also significantly alter the neurological outcome and recovery following TBI [34]. Clinically, homozygous loss of function mutations in TREM2 have been linked to the development of Nasu-Hakola Disease (NHD), a form of progressive, presenile dementia in which patients develop systemic bone cysts and progressive encephalopathy leading to mortality within the fifth decade of life. These studies indicate that TREM2 plays a neuro-protective role in CNS disease/injury [35].

TREM2 is a member of the immunoglobulin (Ig) superfamily and is expressed by cells of the myeloid lineage, including macrophages, dendritic cells, granulocytes and osteoclasts in the periphery and by microglia within the CNS [35]. *In vitro* studies have demonstrated that TREM2 signaling dampens classical/pro-inflammatory microglial activation. shRNA mediated downregulation of TREM2 expression in cultured primary microglia results in increased secretion of TNFα and iNos, without altering expression of pro-inflammatory activation markers like MHC Class II, CD86 and CD11b [36,37]. Subsequent studies have revealed that this occurs through the negative regulation of the toll-like receptor (TLR) pathway [38,39]. TREM2 also affects phagocytosis as demonstrated by increased TREM2 expression by BV2 microglia upon co-culture with apoptotic neurons. Also, transfection of TREM2 into non-phagocytic Chinese Hamster Ovary (CHO) cells increases their phagocytic engulfment of apoptotic neurons [37]. A recent report by *Yeh et al*, suggests that TREM2 dependent uptake of Aβ-lipoprotein complexes results in rapid degradation of phagocytosed Aβ *in-vitro*, thereby providing evidence that TREM2 is necessary for Aβ clearance [40].

Despite compelling in-vitro studies, elucidating the role of TREM2 *in-vivo*, using murine models of injury and/or disease have yielded conflicting results. Analysis of transcriptional

profiles of microglia purified from 8 month old 5XFAD;TREM2$^{+/+}$ and 5XFAD;TREM2$^{-/-}$ mice, show impaired expression of MHC Class II, CD11b, and pro-inflammatory cytokines TNFα and IL1β in the absence of TREM2 [41]. Additionally, while the loss of TREM2 does not alter the capacity of microglia to phagocytose and degrade Aβ and apoptotic cells in 8 month old 5XFAD mice [41], TREM2 is required for the internalization of fibrillar Aβ by microglia isolated from 4 month old 5XFAD;TREM2$^{+/+}$ mice [42] and for the phagocytic clearance of apoptotic cells and myelin debris in murine models of stroke and cuprizone-induced demyelination [43,44]. Furthermore, TREM2 signaling is critical for myeloid cell survival and proliferation in the AD brain, and reduces plaque-associated neurotoxicity by regulating compaction of toxic Aβ deposits [35,42]. Thus, not only does TREM2 play varying roles *in-vivo* depending on the injury and/or disease being studied, but the downstream effects of TREM2 signaling change at different stages of pathology.

Using the lateral fluid percussion injury (FPI) model, *Saber et al* have demonstrated TREM2 dependent worsening of chronic hippocampal atrophy and cognitive decline. While TREM2 deficiency reduces the overall recruitment of peripheral macrophages to the CNS following injury [45], acute lesions (3 dpi) in TREM2$^{-/-}$ mice display an increased number of CD45^{+} and F4/80^{+} cells associated with injury lesions, with no differences in mRNA expression of TNF, IL-1β and IL-6 and lesion volumes between groups. Interestingly, injured, WT control animals display reduced hippocampal volumes in the ipsilateral and contralateral hemispheres as compared to TREM2$^{-/-}$ mice at 120 dpi, a stage with no differences in lesion-associated, pro-inflammatory cellular-immune responses [45]. These results are unique, in that they suggest contrasting roles of TREM2 during acute vs. chronic injury (Fig.1). While, TREM2 may limit acute macrophage recruitment and dampen early inflammatory responses in the injured CNS, it

23

exacerbates neurodegeneration during chronic TBI, by mechanisms that maybe independent on inflammatory dysfunction.

A similar reduction in the recruitment of a population of CD11b$^+$CD45^{hi}Ly6c^{high} peripheral macrophages is associated with TREM2 deficiency in the APPPS1 and 5XFAD mouse models of AD [46]. Reduced macrophage recruitment correlates with ameliorated Aβ plaque pathology in the early stages of disease. However, APPPS1; TREM2$^{-/-}$ animals show increased accumulation of larger Aβ plaques in the cortex in late-stage AD, along with reduced expression of *TNF*, *IL-1β* and *IL6* mRNA as compared to APPPS1; TREM2$^{+/+}$ mice [47]. It is important to note that TREM2 can be expressed on brain-resident microglia as well as the peripherally derived macrophages and the temporal profile of this cell-type specific TREM2 response may shape lesion pathology (Fig.1). A recent elegant study using transcriptome analysis has suggested that attenuated CX$_3$CR1 signaling may drive an increase in microglial-TREM2 expression in the 5XFAD model of AD. This TREM2$^+$ microglial phenotype is associated with an increased Aβ phagocytic capacity, and thereby is hypothesized to be protective in the context of amyloid clearance [48]. It is an interesting hypothesis that while acute macrophage associated TREM2 signaling may exacerbate neuropathology; chronic microglial TREM2 is a critical neuroprotective response (Fig.1). Thus, how distinct cellular sources of TREM2 shape acute vs. chronic TBI pathophysiology remains to be investigated. Taken together, these studies are indicative of common TREM2-dependant mechanisms that may govern TBI mediated AD-like pathology in a disease-stage dependent manner.

24

5. Altered Neuroinflammation Influences Behavioral Recovery

Inflammation in both TBI and AD has been linked to behavioral changes in multiple rodent models but remains controversial. However, recent studies suggest that the infiltration of peripheral macrophages is particularly detrimental. Mice deficient for the receptor CCR2, important for monocyte chemotaxis, or animals treated with CCR2 antagonists, exhibit a reduction in infiltrating macrophages that correlates with improved spatial learning and memory after TBI [13,21,22]. Further studies using reporter mice for CCR2 have reported the presence of CCR2$^+$ peripheral macrophages in areas sensitive to injury-induced pathology and inflammation, namely the CA3/4 region and the dentate gyrus, and depletion of these macrophages correlates with improved learning and cognitive behavior after TBI [22]. Conversely, deficiency in CX_3CR1, a protein important in microglial function, increases cognitive dysfunction after TBI suggesting that microglial activation is necessary for behavioral recovery after TBI [29]. *Febinger et al* found no changes in locomotor activity or anxiety as measured through open field after brain injury in WT control mice. However, CX_3CR1 deficiency inhibits motor and spatial learning after TBI. Reduced deficits in spatial working memory and spatial reference memory in injured TREM2 deficient mice, correlate with reduced macrophage infiltration in the brain [45]. A similar decrease in peripheral macrophage infiltration and Aβ pathology with TREM2 deficiency has also been reported in early disease in the APPPS1 and 5XFAD models of AD [42,46]. Conversely, other studies have found that TREM2 upregulation ameliorates AD related behavioral deficits by modulating microglial function in mouse models of AD [49,50]. These studies together suggest that while controlled microglial activation is beneficial in reducing behavioral deficits related to TBI and AD, the infiltration of peripheral macrophages is detrimental in AD and TBI recovery. More

research is necessary in this field to identify potential therapeutic targets for reducing behavioral deficits associated with both TBI and AD.

6. Conclusions

TBI induces a unique and complicated inflammatory response. A concise overview of this inflammatory response and its effect on behavior can be found in Figure 1. Substantial effort has been spent on identifying therapeutic targets that modulate the acute post-injury neuroinflammatory response in experimental models; however, limited translation to the clinical setting suggests that a multi-target therapeutic approach is needed to improve chronic outcome. Novel advances in the field, particularly imaging techniques and transgenic mouse models such as those with targeted deletion of CCR2 or CX_3CR1, have proved useful in distinguishing microglia and macrophages in brain injury and AD pathophysiology. These studies suggest that microglia and macrophages maintain the ability to be both beneficial and detrimental in disease pathogenesis and their timing of reactivity is critical in mediating outcome. Future studies that consider the cell specific response of microglia and macrophages to TBI will be necessary in improving post-injury recovery. Recent, genetic-based studies have suggested that inflammation, particularly the peripheral macrophage response, may be the underlying link between brain injury and the progression of AD pathology. Thus, determining the role of genetic influences and the progression of neurodegeneration via novel targets such as TREM2 will also be a key component in effectively managing outcome after TBI.

Figure 1. Lesion pathophysiology following TBI is governed by the dynamics between neurotoxic and neuroprotective/regenerative immune responses. Within hours of TBI (top panel), CNS resident microglia and astrocytes are activated as a consequence of the physical injury and in response to DAMPs released by apoptotic/necrotic cells, along with serum proteins released in the CNS from a disrupted BBB. Neuroprotective gliosis that functions to contain injury lesion begins within minutes following injury (top panel), continues to evolve and persists for months/years after initial injury (middle-bottom panel). These neuroprotective glial responses facilitate the stabilization, repair and re-establishment of the BBB and release chemokines and trophic factors that support recruitment of precursor populations and their in-situ differentiation. Furthermore activated microglia can skew astroglial responses towards a neurotoxic or a neuroprotective phenotype. Acute glial activation results in the secretion of chemokines and the recruitment of peripheral immune cells, namely neutrophils and populations of $CCR2^+$ and $CCR2^-$ macrophages. Macrophage recruitment, which peaks within 2-3 days post injury, results in the accumulation of neurotoxic ($TREM2^+$ and $Arginase1^-$) as well as neuroprotective ($Arginase1^+$) populations (middle panel). Phagocytic clearance of apoptotic cells and other debris by $TREM2^+$ macrophages and microglia may further drive their neurotoxic, pro-inflammatory activation. While CNS resident microglia are the primary CX_3CR1^+ population in the lesion, infiltrating macrophages upregulate CX_3CR1 expression in-situ (middle panel). This population of CX_3CR1^+ microglia and macrophages can be modulated via CX_3CL1 mediated neuronal signaling and may represent a neuroprotective response that can allow for controlled phagocytosis and removal of cellular and myelin debris without eliciting bystander immune-pathology (middle-bottom panels). Accumulation of extracellular deposits of amyloid-β (Aβ) and intra-neuronal neurofibrillary tangles (NFTs) of hyperphosphorylated tau (pTau) as lesions

27

become chronic are accompanied by plaque-associated gliosis that may be pro- and/or anti-inflammatory (bottom panel). In contrast to the acute neurotoxic effects of TREM2 (middle panel), prolonged TREM2 expression on microglia and macrophages may be critical for their survival in the chronic lesion microenvironment. Additionally, sustained upregulation of TREM2 expression on plaque associated microglia and macrophages at this stage may be critical for the phagocytic removal of Aβ and the compaction of Aβ plaques, thereby driving neuroprotective responses (bottom panel).

REFERENCES

1. Centers for Disease C, Prevention. CDC grand rounds: reducing severe traumatic brain injury in the United States. MMWR Morb Mortal Wkly Rep. 2013;62(27):549-52

2. McKee CA, Lukens JR. Emerging Roles for the Immune System in Traumatic Brain Injury. Front Immunol. 2016;7:17.

3. Jassam YN, Izzy S, Whalen M, McGavern D, El Khoury J.Neuroimmunology of Traumatic Brain Injury: Time for a Paradigm Shift. Neuron. 2017; 95(6):1246-1265

4. Ramlackhansingh AF, Brooks DJ, Greenwood RJ, Bose SK, Turkheimer FE, Kinnunen KM, et al. Inflammation after trauma: microglial activation and traumatic brain injury. Ann Neurol. 2011;70(3):374-83.

5. Hyder AA, Wunderlich CA, Puvanachandra P, Gururaj G, Kobusingye OC. The impact of traumatic brain injuries: a global perspective. NeuroRehabilitation. 2007;22(5):341-53

6. Scott G, Ramlackhansingh AF, Edison P, Hellyer P, Cole J, Veronese M, et al. Amyloid pathology and axonal injury after brain trauma. Neurology. 2016;86(9):821-8

7. Chakraborty S, Skolnick B, Narayan RK. Neuroprotection Trials in Traumatic Brain Injury. Curr Neurol Neurosci Rep. 2016;16(4):29

8. Louveau A, Harris TH, Kipnis J. Revisiting the Mechanisms of CNS Immune Privilege. Trends Immunol. 2015;36(10):569-77.

9. Romo-González T, Chavarría A, Pérez-H J. Central Nervous System: a modified immune surveilliance circuit?. Brain Behav Immun. 2012;26(6):823-9

10. Plog BA, Nedergaard M. The glymphatic system in CNS health and disease: past, present and future. Annu Rev Pathol. 2018;13:379-94.

11. Eyo UB, Wu LJ. Bidirectional microglia-neuron communication in the healthy brain. Neural Plast. 2013;2013:456857.

12. Abbott NJ RL, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. Nat Rev Neurosci. 2006;7(1):13.

13. Liu S, Zhang L, Wu Q, Wu Q, Wang T. Chemokine CCL2 induces apoptosis in cortex following traumatic brain injury. J Mol Neurosci. 2013;51(3):1021-9

14. Almad A, Sahinkaya FR, McTigue DM. Oligodendrocyte fate following spinal cord injury. Neurotherapeutics. 2011;8(2):262-73.

15. Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, et al. Neurotoxic reactive astrocytes are induced by activated microglia. Nature. 2017;541(7638):481-7.

16. Begley DJ, Brightman MW. Structural and functional aspects of the blood-brain barrier. Prog Drug Res. 2003;61:39-78.

17. Shlosberg D BM, Kaufer D, Friedman A. Blood-brain barrier breakdown as a therapeutic target in traumatic brain injury. Nat Rev Neurol. 2010;6(7):11.

18. Chodobski A, Zink BJ, Szmydynger-Chodobska J. Blood-brain barrier pathophysiology in traumatic brain injury. Transl Stroke Res. 2011;2(4):492-516.

19. Lou N, Takano T, Pei Y, Xavier AL, Goldman SA, Nedergaard M. Purinergic receptor P2RY12-dependent microglial closure of the injured blood-brain barrier. Proc Natl Acad Sci U S A. 2016;113(4):1074-9

20. Saederup N, Cardona AE, Croft K, Mizutani M, Cotleur AC, Tsou CL, et al. Selective chemokine receptor usage by central nervous system myeloid cells in CCR2-red fluorescent protein knock-in mice. PLoS One. 2010;5(10):e13693.

21. Hsieh CL, Niemi EC, Wang SH, Lee CC, Bingham D, Zhang J, et al. CCR2 deficiency impairs macrophage infiltration and improves cognitive function after traumatic brain injury. J Neurotrauma. 2014;31(20):1677-88

22. Morganti JM, Jopson TD, Liu S, Riparip LK, Guandique CK, Gupta N, et al. CCR2 antagonism alters brain macrophage polarization and ameliorates cognitive dysfunction induced by traumatic brain injury. J Neurosci. 2015;35(2):748-60.

23. Gyoneva S, Kim D, Katsumoto A, Kokiko-Cochran ON, Lamb BT, Ransohoff RM. Ccr2 deletion dissociates cavity size and tau pathology after mild traumatic brain injury. J Neuroinflammation. 2015;12:228.

24. Hsieh CL, Kim CC, Ryba BE, Niemi EC, Bando JK, Locksley RM, et al. Traumatic brain injury induces macrophage subsets in the brain. Eur J Immunol. 2013;43(8):2010-22.

25. Loane DJ, Kumar A, Stoica BA, Cabatbat R, Faden AI. Progressive neurodegeneration after experimental brain trauma: association with chronic microglial activation. J Neuropathol Exp Neurol. 2014;73(1):14-29.

26. Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, et al. ATP mediates rapid microglial response to local brain injury in vivo. Nat Neurosci. 2005;8(6):752-8.

27. Rancan M, Bye N, Otto VI, Trentz O, Kossmann T, Frentzel S, et al. The chemokine fractalkine in patients with severe traumatic brain injury and a mouse model of closed head injury. J Cereb Blood Flow Metab. 2004;24(10):1110-8.

28. Holtman IR, Raj DD, Miller JA, Schaafsma W, Yin Z, Brouwer N, et al. Induction of a common microglia gene expression signature by aging and neurodegenerative conditions: a co-expression meta-analysis. Acta Neuropathol Commun. 2015;3:31.

29. Febinger HY, Thomasy HE, Pavlova MN, Ringgold KM, Barf PR, George AM, et al. Time-dependent effects of CX3CR1 in a mouse model of mild traumatic brain injury. J Neuroinflammation. 2015;12:154.

30. Zanier ER MF, Ortolano F, Perego C, Arabian M, Zoerle T, Sammali E, Pischiutta F, De Simoni MG. Fractalkine Receptor Deficiency Is Associated with Early Protection but Late Worsening of Outcome following Brain Trauma in Mice. J Neurotrauma. 2016;33(11):13.

31. Erturk A, Mentz S, Stout EE, Hedehus M, Dominguez SL, Neumaier L, et al. Interfering with the Chronic Immune Response Rescues Chronic Degeneration After Traumatic Brain Injury. J Neurosci. 2016;36(38):9962-75.

32. Chen Z JW, Shpargel KB, Farabaugh KT, Dutta R, Yin X, Kidd GJ, Bergmann CC, Stohlman SA, Trapp BD. Lipopolysaccharide-Induced Microglial Activation and
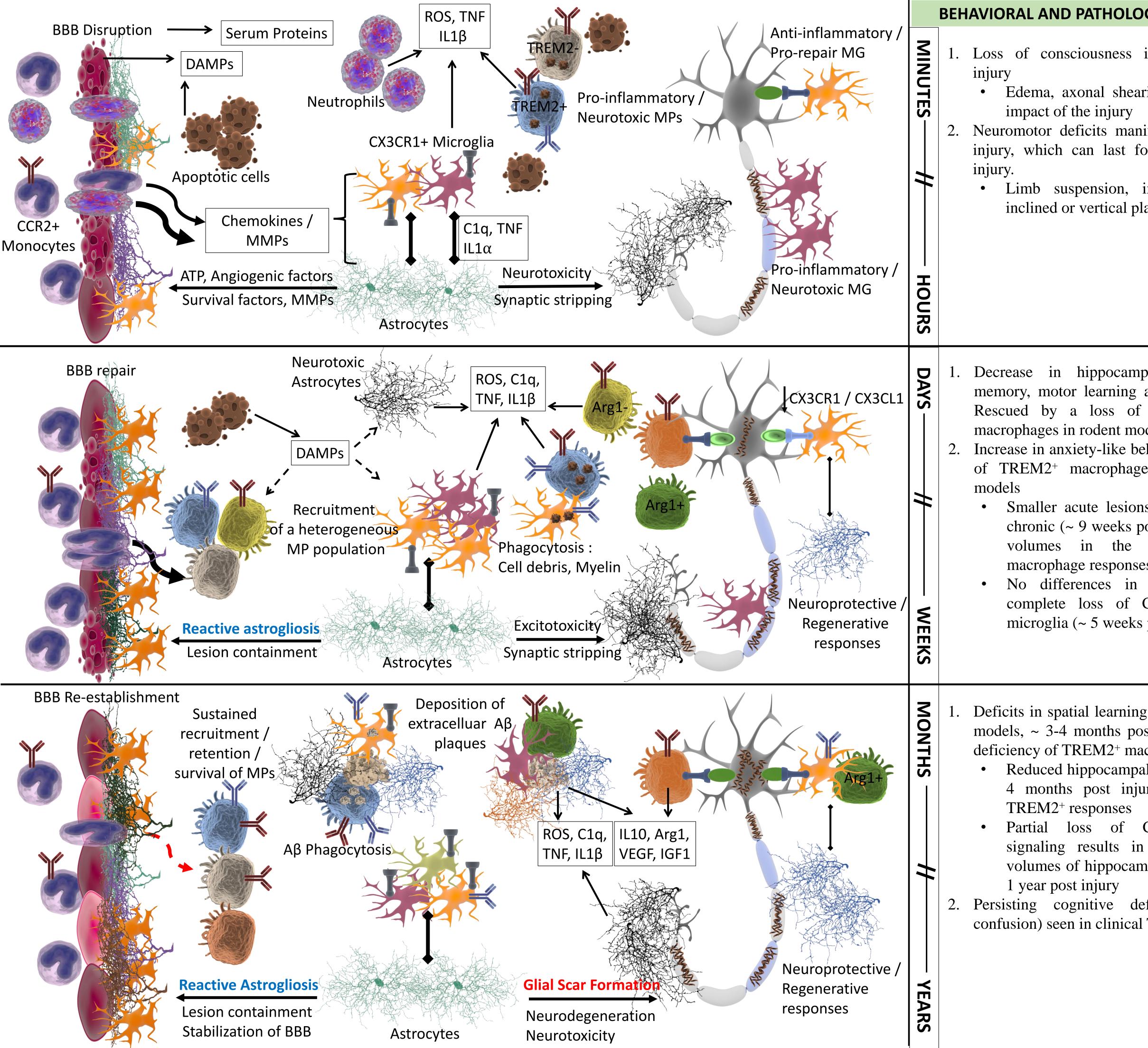
Neuroprotection against Experimental Brain Injury Is Independent of Hematogenous TLR4. J Neurosci. 2012;32(34):10.

33. Roth TL ND, Atanasijevic T, Koretsky AP, Latour LL, McGavern DB. Transcranial Amelioration of Inflammation and Cell Death Following Brain Injury. Nature. 2014;505(7482):20.

34. Guerreiro R, Wojtas A, Bras J, Carrasquillo M, Rogaeva E, Majounie E, et al. TREM2 variants in Alzheimer's disease. N Engl J Med. 2013;368(2):117-27.

35. Kober DL, Brett TJ. TREM2-Ligand Interactions in Health and Disease. J Mol Biol. 2017;429(11):1607-29.

36. Takahashi K, Rochford CD, Neumann H. Clearance of apoptotic neurons without inflammation by microglial triggering receptor expressed on myeloid cells-2. J Exp Med. 2005;201(4):647-57.

37. Hsieh CL, Koike M, Spusta SC, Niemi EC, Yenari M, Nakamura MC, Seaman WE. A role for TREM2 ligands in the phagocytosis of apoptotic neuronal cells by microglia. J Neurochem. 2009;109(4);1144-56.

38. Gao X, Dong Y, Liu Z, Niu B. Silencing of triggering receptor expressed on myeloid cells-2 enhances the inflammatory responses of aveolar macrophages to lipopolysaccharide. Mol Med Rep. 2013;7(3):921-6

39. Ito H, Hamerman JA. TREM-2, triggering receptor expressed on myeloid cells-2, negatively regulates TLR responses in dendritic cells. Eur J Immunol. 2012;42(1):176-85.

40. Yeh FL, Wang Y, Tom I, Gonzalez LC, Sheng M. TREM2 binds apolioproteins, including APOE and CLU/APOJ, and thereby facilitates uptake of amyloid-beta by microglia. Neuron. 2016;91(2):328-40.

41. Wang Y, Cella M, Mallinson K, Ulrich JD, Young KL, Robinette ML, Gilfillan S, Krishnan GM, Sudhakar S, Zinselmeyer BH, Holtzman DM, Cirrito JR, Colonna M. TREM2 lipid sensing sustains the microglial response in an Alzheimer's disease model.Cell. 2015;160(6):1061-71.

42. Wang Y, Ulland TK, Ulrich JD, Song W, Tzaferis JA, Hole JT, Yuan P, Mahan TE, Shi Y, Gilfillan S, Cella M, Grutzendler J, DeMattos RB, Cirrito JR, Holtzman DM, Colonna M. TREM2-mediated early microglial response limits diffusion and toxicity of amyloid plaques.J Exp Med. 2016;213(5):667-75.

43. Poliani PL, Wang Y, Fontana E, Robinette ML, Yamanishi Y, Gilfillan S, Colonna M. TREM2 sustains microglial expansion during aging and response to demyelination. J Clin Invest. 2015;125(5):2161-70.

44. Kawabori M, Kacimi R, Kauppinen T, Calosing C, Kim JY, Hsieh CL, Nakamura MC, Yenari MA. Triggering receptor expressed on myeloid cells-2 (TREM2) deficiency attenuates phagocytic activities of microglia and exacerbates ischemic damage in experimental stroke. J Neurosci. 2015;35(8):3384-96.

45. Saber M, Kokiko-Cochran O, Puntambekar SS, Lathia JD, Lamb BT. Triggering Receptor Expressed on Myeloid Cells 2 Deficiency Alters Acute Macrophage Distribution and Improves Recovery after Traumatic Brain Injury. J Neurotrauma. 2017;34(2):423-35.

46. Jay TR, Miller CM, Cheng PJ, Graham LC, Bemiller S, Broihier ML, et al. TREM2 deficiency eliminates TREM2+ inflammatory macrophages and ameliorates pathology in Alzheimer's disease mouse models. J Exp Med. 2015;212(3):287-95.

47. Jay TR, Hirsch AM, Broihier ML, Miller CM, Neilson LE, Ransohoff RM, et al. Disease Progression-Dependent Effects of TREM2 Deficiency in a Mouse Model of Alzheimer's Disease. J Neurosci. 2017;37(3):637-47.

48. Keren-Shaul H, Spinrad A, Weiner A, Matcovitch-Natan O, Dvir-Szternfeld R, Ulland TK, et al. A Unique Microglia Type Associated with Restricting Development of Alzheimer's Disease. Cell. 2017;169(7):1276-90 e17.

49. Jiang T, Zhang YD, Chen Q, Gao Q, Zhu XC, Zhou JS, Shi JQ, Lu H, Tan L, Yu JT. TREM2 modifies microglial phenotype and provides neuroprotection in P301S tau transgenic mice. Neuropharmacology. 2016;105:196-206.

50. Jiang T, Tan L, Zhu XC, Zhang QQ, Cao L, Tan MS, Gu LZ, Wang HF, Ding ZZ, Zhang YD, Yu JT. Upregulation of TREM2 ameliorates neuropathology and rescues spatial cognitive impairment in a transgenic mouse model of Alzheimer's disease. 2014. Neuropsychopharmacology. 39(13);2949-62.

Highlights:

- Blood-brain-barrier disruption facilitates influx of peripheral cells after TBI

- Peripheral macrophages and resident microglia have distinct roles after TBI

- Macrophage sub-populations possess both beneficial and detrimental properties

- CX3CR1 deletion shows time dependent neuroprotective properties of microglia

- CCR2 and TREM2 deletion reduces monocytes and improves behavioral recovery

BEHAVIORAL AND PATHOLOGICAL OUTCOMES

MINUTES — HOURS

1. Loss of consciousness immediately following injury
   - Edema, axonal shearing due to mechanical impact of the injury
2. Neuromotor deficits manifest within hours post injury, which can last for days to weeks post injury.
   - Limb suspension, inability to stand on inclined or vertical plane in rodent models

DAYS — WEEKS

1. Decrease in hippocampal dependent spatial memory, motor learning and cognitive function. Rescued by a loss of CCR2$^+$ inflammatory macrophages in rodent models
2. Increase in anxiety-like behavior. Rescued by loss of TREM2$^+$ macrophage responses in rodent models
   - Smaller acute lesions, but no difference in chronic (~ 9 weeks post injury) hippocampal volumes in the absence of CCR2$^+$ macrophage responses.
   - No differences in lesion volumes with complete loss of CX$_3$CR1 signaling on microglia (~ 5 weeks post injury)

MONTHS — YEARS

1. Deficits in spatial learning and memory in rodent models, ~ 3-4 months post injury. Rescued by a deficiency of TREM2$^+$ macrophage responses.
   - Reduced hippocampal lesion volumes at ~ 3-4 months post injury in the absence of TREM2$^+$ responses
   - Partial loss of CX$_3$CR1 (CX$_3$CR1$^{+/-}$) signaling results in significantly reduced volumes of hippocampal and cortical lesions 1 year post injury
2. Persisting cognitive deficits (memory loss, confusion) seen in clinical TBI patients.

INFLAMMATORY GLIAL OUTCOMES

MINUTES — HOURS

1. Microglial and Astroglial activation due to serum proteins in the brain as a result of the physical disruption of the BBB
2. Release of pro-inflammatory mediators (IFNγ, ROS, TNF, IL1β etc) in response to DAMPs (DNA, RNA, ATP) released by necrotic and apoptotic cells
3. Increased secretion of chemokines (CCL2, CCL5, CXCL1, CXCL2, CXCL8 etc) and recruitment of inflammatory immune cells
   - Neutrophils : ROS production leading to cellular /neuro-toxicity
   - Macrophages : Neurotoxic CCR2$^+$TREM2$^+$, other CCR2$^+$TREM2$^-$ subpopulations
4. Increased phagocytosis and clearance of cellular debris by microglia and macrophages
5. Increased gliosis to clear apoptotic cells promote BBB repair and contain the injury lesion
6. Active synaptic stripping by pro-inflammatory astrocytes and microglia

DAYS — WEEKS

1. Heightened chemokine synthesis by astrocytes and microglia resulting in increased infiltration of macrophage subsets. In-situ macrophage polarization.
   - CCR2$^+$TREM2$^+$ macrophages (neurotoxic), Arginase1- (increased pro-inflammatory signaling)
   - CCR2$^+$TREM2$^-$ macrophages (prevent neuronal accumulation of NFTs), Arginase1+ macrophages (increased anti-inflammatory signaling), CX$_3$CR1$^+$ macrophages (possibly neuroprotective)
2. Early loss/down-regulation of CX$_3$CR1 on microglia drives protective responses (increased phagocytosis of cellular and myelin debris (via increased TREM2), increased IL-10, Arginase1 etc. Active myelin stripping). Sustained loss of CX$_3$CR1 signaling drives increased pro-inflammatory activation (may result in spread of inflammatory lesion).
3. Pro-inflammatory microglia drive neurotoxic astroglial responses, resulting in aberrant synaptosome engulfment, synaptic stripping, glutamate and excitotoxic neuronal death
4. Anti-inflammatory glial responses resulting in synaptogenesis, recruitment and differentiation of glial precursors, and remyelination/repair

MONTHS — YEARS

1. Re-establishment of a stable BBB, but possible leaky barriers that allow for sustained infiltration of the CNS by peripheral immune cells.
   - Passive infiltration or active recruitment due to sustained/chronic chemokine expression by glial cells in the injured CNS?
2. Extracellular deposition of Aβ plaques resulting in accumulation of microglia, macrophages and astrocytes to plaque vicinity. Increase intra-neuronal NFTs.
   - Varied activation phenotypes of plaque associated immune cells in a region/plaque specific manner? Plaque associated immune response can be both pro- and/or anti-inflammatory
3. Retention and/or survival (TREM2 dependent) of a heterogeneous population of peripheral macrophages.
4. Heterogeneous activation phenotypes of resident microglia that may drive a mixed astrocyte activation. Chronic expression of TREM2 on resident microglia?
5. Reactive astrogliosis driven stabilization of the BBB (neuroprotective) and scar-formation that inhibits nerve regeneration, drives ROS/S100B/glutamate etc. mediated neurodegeneration

---

Figure labels (diagram, left panels)

MINUTES — HOURS panel:
BBB Disruption → Serum Proteins
DAMPs
CCR2+ Monocytes
Neutrophils
Apoptotic cells
Chemokines / MMPs
ATP, Angiogenic factors, Survival factors, MMPs
ROS, TNF, IL1β
TREM2-
TREM2+
Pro-inflammatory / Neurotoxic MPs
Anti-inflammatory / Pro-repair MG
CX3CR1+ Microglia
C1q, TNF, IL1α
Astrocytes
Neurotoxicity
Synaptic stripping
Pro-inflammatory / Neurotoxic MG

DAYS — WEEKS panel:
BBB repair
Neurotoxic Astrocytes
DAMPs
Recruitment of a heterogeneous MP population
Reactive astrogliosis
Lesion containment
Excitotoxicity / Synaptic stripping
Phagocytosis : Cell debris, Myelin
Astrocytes
ROS, C1q, TNF, IL1β
Arg1-
Arg1+
CX3CR1 / CX3CL1
Neuroprotective / Regenerative responses

MONTHS — YEARS panel:
BBB Re-establishment
Sustained recruitment / retention / survival of MPs
Deposition of extracellular Aβ plaques
Aβ Phagocytosis
Reactive Astrogliosis
Lesion containment / Stabilization of BBB
Astrocytes
Glial Scar Formation
Neurodegeneration / Neurotoxicity
ROS, C1q, TNF, IL1β
IL10, Arg1, VEGF, IGF1
Arg1+
Neuroprotective / Regenerative responses