6. Crosstalk between mitochondrial dysfunction and neuroinflammation in TBI

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Abstract. Traumatic brain injury (TBI) affects a growing population of all ages with long-term consequences on health and cognition. Mitochondrial dysfunction and neuroinflammation are two main factors contributing to secondary injury in TBI-associated brain damage, which can ultimately lead to secondary cell death, neurodegeneration, and long-lasting neurological impairment. TBI can cause mitochondrial dysfunction and dynamic changes including reduced mitochondrial biogenesis, oxidative phosphorylation and ATP production, as well as mitophagy impairment, which leads to increased inflammatory responses. In turn, inflammation can also exacerbate the mitochondrial dysfunction. So the mechanisms underlying TBI should entail multiple mechanisms coordinating and interacting with each other. This review focuses on the alterations in mitochondria and inflammatory responses and their connection in TBI.

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Introduction

Traumatic brain injury (TBI) affects millions of people worldwide, and the World Health Organization (WHO) estimates that TBI will be the third leading cause of death and disability by the year 2020 [1]. In the United States, more than 1.7 million Americans seek medical treatment for brain trauma each year [2], and approximately 5-6 million people, or nearly 2% of the American population currently suffer from TBI-related disabilities [3]. TBI is a particularly serious threat to health in military service personnel, athletes involved in contact sports, newborns, children, and the elderly. Trauma to the brain can result in persistent and devastating long-term consequences in cognition, motor function, sensory function and mental health. Moreover, in addition to the physiological and psychological impact, the effects of TBI also extend to the whole society. Across Europe in 2010, the economic cost of TBI was an estimated €33 billion, while the cost exceeds $70 billion per year in the United States [4].

About 25% of the total body glucose is utilized by the brain, and the majority of which is converted to energy to support synaptic transmission through glycolysis and mitochondrial oxidative phosphorylation [5]. Mitochondria not only provide energy through ATP synthesis, but also participate in intracellular calcium buffering, apoptosis, and reactive oxygen species (ROS) production. More and more evidence from both clinical and experimental studies on brain injury has suggested that structural and functional injury of mitochondria is an early event after TBI that contributes to cell death and poor cognitive outcome [6].

Inflammation after TBI is now recognized as a robust and complex interaction between central and peripheral cellular and soluble components, influenced by patient’ age, sex, mechanism of injury (focal, diffuse, blast), degree of injury (mild, repetitive mild, severe), secondary insults (hypoxaemia, hypotension), therapeutic interventions, and genetic variability. TBI leads to early resident microglial activation and peripheral neutrophil recruitment, followed by infiltration of lymphocytes and monocyte-derived macrophages [7]. Simultaneously, proinflammatory and anti-inflammatory cytokines vie to promote or terminate the post-traumatic neuroinflammatory response, and the chemokine signalling results in the activation and recruitment of immune cells towards the lesion [8]. Post-traumatic inflammation could be beneficial, by promoting both debris clearance and regeneration, and/or potentially harmful at the same time by mediating neuronal death and progressive neurodegeneration.
Anti-inflammatory therapies have demonstrated efficacy in preclinical and single-centre trials. Unfortunately, however, these therapies failed to show benefit— and several were even deleterious—in multicentre clinical trials [9, 10].

Mitochondrial dysfunction has been linked to inflammatory responses. Increased mtROS generation, extracellular ATP and mtDNA release can cause inflammatory responses, which, in turn, aggravate mitochondrial dysfunction [11]. So the mechanisms underlying TBI should entail multiple mechanisms coordinating and interacting with each other. This review summarizes our current understanding of mitochondrial dysfunction and inflammatory changes in TBI. Moreover, we highlight the possible crosstalk between the mitochondrial dysfunction and neuroinflammation.

1. Mitochondrial dysfunction in TBI

1.1. Structure and function of mitochondria

Mitochondria are intracellular organelles that work for the important process of triphosphate (ATP) synthesis, known as oxidative phosphorylation (OxPhos). The widely accepted model of mitochondria can be seen in Fig. 1. It includes outer membrane (OM), inner membrane (IM) which is further divided into an inner boundary membrane (IBM) and a cristae membrane (criM), intermembrane space (IMS), the matrix and the additional intracristal space (intracriS). The OM contains large numbers of integral proteins that are called porins. Molecules of less than 5 kDa can diffuse freely through the channels that are formed by these porins. The IM contains several proteins with five types of functions: those that perform the redox reactions of oxidative phosphorylation, ATP synthase, protein import machinery, specific transport proteins, fusion and fission protein. The IM is highly impermeable to all molecules. Almost all molecules enter or exit the matrix through special membrane transporters. The IMS contains cytochrome (cyt) c and the apoptosis inducing factor (AIF) that play important roles in cell death. The matrix contains enzymes that carry out the oxidation of pyruvate and fatty acids, and the citric acid cycle. Mitochondria also have the DNA sequence that contains 16 569 base pairs and encode a total of 37 genes [12].

Mitochondria are in constant fusion and fission dynamically in order to provide enough ATP for the proper function of the cells. Fission1 (Fis1), dynamin-related protein 1 and Endophilin B1 are three mammalian
orthologues which are required for mitochondrial fission. Mitochondrial fusion requires both outer and inner mitochondrial components, such as optical atrophy protein 1 (Opa1) and mitofusin 1 and 2 [13].

Cooperation between mitochondria and other organelles is also important in maintaining normal function of the cells. The mitochondria-associated endoplasmic reticulum (ER) membrane (MAM) is the structure that associates the mitochondria with the ER membrane. The MAM can regulate the communication and functional interactions between ER and mitochondrion [14]. The mitochondrial–lysosomal axis is also involved in the cell death in TBI. Lysosomal membrane permeabilization (LMP) causes the release of cathepsin proteases into the cytosol, which facilitates the release of AIF and EndoG and then results in caspase-independent DNA degradation. Cathepsins can also stimulate mitochondrial outer membrane permeabilization (MOMP) and result in the mitochondrial pathway of apoptosis [15-17].

**Figure 1.** The structure of a mitochondrion. Mitochondrion have three membrane systems. The outer membrane (OM) contains porins that allow free diffusion of small molecules. The inner membrane (IM) contains proteins with different functions, such as those related to respiratory chain, ATP synthase, mitochondria fusion and fission. The permeability transition pore complex (PTPC) is a supramolecular channel which can participated in mitochondrial outer membrane permeabilization (MOMP). The IMS contains many proteins that play important roles in cell death, such as cyt c, Endo G and AIF. Enzymes that involved in the citric acid cycle and mitochondrial DNA (mtDNA) are located in the matrix.
1.2. Mitochondrial dysfunction and cell death

Neuronal and glial cell death contribute to the overall pathology of TBI. Apoptosis, necrosis and autophagy are three major forms of cell death that are widely recognized [18]. All three forms of cell death participate in cell damage after TBI. In TBI pathology, mitochondria serve as signalling platforms for numerous biomolecules that are produced inside and outside the mitochondria. The coordinative role of different regulators and effectors in mitochondria determine the death or life of the cell (Fig. 2). For instance, specific apoptotic proteins target mitochondrial function can cause mitochondria to swell through the formation of membrane pores. Small mitochondria-derived activator caspases are released into the cytosol and initiate the apoptotic processes. In addition, cytochrome c is released from

Figure 2. The role of mitochondria in cell death. Mitochondria is an interactive platform of factors that direct the cell to die in TBI pathology. Mitochondria can amplify the apoptotic signal or are essential for execution of the apoptotic program in the extrinsic apoptotic pathway caused by death receptor. In mitochondrial apoptotic pathway, cyt c is the essential component of caspase-dependent pathway and AIF is critical participant of caspase-independent pathway. MOMP and MPT are two models to change the permeability of mitochondrial membrane. There are synergistic effects between mitochondrial membrane, Ca\(^{2+}\) and ROS regulating cell death after TBI. There is iteration among mitochondria, ER and lysosome. MAM play an important role in Ca\(^{2+}\) exchanges between mitochondria and ER. Cathepsin proteases released Lysosome can improve mitochondria-mediated cell death.
the mitochondria into the outer part of the mitochondrial membrane which binds with apoptotic protease activating factor (Apaf-1) and ATP to generate complex proteins named apoptosomes. The apoptosomes cleave to procaspases that activate caspase 3 [19]. Mitochondrial membrane permeability transition (MPT) (a process of a sudden increase in the permeability of the IM to soluble molecular with a mass of less than 1500 Da.) that occurs following the opening of the permeability transition pore complex (PTPC, a supramolecular channel that is assembled at the junction of the IM and the OM) will lead to profound cellular consequences in necrosis [20]. $\Delta \psi m$ is needed to drive the production of ATP from ADP. MPT result in rapid mitochondrial dysfunction and excessive production of ROS that ultimately lead to necroptosis. Moreover, the swollen mitochondrial induced by MPT could lead to the outright rupture of the OM and severe release of death effectors [21].

1.3. Excitotoxicity and mitochondrial dysfunction

During TBI, synaptic release and impaired/reversed uptake mechanisms will lead to excitotoxicity. The main routes that mediate excitotoxicity include AMPA receptors, NMDA receptors, voltage-dependent Ca$^{2+}$ channels (VDCC) and metabotropic glutamate (mGlu) receptors [22, 23]. In these mechanisms, Ca$^{2+}$-dependent influx is the main factor that is responsible for cell death caused by excitotoxicity. In neurons, the departure of Ca$^{2+}$ from the cell is achieved through the plasma membrane Ca$^{2+}$ ATPase pump (PMCA), H$^+$/Ca$^{2+}$ uniporter and Na$^+$/Ca$^{2+}$ exchangers (NCXs) [24]. The cellular homeostasis of Ca$^{2+}$ is regulated mainly by mitochondria via a calcium uniporter (MCU) on the IM, uncoupling proteins 2 and 3, Letm1 mitochondrial Ca$^{2+}$/H$^+$ antiporter [25], NCXs or via ‘calcium induced-calcium-release’ pathways [26], and the interaction between mitochondria and ER [27].

During brain injury, intracellular Ca$^{2+}$ influx is mediated by many signalling pathways, including the p38 MAPK signalling axis and protein phosphatase 2A activation [28]. Mitochondria take up substantial amounts of cytosolic Ca$^{2+}$ at the expense of $\Delta \psi m$ and as a consequence of Ca$^{2+}$ uptake, which resulting Ca$^{2+}$ overload [29]. Excessive Ca$^{2+}$ uptake by mitochondria through the potential-driven uniporter is a key event in severe excitotoxicity, which leads to depolarization of $\Delta \psi m$, ROS generation, depletion of ATP and the permeabilization of mitochondrial membrane that finally leads to apoptosis or necrosis [30]. Recent studies suggest a link between mitochondrial dynamics and excitotoxic injury. In the research, NMDA-induced toxicity results in an impairment of mitochondrial fusion. Calpain activation by Ca$^{2+}$
overload may impair Opa1 and result in impairment of mitochondrial dynamics. The inhibition of calpains can preserve mitochondrial morphology and protect neurons against excitotoxic cell death [31]. But the detail interaction of mitochondrial dynamics and excitotoxic injury in TBI should be explored more deeply.

1.4. ROS-mitochondrial dysfunction

Overproduction of ROS is one of the major causes of secondary injury in TBI. TBI produces more ROS than the antioxidants, which can lead to lipid peroxidation, DNA and protein damage [32]. The 2 major ROS are superoxide (O\(_2^\cdot\)) and the hydroxyl (OH\(_\cdot\)) radical and another class of endogenous free radicals is reactive nitrogen species, such as nitric oxide (NO\(_\cdot\)). During physiological stress, ROS levels can increase dramatically and cause significant cell damage [33]. The excessive production of ROS is due in part to excitotoxicity, free iron and interactions among ROS. Glutamate mediated excitotoxicity leads to an increase in intracellular Ca\(^{2+}\) and the subsequent induction of enzymes, such as nitric oxide synthase and xanthine oxidase, that produce free radicals. Mitochondrial Ca\(^{2+}\) overload can stimulate the net production of ROS by activation of MPT, respiratory inhibition, release of cyt c, release of pyridine nucleotides and loss of intramitochondrial glutathione necessary for detoxification of peroxides [34]. Approximately 10 potential ROS-generating systems have been identified in mitochondria [35]. Superoxide can be produced by respiratory complexes and individual enzymes on the OM, on both sides of the IM and in the matrix. The respiratory chain complexes I and III are the primary mitochondrial sources of superoxide [36]. Besides being the major source of ROS, mitochondria are also targets of oxidative stress [37]. Overproduction of ROS in the mitochondria is one of the early events that precede the collapse of \(\Delta \psi_m\), release of pro-apoptotic factors and activation of caspases. ROS resulting in oxidative structural changes can impair mitochondrial energy metabolism. For instance, because mitochondrial DNA (mtDNA) lacks introns and is close to an ROS source, it is prone to oxidative damage. In turn, the decreased respiratory function induced by mtDNA damage enhances ROS generation, thus eliciting a vicious cycle that ultimately triggers apoptosis [38]. There are synergistic effects between mitochondrial membrane, Ca\(^{2+}\) and ROS in mediating cell injury after TBI. Ca\(^{2+}\) overload enhances ROS generation [39] and oxidative stress can also aggravate Ca\(^{2+}\) overload [40]. Ca\(^{2+}\)-induced injury can be amplified by the interaction of oxidative stress and Ca\(^{2+}\) [41]. Both Ca\(^{2+}\) and ROS can directly activate caspase or facilitate the release of caspases during apoptosis [42] or oxidize
CL which results in the detachment of cyt c [43]. Ca\(^{2+}\) and ROS are two main players, but not the only determiners of the fate of the mitochondria and the cell.

1.5. Bcl-2 family-critical regulator of mitochondria

The Bcl-2 family are proteins that contain Bcl-2 homology (BH) domains, which are divided into anti-apoptotic Bcl-2-like proteins that carry the BH1-4 domains (e.g. Bcl-2 and Bcl-xL) and pro-apoptotic Bcl-2-like proteins that contain the BH1-3 domains (e.g. Bax and Bak) or just a single BH3 domain (e.g. the so-called BH3-only proteins). In TBI pathology, the anti-apoptotic and pro-apoptotic Bcl-2 family on the OM determines the fate of neurons [44]. The anti-apoptotic proteins, such as Bcl-2, Bcl-xL and Bcl-w, are found on the OM in normal healthy cells where they inhibit both the MPT (through their interaction with the PTPC) and MOMP (by sequestering Bax and Bak) [45, 46]. Bax and Bak undergo conformational modifications and enter the OM fully to create MOMP under pro-apoptotic conditions [47]. Apoptotic signals also up-regulate the expression of BH3-only proteins [48]. The BH3-only protein Bid which can be cleaved as tBid by caspase-8 and promote MOMP is one of the major links between extrinsic and mitochondrial apoptosis [49]. Bcl-2 and Bcl-xL are also cleaved by caspases and promote apoptosis. A self-amplifying feed forward loop is involved in the Bcl-2, caspases and mitochondria that may establish an irreversible commitment to apoptosis [50]. Furthermore, the binding of a pro-apoptotic Bcl-2 family (such as tBid, Bax and Bak) to mitochondria also plays critical roles in other apoptotic associated processes, such as cristae remodelling [51], mitochondrial fission and fragmentation [52]. A number of studies have demonstrated that members of the Bcl-2 family reside in ER where they have opposing actions in regulating the transfer of ER Ca\(^{2+}\) to mitochondria and thus direct the cell to live or die [53].

1.6. Mitochondria-associated ER membranes and TBI

MAMs are structures that can modulate the functions between ER and mitochondria. MAMs were demonstrated to be a signaling hub in the regulation of mitochondrial morphology, autophagy and apoptosis (Fig. 2). Secondary brain injury after TBI is related to mitochondrial dysfunction and mitochondrial dysfunction is closely related to MAMs, which suggested that MAM dysfunction may play an important role in TBI [54]. TBI causes an uncontrolled influx of Ca\(^{2+}\) into neurons induced by
mitochondria which can aggravate mitochondrial dysfunction following TBI [55]. The disruption of Ca\(^{2+}\) homeostasis can result in cell injury and apoptosis, which could be regulated by IP3Rs located in MAMs [56]. Moreover, excessive cytosolic Ca\(^{2+}\) could enhance ROS production and ROS generated by mitochondria contribute to the pathophysiology of TBI [57]. Evidence suggested that the propagation of ROS signals between the mitochondria and ER could be modulated by PERK, which is a MAM component that plays a key role in the regulation of mitochondrial apoptosis and ER–mitochondria juxtapositions following TBI [54]. Nrf2, an anti-oxidative stress factor that is a direct substrate of PERK, could be affected by MAMs and protect against TBI by regulating microglial function [58, 59]. Furthermore, as a chaperone at the MAMs, Sig-1R could reduce microglial activation and oxidative stress and accelerate the recovery of nerve function after TBI [60]. All evidences propose that MAMs may play an important role in the mitochondrial dysfunction of TBI, but many questions remained to be solved.

Figure 3. Main structure and function of mitochondria-associated ER membranes. B-cell receptor-associated protein of 31 kDa (BAP31) is an important regulator of ER–mitochondria crosstalk by interacts with the Fission 1 and phosphofurin acidic cluster sorting protein-2 (PACS-2). Inositol 1,4,5-trisphosphate receptors (IP3Rs), the mitochondrial voltage-dependent anion channel (VDAC1) and glucose-regulated protein 75 (Grp75) form a complex which is the major Ca\(^{2+}\) transporter and channel between the ER and mitochondria. Sigma-1 receptor (Sig1-R) can regulate inflammation. RNA-dependent protein kinase (PKR)-like ER kinase (PERK) is required at the MAMs for tethering the ER to mitochondria and promoting the rapid transfer of ROS signals. So MAMs can regulate the inflammation and cell death by regulating the ROS and Ca\(^{2+}\) of mitochondria.
1.7. Mitochondrial dynamics and TBI

Mitochondria are organelles that continuously undergo fusion and fission. These balanced processes alter mitochondrial morphology and help mitochondria to respond to cellular energy needs efficiently [61]. Fusion allows for an increase in cristae density and results in maximization of ATP production during stress. In contrast, fission results in proliferation and transportation of mitochondria to areas with energy demands, in addition to segregation of injury mitochondria from the network through mitophagy [62]. An imbalance between fusion and fission can be detrimental for energy homeostasis and has been emerged in neurodegenerative diseases [63]. Specifically, excessive fission can lead to reduced mitochondrial respiration and ATP, increased ROS production, and release of apoptogenic factors, changes similar to those seen after TBI (Fig. 4). Dynamin-related protein 1 (Drp1) is a key regulator of mitochondrial fission, through its interactions with the mitochondrial outer membrane [64]. TBI can increase mitochondrial fission accompanied with increased translocation of Drp1 levels in hippocampal

Figure 4. Effects of mitochondrial. Fusion is regulated by Optic Atrophy 1 (Opa1) and Mitofusin1/2(Mfn1/2) which can increase the cristae density and ATP production for functional complementation and repair of damaged mitochondria. On the other hand, fission that is regulated by dynamin-related protein 1 (Drp1) and mitochondria that cannot be repaired can be isolated to degradation by mitophagy. Excessive fission can lead to reduced mitochondrial respiration and ATP, increased ROS production, and release of apoptogenic factors.
mitochondria of brain injured animals. Inhibition of fission improves hippocampal-dependent learning and memory, suggesting that strategies to reduce fission may have translational value after injury [65]. More researches about the relationship and mechanism between mitochondrial dynamics and TBI need to be explored.

2. Neuroinflammation in TBI

2.1. Immune response to brain injury

After brain injury, DAMPs and alarmins are released into the extracellular space and then interact with pattern recognition receptors (PRRs) on CNS resident cells. This promotes the production of cytokines and chemokines that are involved in recruitment of immune cells to the injury sites (Fig. 5). Neutrophils are the first immune cells that are recruited to the brain in response to trauma [66]. As the first responders, they play critical roles in the removal of cellular debris and containment of the cellular damage.

![Immune response to TBI](image)

**Figure 5.** Immune response to TBI. Firstly, cellular damage results in the rapid increase of damage-associated molecular patterns (DAMPs) accompanied with cytokines and chemokines released by resident cells. Then, neutrophils will be accumulated at the injury site and promote the removal of damaged cells. When neutrophil numbers begin to decline, monocytes and activated glia begin to accumulate around the site of injury. Finally, T and B cells can also be recruited to sites of brain at later time points (3–7 days post-injury).
injury lesion. Their numbers diminish greatly between days 3 and 5 post-injury, accompanied with the recruitment of the local activation of microglia and astrocytes and other peripheral immune cells. Although T cells, natural killer (NK) cells, and dendritic cells (DCs) are around the injury site, CCR2-expressing monocytes are the major immune cell population at days 3-5 post-injury [67, 68]. By 2 weeks post-injury, the brain is almost devoid of any infiltrating immune cells. But activated astrocytes and microglia with elevated levels of inflammatory cytokines can be detected for months to years after TBI [69]. The existence of activated glial cells and aberrant regulation of cytokine expression for months to years post-TBI proposed that the immune response to TBI can persist for long periods beyond the initial trauma.

2.2. Immune cell types in TBI

2.2.1. Neutrophils

Neutrophils accumulate at the damaged sites within hours post-injury and the number in the sites of brain trauma correlates with the severity of the injury [70]. Expression of neutrophilic vascular adhesion molecules E-selectin (CD62E) and intracellular adhesion molecule 1 (CD54) increases on the endothelium within 4 h of TBI [71]. Inhibition the CD11d/CD18 integrin with antibody reduced leukocyte infiltration to the CNS and the systemic inflammation to TBI [72]. However, researches on the role of neutrophils in mediating neurodegeneration, BBB breakdown and edema have not been conclusive. In a cortical controlled impact (CCI) model, neutrophil depletion with an anti-Gr-1 antibody led to decreased edema for at least 48 h after injury, but did not ameliorate BBB permeability [73]. Neutrophil depletion was associated with decreased macrophage/microglia activation, cell apoptosis and tissue loss [74]. These data are similar to results from CXCR2 knockout mice with reduced CXCR2-mediated infiltration of neutrophils after TBI [75]. Although the mice did show reduced neutrophil infiltration into the brain and significantly less cell death, BBB damage and functional outcome appeared similar to wild-type mice. These two studies suggest that neutrophil depletion may have neuroprotective effects in TBI, but these effects may not be linked to BBB breakdown. So the relationship between neutrophil activity and BBB breakdown is not as clear as previously thought and remains to be elucidated.
2.2.2. Macrophages and microglia

Activated microglia and macrophages can release pro- and anti-inflammatory factors that promote or resolve the inflammatory response to trauma. Chronically activated microglia and macrophages have been found in experimental models and humans of TBI [76] and are thought to be one of the hallmarks of unresolved inflammation that may affect long-term outcome of patient [77]. Targeted depletion of CD11b-expressing cells with transgenic CD11b-TK (thymidine kinase) and CD11b-DTR (diphtheria toxin receptor) mice [78, 79] were effective in reducing microglia or macrophages post-TBI, but has no effects on attenuating tissue damage such as axonal injury and lesion size. The chemokine receptor CCR2 plays important roles in the recruitment of monocytes/macrophages to the brain. Numerous reports have shown that abrogating CCR2-mediated events can markedly reduce both TBI-induced neuroinflammation and cognitive injury. For instance, the CCR2 antagonist CCX872 ameliorates accumulation of peripheral macrophages in the brain and alters the pro- and anti-inflammatory cytokines associated with less severe hippocampal-dependent cognitive dysfunction after CCI [80]. Another study found that impaired CCR2 signaling prevents monocyte from infiltrating into the brain and reduces cavity volume following fluid percussion injury (FPI) [81]. To understand the role of CX3CR1 in controlling myeloid cell activity in TBI, Zanier et al found a neurological protection 4 days following TBI in CX3CR1 knockout animals [82]. However, CX3CR1 knockout mice still exhibited more seriously impaired neuroscore performance when wild-type mice returned to pre-injury levels by 5 weeks post-injury. This impaired neuroscore performance at later time points in CX3CR1-deficient mice was associated with persistent neuronal death. Further investigation showed that, compared with control group, macrophages and microglia exhibit a more protective, anti-inflammatory phenotype in injured CX3CR1-null mice at early time points. However, CX3CR1-deficient mice showed elevated myeloid cell activation at late time points. So these results indicate that CX3CR1 signaling is necessary at later time points to prevent long-term inflammation and cognitive impairment. The phenotype of macrophage is another interesting issue in TBI field. Hsieh et al found that the subset of macrophages expressing the M2-associated marker arginase-1 (Arg1) had a different transcriptional profile from arginase-1-negative cells, but the genes expressed after TBI did not match traditional M2 markers [83]. They found that although Arg1+ and Arg1− macrophages expressed a variety of M1 and M2 markers, they differed distinctly in their chemokine profiles. These data means that macrophages may have more complicated and special phenotype
differing from traditional M1 and M2 in TBI. Evidence also suggested that macrophage phenotypes may be more flexible than once thought. Wang et al found that at 3 and 7 days after TBI injury, the majority of Iba1+ cells assumed an M1 phenotype, yet at day 5 there was a rise in M2 macrophage/microglial cell numbers [84]. This shift from an M1 state to an M2 phenotype may provide protection from possible detrimental effects of a prolonged state of either phenotype. Clarifying the phenotypes of macrophages and microglia may be important in understanding how unresolved inflammation can lead to long-term detrimental consequences. For example, injection of LPS at 30 days after injury in a FPI model induced more robust inflammatory cytokine production by CD11b-expressing cells associated with decreased social exploratory behavior in TBI animals than in controls [85]. These data indicate that at long-term time points, a second immune challenge can produce further cognitive injury when behavioral deficits appear to have normalized following TBI. All together, these studies show that researchers begin to notice the effects of macrophage and microglia migration, activation, and priming on TBI. But more specifically targeted researches should be done to define the discrete roles of macrophage/microglial subgroups in TBI.

2.2.3. T Cells

The T cell infiltration has been described in TBI [86], but it still remains unclear what role(s) they play in brain trauma. In a study, the influences of absence of B and T cells in brain pathology and neurological injury following experimental TBI was investigated by Rag1−/− mice [87]. But lacking the adaptive immune system did not affect neurological outcome, BBB integrity, apoptotic mediators, hippocampal architecture, or astroglial activation appreciably. In another study, sphingosine-1-phosphate receptor agonist and lymphocyte sequesterer FTY720 were used to inhibit T cell migration after TBI [88]. Although FTY720 decreased the number of circulating lymphocytes, neutrophils and macrophages/microglia in the ipsilateral hemisphere at 1 day after injury, it did not protect TBI animal. So the ability of T cells regulating other immune cells should be further investigated and more specific effects of T cell subsets on TBI progression should be considered. T cells have been found to confer neuroprotection in other models of CNS injury [89]. For instance, evidence has shown that protection after spinal cord injury (SCI) is mediated by specific T cell-derived cytokines, particularly IL-4 [90]. This insight into T cell subsets in injury protection may apply to the TBI, and thus warrants more specific investigations.
2.3. Inflammatory mediators in TBI

2.3.1. Interleukin-1

Interleukin-1, including IL-1α and IL-1β, is a potent pro-inflammatory cytokine that has been implicated in numerous neurological disorders. Two distinct forms both can induce similar levels of inflammatory signaling though IL-1 receptor (IL-1R). Caspase-1 activation in inflammasome complexes is a major mechanism for IL-1β cleavage and IL-1α release [91] and inflammasome-independent pathways that promote IL-1 production also emerged [92]. Interleukin-1β has been shown to be increased after TBI in humans and mouse models [93]. During neuroinflammation, IL-1β is known to have profound effects on glial activation, immune cell recruitment, BBB permeability and neurodegeneration [94]. IL-1β is known to strongly enhance inflammatory responses after TBI [95]. Clausen et al administered CCI-injured animals with anti-IL-1β neutralizing antibody [96, 97]. And the studies show that IL-1β neutralization result in a decrease in the numbers of microglia/macrophages, neutrophils and T cells in the brain accompanied with better performance during learning trials, as well as decreased tissue loss. In other research of CNS injury, IL-1α deletion limits neuronal damage and improves functional recovery [98]. In humans, the recombinant IL-1 receptor antagonist anakinra is currently being tested to treat severe TBI [99]. By anakinra with PCA analysis, evidence has demonstrated that IL-1 signaling is a pivotal upstream regulator of TBI-induced cytokine production [100]. And this signaling may promote macrophages to express higher levels of pro-inflammatory cytokines [101]. Inflammasomes are multiprotein complexes that coordinate caspase-1-mediated inflammatory cytokine production and cell death. Activated caspase-1 can then cleave both pro-IL-1β and pro-IL-18, which is required to elicit their inflammatory properties and for their secretion. Since the finding that inflammasome proteins are upregulated after TBI in human patients [102], significant attention has been paid to identify the inflammasome-associated signaling events that are engaged in response to brain trauma [103]. In a TBI model, inflammasome components, such as caspase-1, were shown to be upregulated in cortical neurons post injury [104]. Inhibition of inflammasome with neutralizing antibody reduced caspase-1 activation and IL-1β production accompanied with decreasing lesion volume, suggesting beneficial effects of targeting inflammasome activity. IL-1β peaks around 6 h after injury and subsequently decreases over time, however IL-18 expression remains elevated through 7 days post injury. Another research also shows that IL-18 production elevated for at least a week post-TBI [105].
These data suggest that IL-1β and IL-18 may be involved in acute inflammation and the perpetuation inflammation respectively in TBI. Until now, the contributions of inflammasome activation to TBI pathogenesis need to be investigated more and multiple questions remained. For instance, the individual contributions of inflammasome-derived cytokines (i.e., IL-1α, IL-1β, and IL-18) and caspase-1-mediated cell death in TBI pathogenesis still remain poorly characterized.

2.3.2. Interleukin-6

Interleukin-6 has frequently been associated with TBI outcome in humans. Though parenchymal IL-6 production has been associated with improved survival in TBI patients [106], more evidence points to a detrimental role for IL-6 in TBI [107]. In the studies, IL-6 level of plasma was significantly higher in severe TBI patients than the level in moderate TBI patients and subacute and chronic serum levels of IL-6 have been associated with unfavorable short and long-term outcomes [95]. In human patients, high cerebrospinal fluid (CSF) IL-6 of patients is much more likely to have unfavorable clinical outcomes [107]. Early animal studies suggested that IL-6 is elevated in CSF and serum after TBI [108]. Evidence has also proved that IL-6 could recruit activated glia and immune cells to sites of injury. Indeed, genetic defects of IL-6 reduced reactive astrocytes and macrophages and increased neuronal death [109]. Conversely, overexpression of IL-6 enhanced recruitment of glia and immune cells and decreased oxidative stress and neuronal death [110]. IL-6-induced inflammation and glial scar formation are important in reducing cell death. A CCI study also showed that IL-6 deficiency leads to deteriorative performance as well as higher IL-1β protein levels in the cortex, which suggested that IL-6 may be important in regulating IL-1β expression in TBI [111]. But another study showed that neutralization of IL-6 mitigates some of the inflammatory and behavioral effects of hypoxia on exacerbating post-injury responses in a weight drop model [112]. Considering these types of studies, it is likely that some level of IL-6 is necessary for a proper inflammation that positively affects outcome but complete elimination or overexpression of IL-6 can be detrimental.

2.3.3. Tumor necrosis factor-α

Literature on TNF-α in TBI consistently shows an upregulation after injury [113], which suggested an important role of TNF-α in TBI pathophysiology. Evidences have suggested that tumor necrosis factor
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(TNF-α) has early deleterious effects in TBI mouse models and exists more protective effects in chronic stages [114]. However, other research suggested that TNF-α is important to protect from early mortality in TBI [115]. And in a weight drop model, mice with administration of a TNF-α inhibitor at 1 and 12 h after injury showed improved outcome and fewer apoptotic neurons and less astrogliosis post-injury, but mice received the inhibitor at 18 h post-injury did not. These results imply a very short window for TNF-α-targeting therapeutics after TBI [116]. TNF-α is known to induce both cell proliferation and apoptosis through several signaling pathways. Research showed that deletion of either TNF receptor 1 (TNFR1) or 2 (TNFR2) can have beneficial effects on cell survival and behavioral deficits [117]. Using p55 (TNFR1) and p75 (TNFR2) knockout mice, researchers found that TNFR1 deletion attenuated neuroscore deficits and led to a shift to pro-survival signaling along with attenuated neuronal death. However, TNFR2 knockout worsen neuroscore with no signs of pro-survival signaling. Compared to the TNFR2 knockout group, the TNFR1 knockout mice also showed more anti-inflammatory macrophage phenotypes. These data showed opposite roles of the two receptors in regulating macrophages and microglia phenotype. A paper by Yang et al also showed that TNFR2/Fas knockout mice showed worse motor and cognitive performance after CCI TBI [118]. All together, these studies implied that the TNF receptors may play different roles post TBI. TNFR2 may have a neuroprotective role and TNFR1 may have a detrimental one. The signal of TNFR1 through NF-κB, JNK, and caspase-mediated apoptotic pathways may be more common for the pro-inflammatory and pro-survival signaling of TNFR2 [119,120]. Thus, more research about the role of TNF-α in TBI should consider the different receptors with survival or death pathways and the timeline on which this signaling can occur.

2.3.4. Granulocyte colony-stimulating factor/granulocyte macrophage colony stimulating factor

Some evidence suggests that both granulocyte colony-stimulating factor (G-CSF) and GM-CSF may play a protective role in TBI. G-CSF injection improves cognitive recovery and increases neurogenesis in the hippocampus accompanied by higher activation of astrocytes and microglia as well as higher levels of neurotrophic factors [121]. These data indicated that G-CSF may regulate the production of neurotrophic factors by activated glia post-TBI to promote neurogenesis. Similarly, a study proved that GM-CSF deficiency in TBI results in more cognitive deficits with higher neuronal loss after FPI by using GM-CSF knockout mice [122]. GM-CSF deficient mice
also showed reductions in astrogliosis, which may suggest that GM-CSF plays a role in protecting cells and boosting tissue repair. However how both of these molecules interact with glia to promote neuronal protection and regeneration need to be research more deeply.

2.3.5. Type 1 interferon

A study by Karve et al found that deficiency in type 1 IFN signaling by using type 1 IFN receptor (IFNAR) knockout or an anti-IFNAR antibody reduces lesion volume associated with a shift toward more anti-inflammatory cytokine signaling [123]. In addition, they found that IFNAR deficiency staining through a bone marrow chimera in hematopoietic cells alone was sufficient to confer lesion volume protection and elevated GFAP and IBA1. Importantly, they also found that brain trauma in humans promotes enhanced expression of type-1 IFN. This research implies that type-1 IFN signaling may potentially influence clinical outcome and TBI pathogenesis.

2.3.6. Interleukin-10

Although interleukin-10 has been shown to be elevated in TBI patients and has been associated with unfavorable outcome and mortality [124], IL-10 has emerged neuroprotection with hyperbaric oxygen (HBO) treatment [125]. HBO in TBI could reduce lesion volume and edema, improve cognitive performance, inhibit pro-inflammatory cytokine production, lead to a shift from apoptotic to cell survival pathways and protect BBB integrity. However evidences showed that these positive effects were dependent on IL-10 by using IL-10-knockout animals. It implies the potential protective role of IL-10 in TBI. It is possible that the association between IL-10 and poor outcome is primarily due to a widespread upregulation of cytokines after TBI. A more informative approach to understanding the role of IL-10 after TBI is needed.

2.3.7. Transforming growth factor-β

Transforming growth factor β (TGF-β) has been reported to increase acutely in the serum and CSF of TBI patients [126]. And several mediators of TGF-β signaling have also been shown to be upregulated in TBI models [127, 128], including transforming growth factor beta-activated kinase 1 (TAK1) detected in cortical neurons and astrocytes [129]. Inhibition of TAK1 improved neuronal survival, ameliorated motor function and decreased NF-κB activity and inflammatory cytokine release. As a
transcriptional co-repressor, transforming growth-interacting factor (TGIF) can inhibit the transcriptional activation of TGF-β and was shown to be upregulated in neurons and microglia of TBI animals. Knockdown TGIF in the brain led to a decrease in microglia number around the lesion and a change in microglia morphology accompanied with motor function improvement. These data indicate that TGF-β signaling may have important effects on the neuroinflammation.

3. Mitochondria and neuroinflammation

3.1. Mitochondria as platforms for inflammation

Mitochondria can not only generate ligands but also serve as signaling platform for innate sensing receptors [130]. For instance, mitochondrial N-formyl peptides can activate receptors such as formyl peptide receptor-1 to promote cytokine production. And mitochondrial DNA (mtDNA) can trigger TLR9 activation [131] or access the cytosol through an injured mitochondrial membrane to activate the NLRP3 inflammasome. Another example of how the outer mitochondrial membrane acts as a platform for inflammatory response is the effect of mitochondrial antiviral-signaling protein (MAVS) [132]. MAVS can aggregates in the outer mitochondrial membrane and lead to the localization to mitochondria of signaling and adaptor proteins, which trigger the downstream inflammatory signaling of pattern recognition receptor (PRR) and the possibility that PRRs modulate mitochondrial functions [133, 134]. Mitochondrial dysfunction can initiate inflammation across various models. For example, mitochondria-derived ROS can activate the NLR family pyrin domain containing 3 (NLRP3) inflammasome pathway. After activation, NLRP3 is redistributed to nuclear and mitochondrial membranes from the endoplasmic reticulum membrane, then oligomerizes with apoptosis-associated speck-like protein containing a CARD (ACS) and pro-caspase 1 to form the NLRP3 inflammasome [135, 136]. Inhibition of complex II with 3-nitropropionic acid (3NP) can induces microglial activation, increase ROS production and cell death rate. Similar results were observed following intrastratial 3NP injection in adult rats [137]. Rats treated with rotenone (a complex I inhibitor) revealed increased IL-1β within the hypothalamus, decreased number of tyrosine hydroxylase positive neurons, perturbed locomotion and sleep abnormalities [138]. In turn, pro-inflammatory cytokines also modulate mitochondrial function. For example, TNF-α can decrease complex I activity, reduce ATP production, depolarize the mitochondrial membrane potential, increase ROS, and lower activities of complexes II and IV [139,140].
hippocampal cell line (HT22), TNF-α and IL-1β can induce mitochondrial respiration deficit and a loss of mitochondrial membrane potential [141, 142]. In human retinal pigment cells, TNF-α, IL-1β, and IFN-γ could increase the production of both mitochondrial and NADPH oxidase-derived ROS [143]. So it's reasonable to believe that mitochondrial dysfunction and neuroinflammation can impact on each other. The crosstalk between them in TBI still needs to be expounded more.

3.2. OXPHOS and immune cells

The major function of mitochondria is to generate ATP by the process of oxidative phosphorylation (OXPHOS). OXPHOS is differentially regulated in M1 and M2 macrophages, and this is associated with their various function in the immune response. Stimulation of dendritic cells (DCs) and M1 macrophages can decrease OXPHOS, with increase in glycolysis and pentose phosphate pathway [144,145]. As an inflammatory mediator, NO can inhibit mitochondrial respiration by nitrosylating iron-sulfur-containing proteins of complex I, complex II and complex IV, thus inhibiting electron transport and ATP production [146]. Unlike DCs and inflammatory macrophages, adaptive immune cells do not shut down OXPHOS. The metabolism of T cells varies with function and developmental state [147]. Metabolites derived from non-immune cells in the surrounding microenvironment can also affect the ability of immune cells to carry out glycolysis and OXPHOS, and alter their phenotype [148]. So different immune cells with different functional state have different ability of OXPHOS and changes of OXPHOS can also affect the immune cells' function. As described above, different phenotypes of macrophages and T cells may play an important role in TBI pathological mechanism, so more researches are needed to explain the relationship between OXPHOS and specific immune cells, as well as the effects of non-immune cells on the immune cells in TBI.

3.3. Mitochondrial dynamics and immunity

Mitochondria undergo both fusion and fission processes in T cells (Fig. 6a). For instance, naive T cells have fragmented, round mitochondria, however memory T cells (Tm cells) exhibit increased total mitochondrial mass and have elongated mitochondria as a result of decreased fission and effector T cells (Teff cells) have increased fission and more punctate mitochondria with looser cristae [149]. These characteristic differences between the cell types may be central to their functions. Fused mitochondria tend to have efficient electron-transport chain (ETC) supercomplex formation.
Figure 6. Effects of mitochondrial dynamics on immune-cell function. (a) Teff cells with decreased fission have small, round, fragmented mitochondria and less cristae organization. The decreased supercomplex formation leads to less ATP synthesis and more ROS production. On the other hand, Tm cells exhibiting fused, elongated mitochondria and tight cristae that promotes supercomplex formation and decrease the ROS production. (b) In resting macrophages, supercomplexes can promotes ATP synthesis. However, activation of macrophage may destabilize complex I and enhance complex II activity, which finally increased mtROS production.

and OXPHOS [150], which may be benefited to cell survival [151]. Fission resulted in fragmented mitochondria with increased ROS production [152], which may be important during Teff cell activation, and enhanced mitophagy [153]. In addition, looser cristae may lead to the dissociation of ETC supercomplexes [154], which could result in increased mtROS production and less efficient ETC and OXPHOS activity. Mitochondrial morphology is also functionally important in CD4+ T cells. Deficiency of the mitochondrial transcription factor TFAM drastically disrupts mitochondrial morphology in CD4+ T cells, leading to impaired cristae organization [155]. ETC function is impaired in TFAM-deficient CD4+ T cells, as indicated by reduced amounts of complexes I and III, but not complex II. TFAM-deficient T cells are more inflammatory, with increased transcription and secretion of IFN-γ and IL-6, but reduced amounts of IL-10.
Mitochondrial dynamics are less well characterized in macrophages, although a study has showed that stimulation of macrophages with bacteria alters ETC architecture in a ROS-dependent manner [156], and that such ETC adaptations are important for the appropriate response to bacterial infection (Fig. 6b). As reviewed above, mitochondrial dynamics is abnormal in TBI and alterations of mitochondrial dynamics can regulate the immune function of both innate and adaptive immune cells. So further research is needed to explore the relationship between mitochondrial dynamics and specific cells in TBI (including non-immune cells and immune cells).

3.4. Mitochondria-associated ER membranes and inflammation

MAMs have been shown to be critical in inflammation. Recent studies have proved that NLRP3 inflammasome and subsequent pro-inflammatory cytokines were activated in human brains after TBI. More importantly they found that the protective effect of Omega-3 fatty acids (omega-3 FAs) on TBI by inhibition of NLRP3, which implies the important role of NLRP3 in TBI [157]. The role of MAMs in the activation of NLRP3 inflammasome is still unclear. But recent studies indicated that ROS could promote the activation of the NLRP3 inflammasome [158]. So it's reasonable to speculate that ROS may be one of the association between MAMs and NLRP3 in TBI. VDAC1 is a critical regulator of mitochondrial metabolic activity through the uptake of Ca$^{2+}$ into the mitochondria from MAMs and is essential for the production of mitochondrial ROS. When the activity of VDAC was inhibited, the formation of NLRP3 inflammasome was selectively abrogated. Thioredoxin-interacting protein (TXNIP) is another bridge between oxidative stress and NLRP3, because when TXNIP was silenced, the activation of NLRP3 inflammasome was blocked [159]. PERK belongs to the eukaryotic translation initiation factor 2a (eIF2a) kinase subfamily which is particularly enriched at MAMs. The activation of the PERK/JAK1/STAT3 signaling pathway could elicit a feed-forward inflammatory loop that involves astrocytes and microglia to drive neuroinflammation and the activation of microglia could be abolished by silence of PERK [160]. Moreover, PERK is required for ROS generation and is involved in the activation of NF-κB in astrocytes [161]. Sig-1R, as a chaperone of the MAMs, could form a complex at MAMs to participate in the regulation of Ca$^{2+}$ mobilization from ER stores [162]. The neuroprotective effects of Sig-1R are attributed to anti-inflammatory actions in various disease models. For instance, scientists found that PRE-084, a Sig-1R agonist, could significantly reduce the number of
Mitochondrial dysfunction and neuroinflammation in TBI

active microglial cells, which indicated that Sig-1R regulated inflammation [163]. Consistent with previous studies, Dong et al. found that PRE-084 could reduce microglial activation and nitrosative and oxidative stress to proteins after TBI [164]. Autophagy may be another association between MAMs and neuroinflammation. It's a lysosomal degradation pathway that degrades damaged organelles and can be induced by TBI [165]. MAM–mitochondria contact are required for the formation of autophagosomes [166]. A recent study indicated that autophagy is a negative regulator of NLRP3 inflammasome activation [167]. The activation of the NLRP3-inflammasome could cause the processing and release of IL-1b and IL-18 and enhance the progression of the inflammatory response after TBI, which links the MAMs and the inflammatory response after TBI [168]. All of these results suggest that MAMs may play an important role in initiating the inflammatory response, however the mechanisms of crosstalk between MAMs and inflammation after TBI are yet to be investigated.

Summary and conclusions

In a word, it's a consensus that mitochondrial dysfunction and neuroinflammation are two critical factors that influence outcomes of TBI. More and more evidences have implied that there is extensive interaction between mitochondrial dysfunction and neuroinflammation. Mitochondria can not only generate ligands but also serve as signaling platform for innate sensing receptors, which in turn inflammation also can exacerbate the mitochondrial dysfunction. Some common signal pathways, such as p38, that regulate mitochondrial dysfunction and neuroinflammation also have been revealed recently [169, 170]. So, the mechanisms underlying TBI should entail multiple mechanisms coordinating and interacting with each other. The roles of MAMs and mitochondrial dynamics in TBI are the new focus of recent research, and they may associate neuroinflammation and mitochondria with new mechanism in TBI. Until now, the crosstalk between mitochondrial dysfunction and neuroinflammation after TBI are still many questions remained to be solved. For instance, what are the mitochondrial changes in specific immune cells, such as macrophages/microglial cells, respectively after TBI? Does neuroinflammation have effects on mitochondria of neuron? Can TBI change the protein composition or stability of MAMs? What are the mechanisms underlying the regulation of MAMs in TBI? So if we answer these questions, we may find potential treatments for TBI by combination of mitochondrial protection and neuroinflammatory regulation.
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