Chapter 9

Roles of Pro- and Anti-inflammatory Cytokines in Traumatic Brain Injury and Acute Ischemic Stroke

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Additional information is available at the end of the chapter

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Abstract

This chapter will introduce the reader to the pathophysiology of two devastating neurologic events, traumatic brain injury (TBI) and acute ischemic stroke (AIS). Here we focus on the role of key pro-inflammatory and anti-inflammatory cytokines. Several experimental interventions have been found to modulate cytokine production and brain injury after AIS or TBI. Here minocycline, biological response modifiers, hormonal therapies, omega-3 fatty acids, N-acetylcysteine, and cannabinoids will be discussed. In addition, the role of cytokine-induced inflammasomes in both TBI and AIS will be addressed and followed by discussion of pro-inflammatory cytokines (e.g., TNF-α, IL-1β, IL-18, and IFN-γ). Finally, the main anti-inflammatory cytokines, IL-33, IL-10, IL-6, and IL-4, will be discussed in the context of both TBI and AIS. It should be noted that the role of these cytokines is diverse and the dichotomization of classically pro-versus anti-inflammatory cytokines is being re-examined, as many of these cytokines have been found to play dual roles in TBI and AIS brain injury.

Keywords: traumatic brain injury, ischemic stroke, cytokines, interleukins, inflammasome

1. Pathophysiology of traumatic brain injury

Traumatic brain injury (TBI) is a major cause of death and disability worldwide [1, 2]. It is one of the most commonly diagnosed neurological disorders in the United States, impacting people of a variety of ages and segments of society [3]. In Europe, the economic cost of

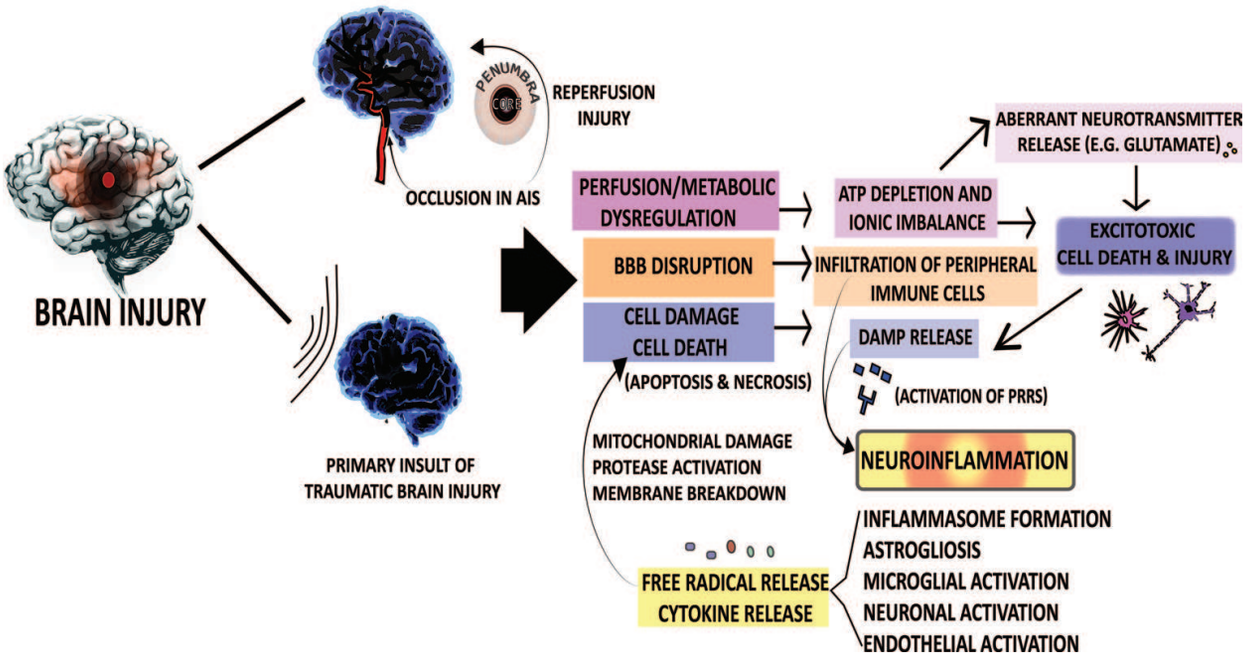

Figure 1. AIS and TBI pathophysiology. The pathophysiology of AIS and TBI share common mechanisms. The initial insult in AIS, an occlusion of blood flow resulting in a core infarct zone surrounded by a poorly perfused penumbra, versus the initial primary physical impact in TBI both result in perfusion and metabolic dysregulation leading to decreased glucose delivery and resultant adenosine triphosphate (ATP) depletion. The depletion of ATP prevents ATP-dependent ion pumps from regulating ionic gradients across cell membranes. As a result, there is aberrant neurotransmitter release and fluxes of ions like calcium causing excitotoxic cell death and cell injury via the activation of downstream molecules, like proteases. Cell damage leads to the release of damage-associated molecular patterns (DAMPs) which bind to pattern recognition receptors (PRRs) to exacerbate neuroinflammation via inflammasome formation, and astroglial, microglial, neuronal, and endothelial cell activation; these cells orchestrate the release of numerous cytokines, both pro-and anti-inflammatory. Blood-brain barrier disruption also occurs following the initial brain injury in TBI and AIS, permitting the influx of peripheral immune cells that exacerbate the inflammatory response through cytokine release, free radical release, and complement cascade activation. Further oxygen free radical production and reperfusion cellular injury occurs due to re-establishment of blood flow after its blockade in AIS. **Abbreviations**: AIS, acute ischemic stroke; ATP, adenosine triphosphate; BBB, blood-brain barrier; DAMP, Damage-associated molecular patterns; PRRs, pattern recognition receptors; TBI, traumatic brain injury.

TBI is over 33 billion euros per year [4]. Continued surveillance and research is being done to reduce primary and secondary TBI [as well as acute ischemic stroke(AIS)]–induced brain injury [1].

The pathophysiology of TBI begins with the initial brain trauma (i.e., the primary injury). This primary injury results from mechanical damage that disrupts the blood-brain barrier (BBB), alters the vasculature and damages brain tissue. The resulting injured glia and neurons release their intracellular contents (i.e., damage-associated molecular patterns; (DAMPs)) into the extracellular space and activate neighboring glia and neurons. Activated glia and neurons then produce molecular signals that can both exacerbate and mend the acute injury and contribute to long-term recovery [5–9]. These downstream molecular and cellular processes (i.e., the secondary injury in TBI) are the focus of many pre-clinical and clinical therapeutic studies. Secondary injury in TBI involves a host of molecular and cellular responses to the

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primary impact including: (a) an influx of peripheral inflammatory cells through the disrupted BBB leading to the release of reactive oxygen species (ROS), cytokines, chemokines, and free radicals; (b) the excessive release of excitatory neurotransmitters in response to ion imbalance across the cell membrane following adenosine triphosphate (ATP) depletion and metabolic dysregulation; and (c) significant increases in intracellular calcium concentration that contribute to protease, nuclease and lipase/phosphatase activation [10]. All of these factors culminate in cellular dysfunction and cell death/loss via rapid necrotic and more delayed (e.g., apoptotic) cell death pathways (See **Figure 1**).

The spatiotemporal distribution of pro- and anti-inflammatory cytokine production in secondary injury is a key feature of TBI pathophysiology and the development of post-TBI acute, sub-acute and chronic disability and recovery. By examining the role of individual cytokines in these processes, we can expect to identify novel approaches to TBI intervention/therapy.

2. Pathophysiology of ischemic stroke

Stroke is the fifth leading cause of death worldwide. Each year it affects approximately 800,000 people [11, 12]. Of all the people affected by stroke, two-thirds either die or are disabled [13]. Ischemic stroke constitutes 87% of all stroke cases [14]. The initial acute insult occurs when a thrombus or embolus lodges in one of the cerebral arteries. This blockage produces cellular and chemical changes in the ischemic core and the ischemic penumbra (the periphery of the lesion which receives some collateral blood flow from other arteries). The lack of perfusion to the ischemic core causes brain cells to die and release their intracellular contents due to a lack of ATP. The intracellular contents act as DAMPs to trigger neuroinflammatory cascades, while decreased perfusion in the ischemic penumbra leads to aberrantly functioning brain cells [15]. Macrophage scavenger receptor 1 and other macrophage receptors clear DAMPs and when deleted in a mouse model of AIS exacerbated neurologic deficits and infarct size [16]. Congruently, increased expression of *Mafb*, a transcription factor that promotes the expression of macrophage scavenger receptor 1, decreased the severity of post-AIS deficits [16].

Glutamate is also released in AIS and interacts with glutamate receptors in the penumbra resulting in excitotoxicity. This along with a state of energy depletion increases influx of sodium and calcium into the cells, resulting in membrane and cytoskeletal disintegration, enzyme activation, and eventual cell death [17]. Neuroinflammation, driven by cytokine production and complement activation, leads to the recruitment and adhesion of leukocytes into the CNS and endothelial surface and increases BBB disruption [15]. Subsequent reperfusion, although essential to protect brain tissue from further ischemic injury, is responsible for "reperfusion injury" by initiating additional inflammatory cascades; cytokines, free radicals and degradation enzyme activation as well as recruitment of leukocytes from the periphery further aggravate these processes [18]. This additionally causes mitochondrial

damage, phospholipid membrane breakdown, cytoskeletal disintegration and cell death (See **Figure 1**) [18].

There is increasing emphasis on the importance of ongoing inflammatory processes in the pathophysiology of stroke. Neuroinflammation is central to ischemic stroke pathophysiology from the initial endothelial activation within minutes to hours post-insult to the post-injury reparative phases occurring over days to months [8, 15]. Cytokine signaling plays an especially extensive role in the pathophysiology of stroke and in the reparative mechanisms and residual deficits detected post-stroke. They also contribute to behavioral changes following AIS that include post-stroke depression, apathy, fatigue, as well as post-traumatic stress and anxiety (i.e., related to the presence of various cytokines and neuroinflammatory changes) [19–25].

3. The role of cytokines in central nervous system injury

3.1. What are cytokines?

Cytokines are small, secreted proteins released by many brain parenchymal cells and infiltrating immune cells (i.e., via autocrine, paracrine or endocrine actions) that influence the interaction and communication between cells. Historically, cytokines have been defined as lymphokines (when secreted by lymphocytes), monokines (when secreted by monocytes), chemokines (having chemotactic/attractant properties) and interleukins (cytokines made by one leukocyte that act on other leukocytes) [26]. Importantly, many brain cells (i.e., other cell types) can release cytokines. Cytokines have been broadly classified based on their receptor homology, their overall action as pro-versus anti-inflammatory, or their membership into the tumor necrosis factor, lymphokine, interleukin (IL-) and interferon (IFN-) families [9]. Cytokine receptor classes include the tumor necrosis factor receptor family, interleukin-1 receptor family, Class-II cytokine receptor family which includes interferon receptors and the IL-10 receptor, and Class-I or hematopoietin cytokine receptors which includes receptors of the IL-2, IL-3, and IL-6 family, as well as homodimeric receptors [9, 27–30].

Similar to their effects in the periphery, CNS cytokines are known to regulate the production of other cytokines. They can also alter the BBB, recruit inflammatory cells and influence neurotransmitter metabolism (monoamines, serotonin, dopamine and glutamate) [31–33]. In AIS and TBI, activated glial and neuronal cells both produce and respond to anti- and pro-inflammatory cytokines, influencing reparative and destructive mechanisms after brain injury has occurred. The balance of these reparative and destructive processes influences post-stroke and post-TBI outcomes.

3.2. Pro-inflammatory cytokines

In AIS and TBI, tissue injury and hypoxia activate microglia, the endogenous brain immune cells and a major source of pro-inflammatory cytokines in the CNS [8]. Pattern recognition

receptors (PRRs) on microglia detect DAMPs triggering microglia to transition to various phenotypes, some of which promote the production of pro-inflammatory cytokines or anti-inflammatory cytokines. Classically, microglia have been characterized as M1 (i.e., pro-inflammatory) versus M2 (anti-inflammatory) phenotypes [34]. However, the classic nomenclature is under scrutiny, as many studies have demonstrated results incongruent with the simple categorization of M1 versus M2. Microglia are dynamic cells, continuously changing and responding to local stimuli. The M1/M2 polarization scheme does not necessarily fully capture the versatility of microglia behavior along a pro- to anti-inflammatory continuum [35]. Microglia contribute to neurodegeneration through the release of pro-inflammatory cytokines such as IL-1β, TNF-α, and IFN-γ, and via promotion of cytotoxic levels of ROS, reactive nitrogen species, nitric oxide, glutamate, and histamine [27, 34, 36]. Additionally, other CNS effector cells such as astrocytes, neurons, oligodendrocytes, CNS-derived macrophages and mast cells contribute to the pro-inflammatory cytokine milieu post-injury [37–39]. The pro-inflammatory cytokines TNF-α and IL-1β, as well as IL-6, are also involved in the initiation of sickness behaviors and may be related to post-AIS and post-TBI recovery responses [40].

Post-AIS and post-TBI inflammatory changes may be deleterious or beneficial. Several studies show that the inflammatory response in AIS relates to infarct volume and the inflammatory response in TBI to injury severity and contusion volume. While some studies consistently demonstrate deleterious roles of cytokines such as TNF-α, others are inconsistent in polarizing cytokines as pro-versus anti-inflammatory [41–48]. Thus, the roles of cytokines in TBI and AIS are still unclear and while categorization of cytokines as pro-versus anti-inflammatory is helpful, it is not definitive in all cases.

3.3. The inflammasome and pro-inflammatory cytokine release

Inflammasomes play a major role in the release of pro-inflammatory cytokines and the induction of cell death in TBI and AIS. Multiple molecules join together due to the activation of PRRs by DAMPs to form inflammasomes specific to their PRR. Inflammasomes can be formed in the cytoplasm of a variety of cells (neurons, microglia, macrophages, brain endothelial cells) [49] and upregulate cytokines that augment the inflammatory response [50]. The standard inflammasome is composed of a PRR in the cell cytosol connected to the protease caspase-1 by an adaptor protein. In TBI and AIS, NLRP1 and NLRP3 inflammasomes are formed in microglia and neurons in response to DAMPs [51, 52]. NLRP1 and NLRP3 are both formed via cytosolic NOD-like PRRs (i.e. NLR) with a carboxy-terminal leucine-rich repeat, nucleotide-binding domain, and pyrin domain; these components permit interactions with an adaptor apoptosis-associated speck-like protein (ASC) which contains a caspase activation and recruitment domain to permit the activation of caspase-1 (See **Figure 2**, Key concepts box) [50, 53]. Activated caspase-1 goes on to activate cytokines IL-1β and IL-18 while triggering apoptotic cell death. The NLRP1 inflammasome can additionally recruit various cell molecular responses including the membrane channel pannexin-1, the X-linked inhibitor of apoptosis protein (XIAP), caspase-5, caspase-11, and P2X purinoreceptor 7 to guide its activation and actions in various cell types [50].

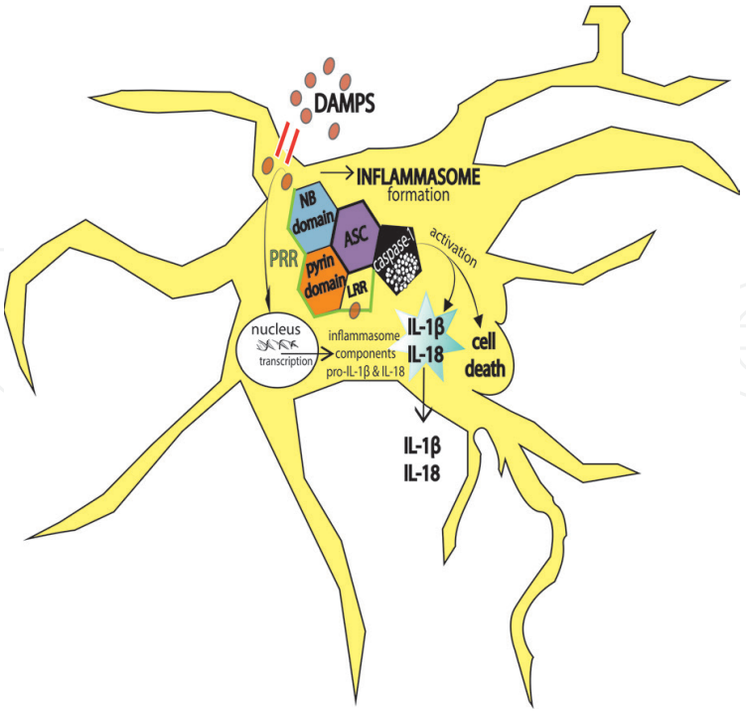

Figure 2. The Inflammasome. Inflammasome complexes form in response to activation via DAMPs or cell signaling molecules produced in states of cell stress and injury. Cytoplasmic or transmembrane PRRs recognize DAMPs and initiate the assembly of the inflammasome complex and the transcription of cytokine precursors. The typical inflammasome complex is composed of a PRR connected via an adaptor protein to caspase-1. Through PRR activation by DAMPs, caspase-1 is activated and initiates an inflammatory cascade involving the activation of the cytokines IL-18 and IL-1β as well as cell death mechanisms. The inflammasome depicted above is representative of the NLRP3 inflammasome of the NLR family of pattern recognition receptors. The NOD-like receptor pattern recognition receptor is a cytoplasmic receptor complex containing a leucine rich repeat, pyrin domain, and nucleotide-binding domain. It is activated by DAMPs/molecular pattern signals to activate caspase-1 which it is connected to via the apoptosis-associated speck-like protein, an adaptor protein. Activated caspase-1 cleaves pro- IL-18 and IL-1β to their active forms to promote neuroinflammation and cell death mechanisms. **Abbreviations**: ASC, apoptosis-associated speck-like protein; DAMP, Damage-associated molecular patterns; LRR, carboxy-terminal leucine-rich repeat, NB domain, nucleotide-binding domain; NLR, NOD-like receptor; PRR, pattern recognition receptor. See Key Concept Box.

Key Concept Box:

Key components of the inflammatory response in AIS and TBI

DAMPs (**Damage-associated molecular patterns**)-intracellular contents and products released from damaged cells that act as molecular signals to trigger neighboring and infiltrating cells to respond, thus promoting inflammation. DAMPs interact with PRRs on cells to recruit additional cells and organize an inflammatory response.

PRRs (**Pattern recognition receptors**)-a receptor that recognizes the molecular signatures associated with damage or pathogen invasion to activate downstream signal transduction and the formation of the inflammasome complex. DAMPs or pathogen-associated molecular patterns (PAMPs) bind to their respective PRR, triggering the downstream inflammatory response.

Inflammasome-large multiprotein complexes named after their respective PRR (e.g. NLRP1, named for the NOD-like receptor, NLR). These complexes have been observed in astrocytes, microglia, and neurons. The standard inflammasome is composed of a PRR attached to an adaptor protein linking it to a protease, classically, caspase-1, which goes on to cleave and activate cytokines IL-1β and IL-18. Cell death is triggered via activation of these cytokines and pyropoptosis.

Cytokines-small proteins secreted by immune cells both centrally and peripherally to relay information between cells and trigger immune responses and cell interactions to be protective or damaging. Cytokine actions include the recruitment of immune cells, alteration of the BBB, alteration of neurotransmitter metabolism, angiogenesis, astrogliosis, the promotion of other cytokines, protease activation, activation of apoptotic cell death mechanisms, and many more.

NLRP3 inflammasomes were found in astrocytes, microglia and cortical neurons in a weight-drop model of TBI in rats [54]. Protein levels of NLRP3, active caspase-1, and IL-18 all gradually increased over the course of 7 days in cortical tissue ipsilateral to the contusion, while levels of IL-1β rose at 6 h post-injury and declined over the course of 7 days to sham levels [54]. In support of the role of NLRP3 in TBI, another animal study showed that the expression of NLRP3, caspase-1, and thioredoxin-interacting protein (TXNIP), a regulator of NLRP3 activity, were all increased in the cerebral cortex of rats at 12 and 24 h post-blast injury [55]. In a fluid-percussion model of TBI in rats, NLRP1 inflammasome complexes containing ASC, caspase-1, caspase-11, XIAP, and pannexin-1 with resultant caspase-1 activation and XIAP cleavage were present in injured cortical lysate at 4 h post-injury. These complexes were also localized to cortical neurons [52]. Thus, the NLRP3 inflammasome is implicated in multiple mechanisms of brain injury ranging from diffuse blast injury to localized fluid percussion injury in animal models and may translate to patients with different mechanisms of brain injury as well.

A single immediate intracerebroventricular post-TBI dose of anti-ASC antibody, an antibody targeting the ASC component of the NLRP1 and NLRP3 inflammasome complex, decreased the activation of caspase-1, IL-1β and XIAP cleavage at 24 h post-injury. This same intracerebroventricular dose followed by booster anti-ASC antibody injections intraperitoneally (i.p.) at 24 and 48 h post-TBI significantly decreased the lesion volume at 3 days post-injury [52]. In contrast, a study using the controlled-cortical impact (CCI) TBI model in NLRP1 knockout mice and ASC knockout mice found no differences in the recovery of motor function up to 14 days post-injury in wild-type injured mice versus knockout mice; there were also no differences in lesion volume or the number of dead cells in the cortex or dentate gyrus ipsilateral to the injury at 3 days post-injury, despite an observed decrease in IL-1β at 1 day post-injury. Interestingly, the level of IL-6 also decreased in NLRP1 knockouts [56]. These findings suggest a role for additional inflammatory mediators in determining histological and behavioral outcomes post-injury. The baseline versus injury-induced inflammatory environment may influence post-injury outcomes, dictating the amount and type of cytokines necessary to produce a certain outcome. Knockout animals have a completely different baseline inflammatory environment with potentially alternate mechanisms of immune activation.

In humans, higher cerebrospinal fluid (CSF) levels of inflammasome complex components caspase-1, ASC, and NACHT leucine rich repeat protein-1 (NALP-1) were associated with significantly poorer outcomes as determined by unfavorable versus favorable Glasgow outcome scores at 5 months post-injury in moderate-severe TBI patients [57]. Similiarly, increased levels of inflammasome proteins, NLRP1, NLRP3, as well as caspase-1 and IL-1β have been detected in ipsilateral brain tissue samples of stroke patients [58].

Preclinically, in a rodent and neuronal culture model of AIS, Fann et al. showed an upregulation of NLRP3, NLRP1, caspase-1, IL-18 and IL-1β. The authors then used a caspase-1 inhibitor to thwart detrimental post-ischemia effects, reducing neuronal cell death in the culture model and functional deficits and infarct volumes in the mouse model [58]. These findings showcase the role of caspase-1 in post-ischemic deficits, a downstream component of many inflammasome complexes.

The NLRP1 inflammasome also plays a role in AIS. In a study by Abulafia et al. using a thromboembolic mouse model of stroke, NLRP1 was detected in microglia post-stroke as early as

6 h as compared to sham mice (NLRP1 was expressed in neurons and astrocytes, but not in microglia in sham mice). Despite this finding, the overall concentration of NLRP1 in cortical lysates was no different than shams [51]. In this same study, intracerebroventricular injection of anti-NLRP1 antibody 15 min post-thromboembolic stroke reduced caspase-1 and IL-1β activation at 24 h, but did not affect infarct volume. These results are similar to the results from the NLRP1 knockout mice studies in TBI. While the reduction in caspase-1 and IL-1β activation via anti-NLRP1 in Abulafia's study evidences the role of NLRP1 in inflammasome formation post-stroke, it does not distinguish NLRP1's relative importance to other inflammasomes formed post-stroke. In a rodent model of stroke, the NLRC4 (NLR family, CARD domain containing 4) and AIM2 (absent in melanoma 2) inflammasomes were shown to contribute to brain injury without NLRP3 involvement (i.e., without this inflammasome commonly involved in brain injury) [58–61]. Furthermore, knock-out mice (NLRC4$^{-/-}$, AIM2$^{-/-}$, ASC$^{-/-}$) studies all show an improvement in function following AIS with decreased microglia activation, decreased leukocyte recruitment and decreased infarct volume [59]. These discoveries identify the NLRC4 and AIM2 inflammasomes in addition to the NLRP3 inflammasome as potential therapeutic targets for stroke and provide new insights into how the inflammatory response is regulated post-stroke.

3.4. Inflammatory cytokine–tissue necrosis factor-α (TNF-α)

TNF-α can interact with two receptor subtypes: TNF receptor type 1 (TNFR1) or type 2 (TNFR2). TNF-α binds to TNFR1 or TNFR2 triggering the formation of intracellular complexes (complex 1, 2a, 2b, and 2c in TNFR1 and complex 1 in TNFR2) to promote inflammation, apoptosis, neurodegeneration, necroptosis, as well as some aspects of cell survival and proliferation through respective signal transduction pathways [62]. TNFR1 activation is classically associated with the exacerbation of cell injury and the promotion of cell death, while TNFR2 is associated with cell survival and proliferation, but has also been associated with inflammation and apoptosis [62–64].

TNF-α is among the first cytokines upregulated following TBI and AIS and is involved in the regulation of microglia activation as well as glutamatergic synaptic and glial signaling [64, 65]. Two biologically active forms of TNF-α, a soluble form and a transmembrane form, can be released primarily by microglia in inflammatory conditions, in addition to astrocytes, endothelial cells, and neurons. Many studies in a variety of animal models of mild to severe TBI have detected increased levels of TNF-α post-injury [66–69].

3.5. TNF-α in TBI

In TBI patients, increased levels of TNF-α have been detected in the CSF for up to 22 days after injury and have been noted in varying degrees in post-mortem tissue from TBI brain samples taken early post-injury (less than 17 min survival time) versus late post-injury (6–122 h survival time) [70, 71]. Tissue from the cortex ipsilateral to injury in both the early group and late group showed higher levels of TNF-α than controls. Specifically, the late group had TNF-α concentrations approximately five times higher than the early group, and higher levels of TNF-α in the contralateral cortex as compared to controls [71].

In animal models of TBI, TNF-α inhibition has resulted in protective effects. The lipophilic analog of thalidomide, 3,6′ dithiothalidomide, an inhibitor of TNF-α synthesis, ameliorated Y-maze spatial memory deficits and deficits in novel object recognition at 7 days post-injury when dosed up to 12 h post-mild TBI injury in mice [69]. Another TNF-α synthesis inhibitor, pentoxifylline, and a TNF-α activity inhibitor, TNF-α binding protein, improved edema at 24 h and motor deficits at up to 14 days post-injury as measured by rats' neurologic severity score in a closed head injury weight drop model of TBI in the rat [72]. The effect of pentoxifylline on motor recovery being reversible with administration of recombinant TNF-α [72].

TNF-α has demonstrated dual effects based on timing post-injury. In a study examining motor function and lesion severity in TNF-α knockout mice at 48 h post-injury, TNF-α knockouts had better motor function [73]. However, at 2 and 4 weeks post-injury, TNF-α knockouts had worse motor function and more cortical tissue loss than wildtype mice [73]. In another study, mice lacking the mitogen activated protein kinase (MAPK), p38α, a downstream signaling mechanism in microglia known to promote TNF-α and IL-1β cytokine release (p38α knockout mice) were examined in a model of diffuse TBI using fluid percussion injury causing massive microglia activation. Interestingly, in the TBI-injured p38α knockout mice, TNF-α levels were actually higher than wildtype TBI-injured mice at 6 h post-injury and returned to baseline levels by 7 days post-injury, alongside the reversal of motor deficits on rotarod and a decrease in activated microglia morphology in p38α knockout TBI-injured mice [74]. These findings support additional sources of TNF-α post-TBI and contrast the role of other microglia-induced cytokines to TNF-α in aspects of functional recovery post-TBI.

In a closed head injury model of TBI in mice, the expression of the complement system's C5a receptor was examined in TNF/lymphotoxin-α knockout mice. C5a is an anaphylatoxin involved in neutrophil and glial cell chemotaxis to the site of injury as well as neuronal apoptosis [75, 76]. The TNF/lymphotoxin-α knockout TBI mice experienced high levels of C5a receptor in neurons, neuroglia, and neutrophils at 24 and 72 h, similar to wild-type TBI mice, but displayed lower C5a receptor levels than the wild-type TBI mice by 7 days post-injury; sham-injured knockout mice had low levels of C5a receptors [77]. These findings emphasize the importance of the timing of cytokines in the regulation of the immune response post-TBI, as lack of TNF-α decreased C5a receptor expression at only 7 days post-TBI.

3.6. TNF-α in AIS

In AIS patients, TNF-α has been demonstrated in neurons and astrocytes in brain tissue within the first 24 h and for up to 18 days post-stroke; TNF-α immunoreactivity overlapped many TUNEL stained dying cells in the infarct core and peri-infarct region within the first days post-stroke, spreading as far as the contralateral hemisphere by 1.6 days in one case [78]. In patients, TNF-α was also found to be a good marker of ischemia in peripheral blood at 24 h post-stroke [79]. Peripheral TNF-α can induce the production of MCP1/CCL2 which not only help to recruit monocytes into the CNS [80], but also induce leukocyte rolling and adhesion to cerebral vasculature via E- and P-selectins [81].

In a pre-clinical study by Botchkina et al. examining AIS and TNF-α expression, TNF-α was increased locally in astrocytes, microglia, choroid plexus cells, endothelial cells, and in

infiltrating polymorphonuclear cells; neurons expressed maximal levels of TNF-α by 6 h post-stroke, and were surrounded by activated microglia. TNF-α regulates microglial activation as well as glutamatergic glial and synaptic transmission [82]. Apoptotic neurons were also found to express TNF-α at 24 h post-stroke [83]. TNF-α expression in AIS results in the upregulation of MMP-9 and other metalloproteinases that increase BBB permeability; the increased BBB permeability permits entry of leukocytes, proteases, immunoglobulins and thrombin into the CNS, facilitating cell injury [84–86]. Higher baseline peripheral levels of MMP-9 were correlated with larger lesion volumes in stroke patients [79].

Higher levels of TNF-α are generally related to worse outcomes in AIS. Mice genetically modified to overexpress TNF-α have larger infarct volumes post-stroke, as well as increased neuronal apoptosis [87]. Barone et al. showed that administration of TNF-α prior to AIS resulted in worse functional deficits and larger infarcts that were reversed via neutralization with anti-TNF-α antibody. The pre- and post-stroke intracerebroventricular administration of anti-TNF-α antibody or soluble TNF-receptor I also decreased infarct size in this study [88]. The soluble TNFR1 receptors sequester the TNF-α that has already been released due to AIS, thus helping reduce ischemic injury. Additional studies administering TNF-α neutralizing antibodies or soluble TNF-α receptor post-stroke result in smaller lesion volumes and less cerebral edema [86, 89]. A study by Pan et al. on TNF-α trafficking across the BBB post-stroke found that mice who underwent AIS had higher levels of TNF-α transported across the BBB on day 5 post-stroke in both hemispheres, cortically and subcortically without an increase in overall BBB permeability. This finding was substantiated by a peak number of TNFR1 and TNFR2 receptors in endothelial cells ipsilateral to the ischemic site at 5 days post-stroke that would permit this selective uptake across the BBB. However, interestingly, these increases in TNF-α transport peaked while functional deficits began to improve [90]. Thus, the observed time course of increased TNF-α levels in this study may additionally implicate TNF-α in post-stroke repair and neuroplasticity. Further evidence of TNF-α's potential benefit is seen via its ability to activate the formation of the TNFR1-TRADD (TNF receptor associated protein death domain) complex to induce NF-κB mediated transcription of anti-apoptotic proteins, contributing to cell survival. However, TNF-α can also induce cell death via recruitment of caspases and proteins like Fas-associating protein with a death domain (FADD)[64].

3.7. TNF-α polymorphisms

In both AIS and TBI, single-nucleotide polymorphisms (SNPs) in TNF-α have been correlated to different risk profiles for disease severity [91–93]. A meta-analysis on AIS risk in individuals with TNF-α-308G/A gene versus-238G/A gene polymorphisms, both of which lead to high TNF-α production, suggests differences in AIS risk associated with these SNPs in Caucasians versus Asians; the TNF-α-308G/A gene polymorphism was protective in Asians, while the TNF-α-238G/A gene polymorphism was associated with a higher risk of AIS in Caucasians [92]. In a study of the TNF-α-308G/A gene polymorphism in TBI patients, those with this polymorphism had worse clinical outcomes as measured by the Glasgow outcome scale at 6 months post-injury [93].

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3.8. Inflammatory cytokine–interleukin-1β

IL-1β is an essential mediator in the neuroinflammatory response, is constitutively expressed in the CNS, and is upregulated minutes after a neuronal insult [94]. As previously mentioned, pro- IL-1β is cleaved by caspase-1 into its active form [94]. The transcription and translation of pro-IL-1β is modulated by molecules that are altered in neuronal injury and infection, such as prostaglandins, lipopolysaccharide, and glucocorticoids; glucocorticoids decrease the production of pro-IL-1β, while lipopolysaccharides and prostaglandins, as well as intercellular adhesion molecules increase it [94].

The type 1 IL-1 receptor (IL-1R1) and type 2 IL-1 receptor (IL-1R2) bind to active IL-1β to regulate cytokine concentration. IL-1R2 acts as a decoy receptor, as it does not induce downstream effects upon binding to IL-1β [94]. In contrast, the type 1 IL-1 receptor induces signal transduction pathways in multiple cell types (endothelial cells, oligodendrocytes, neurons, astrocytes, microglia, leukocytes). For example, when IL-1β binds to IL-1R1 on microglia, cytoplasmic GTPases signal to MAPK p38α downstream to induce the transcription and release of other cytokines, such as TNF-α and the phagocytosis of axonal and cellular debris; IL-1β also induces the expression and secretion of heat shock proteins that activate other PRRs and expand the neuroinflammatory response [95, 96]. IL-1 receptors can also circulate in a soluble form to bind and impact the concentrations of IL-1 cytokines [94, 95]. The IL-1 receptor antagonist, IL-1Ra, is an innate competitive antagonist to other IL-1 receptors and does not induce a downstream biological response [94, 95].

3.9. Inflammatory IL-1β in TBI

Numerous studies demonstrate the rapid rise of IL-1β post-injury associated with increased cell death. The exogenous addition of IL-1β or increased production of IL-1β post-TBI is associated with an exacerbation of injury [97–100]. For example, Lu et al. observed increased levels of hippocampal IL-1β as early as 3 h, peaking at 12 h and remaining for 48 h post-injury in a weight drop model of TBI in rats associated with severe hippocampal neuronal loss [101]. Lawrence et al. demonstrated an exacerbation of neuronal loss in the cortex when IL-1β was co-administered into the ipsilateral or contralateral striatum with excitotoxin infusion in the cortex. These studies provide examples of IL-1β's global influence on cell death post-injury [102].

Accordingly, studies ablating the expression of IL-1β or inhibiting its biological effect via anti-IL-1β antibodies, upregulation of the endogenous IL-1β receptor antagonist, IL-1Ra, or interleukin-1 receptor antagonists show improvements in TBI outcomes in rat models [103–105]. Injury-induced neuron loss in the rat hippocampus was also significantly improved with pre-injury intracerebroventricular administration of IL-1β antibody [101]. Post-CCI i.p. administration of anti-IL-1β antibody decreased edema at 48 h post-injury, as well as microglia activation, lesion size, and visuospatial learning deficits in the Morris water maze (MWM) task (decreased latency to the hidden platform on 2 out of 4 training days, but did not improve memory probe trial performance) at up to 20 days post-injury [106]. Anti-IL-1β antibody administration via osmotic minipump in this same CCI model resulted in decreased neutrophil and activated T cell penetration across the BBB into the cortex at 7 days post-injury [107].

IL-1β neutralizing antibodies were also tested in a central fluid percussion injury mouse model of diffuse axonal TBI; IL-1β neutralizing antibodies were administered i.p. 30 min post-injury, improving the latency to hidden platform times in the probe trial (long-term memory) of the MWM task at 21 days post-injury to near sham-injured levels [108]. Decreases in the number of stereotypies in a multivariate concentric square field test were also seen in IL-1β neutralizing antibody-treated mice 2 and 9 days post-injury. Histologically, the number of microglia and macrophages were unchanged in treated mice [108]. The difference in IL-1β neutralizing antibodies' effect on cognitive outcomes post-TBI in these models could be related to the different injury models used.

The genetic overexpression of the endogenous receptor antagonist to IL-1β, IL-1Ra in a closed head injury mouse model of TBI resulted in an improved neurologic severity score, lower cortical levels of harmful TNF-α, and a delayed rise in IL-1β and IL-6 at 6 versus 4 h post-injury in wildtype mice [104]. However, in a study examining the use of the recombinant human IL-1Ra (i.e, anakinra), in patients with severe TBI, treatment, while safe without serious adverse effects, was found to induce a shift in cytokine levels towards an unexpected phenotypically M1 microglial response in comparison to non-treated controls [109, 110]. These findings evidence a more broadly defined role for IL-1Ra in TBI and the effect of IL-1Ra on microglial activation.

In a study of post-mortem TBI cortical tissue ipsilateral and contralateral to the lesion site, IL-1β levels were significantly higher in brains from patients with survival times ranging from 6 to 122 h post-injury, signifying a later peak of action [71]. IL-1β CSF samples were higher than extracellular plasma levels of IL-1β and IL-1Ra following severe TBI in 12 patients, with peaks occurring 1 day and 2 days post-injury, respectively [111].

3.10. Inflammatory IL-1β in AIS

IL-1β is released from activated microglia within 30 min of ischemic stroke and appears to be the main IL-1 agonist induced in the brain in response to systemic or local insults [112, 113]. In the acute phase of injury, IL-1β interacts with its receptors to enhance microglial activation, stimulate astrocytic production of vascular endothelial growth factor (VEGF), and MMP-9 from NG2-oligodendrocyte precursor cells (NG2-OPC) [114, 115]. Evidence from human culture systems suggests that hypoxia itself induces the production of IL-1β in endothelial cells, which then upregulates leukocyte adhesion molecules by an autocrine mechanism [116]. Additional preclinical studies have further clarified IL-1β participation in AIS pathophysiology noting that neither IL-1β nor IL-1Ra influence glutamate release or reuptake [117, 118].

A study by Clausen et al. showed that IL-1β and TNF-α are produced by largely segregated populations of microglia and macrophages after AIS in mice, providing evidence of the functional diversity among microglia and macrophages induced post-stroke [119]. This information may inform the design and characterization of anti-inflammatory therapies in stroke. The mRNA of the natural receptor antagonist, IL-1Ra was also much higher at 12 h after permanent middle cerebral artery occlusion and remained elevated for up to 5 days post-stroke in the ischemic cortex and may reflect its effort to dampen the influence of IL-1β in the acute phase of AIS [120]. Multiple laboratories have examined IL-1Ra as a therapy in preclinical

models of AIS in mice. In a multicenter international project examining the short- and long-term effects of IL-1Ra therapy in preclinical models of AIS, consistent decreases in lesion size on days 1 and 7 were noted via histology and MRI after treatment with subcutaneous IL-1Ra [121]. Improvements in neurologic deficits/function ("sensorimotor asymmetry") for up to 28 days post-treatment were also noted in this study across AIS models [121].

The potentially noxious role of IL-1β in AIS is supported by the finding that inhibition of IL-1β converting enzyme (ICE) decreases infarct volumes in mice [122] and rats [123]. Moreover, transgenic mice with a mutant ICE gene developed smaller infarcts, fewer neurological deficits, lower IL-1β levels and decreased DNA fragmentation after transient and permanent middle cerebral artery occlusion [124, 125]. Furthermore, many studies testing therapeutics in animal models of AIS correlate declines in IL-1β levels post-drug administration to improved functional and histologic outcomes post-stroke [115, 126, 127]. Thus, levels of IL-1β and IL-1Ra can be important predictors of the degree of neuroinflammation following ischemic stroke, as increased levels of IL-1β worsened AIS whereas IL-1Ra provided brain protection [114].

Clinically, a longitudinal study of patients with ischemic stroke revealed acutely increased mRNA levels of IL-1β, IL-8, and IL-17 in peripheral blood samples, with IL-1β and IL-8 correlating with Scandinavian stroke scale scores [128]. IL-1β levels were higher in those with more severe neurologic impairment [128]. Increased intrathecal production of several cytokines, including interleukins IL-1β, IL-6, IL-8, IL-10, TNF-α, and granulocyte-macrophage colony-stimulating factor, has also been demonstrated in patients with AIS [42, 129, 130]. In a clinical study involving 30 stroke patients, an early increase in intrathecal, but not systemic levels of IL-1β were observed post-stroke [42]. Ormstad et al. noted an association between high acute serum levels of glucose and IL-1β, and low IL-1Ra and IL-9 to post-stroke fatigue [21]. These findings support the involvement of cytokines in fatigue after stroke [21].

3.11. Inflammatory cytokine–interleukin-18

IL-18 (previously known as IFN-γ inducing factor) is a pro-inflammatory cytokine of the IL-1 family, namely produced by microglia in the CNS [131]. IL-18 also regulates IFN-γ signaling in T-cells and Natural Killer (NK) cells [60, 76]. As previously mentioned, IL-18 can be activated through caspase-1 cleavage via inflammasome formation in addition to other proteases such as proteinase-3[94]. Activated IL-18 binds to IL-18 receptors on a variety of cell types to trigger downstream signal transduction pathways; the release of glutamate at the synapse as well as the upregulation of postsynaptic AMPA receptors in hippocampal neurons is induced via IL-18 and has been shown to inhibit long-term potentiation in the dentate gyrus [132]. IL-18 can also induce apoptotic pathways (Fas-Fas ligand binding via induction of FasL expression on glia), cytotoxic immune cell activation, the extravasation of polymorphonuclear cells, their respiratory burst response and degranulation, as well as the release of matrix metalloproteinases and cytokines like TNF-α, IL-1β and IFN-γ [131].

3.12. Inflammatory IL-18 in TBI

IL-18 is heavily involved in neuroinflammation and neurodegeneration in TBI and AIS. IL-18 levels were found to be elevated for up to 10 days post-TBI in the CSF of patients who had

experienced a severe head injury [133] and have been associated with more severe disability [134]. In a study examining serum levels of IL-18 in TBI patients, plasma IL-18 levels were elevated at 7 days, 3 months, and 6 months post-injury and were noted to decrease over time in parallel to cognitive improvement as measured by the mini mental state examination (MMSE). Those with higher MMSE scores had lower levels of IL-18 at all time points [135]. In mice, IL-18 was elevated above control levels at 7 days post-weight drop TBI. The administration of the IL-18 inhibitor, IL-18-binding protein, at 1 h post-injury improved injury-induced deficits involving motor function, reflexes, and normal behaviors tallied via a neurologic severity score at 7 days, but did not improve cerebral edema or behavioral deficits acutely at 24 h post-injury [133]. This evidences a role for IL-18 in the later phase of inflammation post-injury.

3.13. Inflammatory IL-18 in AIS

IL-18 shows a delayed rise (24–48 h) and peak (7–14 days) following ischemic stroke in mice [136]. However, IL-18 knockout mice showed no difference in infarct size at 24 or 48 h post-AIS, suggesting its limited effect on lesion severity early post-AIS [137, 138].

However, examination of human atherosclerotic plaques from carotid arteries show higher levels of IL-18, IL-18 receptor and caspase-1 expression, with IL-18 levels highest near macrophages and higher in ulcerating, unstable plaques [139]. This supports a pathogenic role for IL-18 early in the pathophysiology of AIS, at stages of thrombus formation. In AIS patients, plasma IL-18 levels obtained from blood samples taken by venous access at 48 h following AIS in 217 patients were significantly higher than in control groups. Patients with high IL-18 had significantly higher incidences of 90-day recurrent stroke and death. Thus, plasma IL-18 levels could be a major independent inflammatory predictor of 90-day morbidity and mortality in AIS patients [140]. However, these observations are incongruent with a larger study in 2008 where IL-6, IL-18 and TNF-α levels were examined in relation to recurrent stroke risk. The data was obtained from the perindopril protection against recurrent stroke study (PROGRESS) study. It was found that IL-6 and TNF-α, but not IL-18, were associated with risk of recurrent ischemic stroke independent of conventional risk markers [141].

Yang et al. explored IL-18 as a potential marker for post-AIS depression. It was observed that serum IL-18 levels on both days 1 and 7 post-AIS were significantly higher in post-stroke depression patients and non-post-stroke depression patients than in non-stroke controls. Serum IL-18 on day 7 was significantly higher in post-stroke depression patients than in non-post-stroke depression patients, suggesting a role for IL-18 in post-AIS changes in mood [25].

3.14. Inflammatory cytokine–interferon-γ

Interferon-γ is a classic pro-inflammatory cytokine released peripherally by activated T cells and natural killer cells to activate macrophages, monocytes, and microglia [33]. In any type of brain injury, compromise of the BBB permits the influx of peripheral T cells and NK cells, subjecting CNS cells to the effects of IFN-γ. IFN-γ may exacerbate BBB permeability to peripheral immune cells post-injury through the upregulation of vascular cell adhesion molecule in astrocytes of the BBB [142]. IFN-γ then directs microglia to express neuroprotective versus

cytotoxic features depending on the cytokines' concentration [143]. Microglia activated by low levels of IFN-γ can actually induce neurogenesis and oligodendrogenesis [143]. IFN-γ was also recently discovered to be released by microglia in response to IL-12 or IL-18 [144].

3.15. Inflammatory interferon-γ in TBI

In biopsies from the brains of severely injured patients, IFN-γ was detected within the first 24 h post-injury and was found to be higher than IL-4 and IL-6 at 3–5 days post-injury, indicating a robust pro-inflammatory response at up to 5 days post-TBI [145]. Post-mortem TBI brain analyses show significantly increased levels of IFN-γ in brains with survival times less than 17 min with even higher levels in tissue ipsilateral and contralateral to the injury in brains with survival times ranging from 6 to 122 h post-injury [71]. In a study examining cytokine levels in patients with severe TBI with post-traumatic hypoxia, the duration of elevated IFN-γ levels was longer, persisting 5 days post-TBI, as compared to severe TBI patients without hypoxia, indicating more persistent neuroinflammation in TBI patients with hypoxia [146]. Experimental models of TBI have also investigated the time course of IFN-γ expression post-injury and have detected variations according to the injury type and sex. A penetrating ballistic injury model of TBI in male rats demonstrated rises in IFN-γ within 4 h post-injury, while a post-craniotomy weight drop model of TBI in female rats did not detect IFN-γ by post-injury day 2 [147–149]. CCI injury versus craniotomy alone in mice also showed significant increases in IFN-γ expression with different cytokine expression time courses, supporting the impact of injury severity on cytokine expression; the mild injury via craniotomy resulted in a shorter lived cytokine response, while the severe CCI injury resulted in a response persisting for at least 21 days. IFN-γ expression peaked at 3 and 7 days post-injury in CCI-injured and craniotomy mice, respectively, with CCI-injured mice expressing higher peak levels of IFN-γ [150].

3.16. Inflammatory interferon-γ in AIS

IFN-γ has been strongly detected in autopsied human brains for up to 28 days post-ischemic stroke and is substantially expressed by inflammatory glia [151]. However, the rise of IFN-γ post-stroke may be largely facilitated by infiltrative lymphocytes, like $CD4^+$ and $CD8^+$ T cells, as IFN-γ is not present in normal brain tissue [152]. A study by Yilmaz et al. utilized the transient middle cerebral artery occlusion model of AIS in mice and knockout mice lacking various lymphocyte populations to assess the role, interaction, and contribution of lymphocytes and IFN-γ to infarct severity and functional deficits post-stroke. AIS-induced increases in platelet and leukocyte adhesion were significantly attenuated in CD4+ T cell knockout mice and CD8+ T cell knockout mice. Leukocyte adhesion also decreased in neutrophil deficient mice. Similarly, lymphocyte deficient mice ($Rag^{-/-}$ mice) and IFN-γ knockout mice had significantly lower levels of leukocyte and platelet adhesion post-stroke. Ischemic infarct volume was also lower in IFN-γ knockout mice and lymphocyte deficient ($Rag1^{-/-}$) mice. In contrast, neurologic deficits were only improved in lymphocyte deficient $Rag1^{-/-}$ mice [152]. These effects were reversed when splenocytes were added to restore lymphocytes in $Rag^{-/-}$ mice [152]. While the absence of IFN-γ alone was enough to significantly impact infarct volume post-AIS, it was not sufficient to significantly change outcomes in neurologic deficits post-AIS [152].

IFN-γ mRNA expression is also systemically increased in blood monocytes, splenocytes, and lymph node cells in AIS [153]. In a study by Li et al., systemic IFN-γ mRNA expression was increased as early as 1 h and remained elevated at 6 days, while expression levels in the ischemic hemisphere had a more delayed onset, rising at 12 h and remaining elevated at 6 days post-stroke in rats; the expression levels correlated with the infarct size [153].

IFN-γ employs the induction of adhesion molecule expression, stimulation of NADPH oxidase and activation of microglial cells and other immune cells to promote neuroinflammation [154–156]. Furthermore, IFN-γ may directly induce arteriosclerosis, increasing the risk of ischemic stroke. A study by Tellides et al. using porcine and human artery grafts transplanted into immunodeficient mice showed that arteriosclerotic changes could be induced by IFN-γ administration alone through its interaction with vascular smooth muscle cells, without the presence of immune cells [157]. IFN-γ may therefore be a chief mediator of inflammatory and thrombogenic responses in the microvasculature.

3.17. Anti-inflammatory cytokines in traumatic brain injury and ischemic stroke

The pro-inflammatory response of effector cells in the CNS to tissue injury is opposed by cytokine-induced anti-inflammatory effects that initiate repair processes and curb excessive inflammation. Some examples are provided below.

3.18. Anti-inflammatory cytokine interleukin-10

IL-10 binds to IL-10 receptors (IL-10R) which contain two receptor subunits, IL-10R- α and IL-10R-β [158]. Through activation of its receptor, IL-10 induces the JAK/STAT pathway to decrease inflammation and the PI3K/Akt pathway, to decrease apoptosis through the upregulation of anti-apoptotic factors and downregulation of caspase-3 expression [159]. Astrocytes, neurons and microglia generate IL-10 in the CNS, while lymphopoietic cells are responsible for its production outside of the CNS [160–162]. Regulatory T-cells produce IL-10 to decrease the activity of other T-cells and are involved in suppressing the immune response contributing to CNS injury [40]. IL-10 is involved in astroglial activation and microglia suppression to promote anti-inflammatory and immunosuppressive actions; microglia stimulated via TLR activation produce IL-10 and can have enhanced production in the presence of other signaling molecules like adenosine [163]. IL-10 inhibits macrophage production of NO and ROS [70] and also inhibits leukocyte adhesion to the endothelium [164]. It also decreases macrophage and lymphocyte production of IL-1, IL-6, IL-8, TNF-α, and IFN-γ [165, 166]. Furthermore, IL-10 curbs inflammatory processes such as T cell generation and MHC class II antigen upregulation [167, 168].

3.19. Anti-inflammatory IL-10 in TBI

In the context of TBI, IL-10 is demonstrably higher intrathecally and has been shown to activate the anti-inflammatory subtype of microglia (phenotypically referred to as M2 microglia) involved in matrix formation and the remodeling of tissue [169–171]. In a study examining IL-10 in TBI, Knoblach et al. administered intravenous IL-10 at 30 min prior to and 1 h after

lateral fluid percussion TBI in rats. IL-10 administration improved motor function at 7 and 14 days post-injury and decreased TBI-related cortical TNF-α and IL-1 expression, as well as hippocampal IL-1 expression at 4 h post-injury [165]. In contrast, intracerebroventricularly administered IL-10 did not result in the same improvements, highlighting the systemic involvement of IL-10 on TBI pathophysiology [165]. Furthermore, the higher intracerebroventricularly administered dose trended towards a lower survival rate than the lower dose and control groups [165].

In a 21-day analysis of cytokine expression post-CCI in mice, IL-10 was modestly elevated by day one post-injury with peak expression at 3 days post-CCI [150]. A weight drop model of TBI in rats showed an acute rise of IL-10 brain levels beginning 2 h post-injury followed by a progressive rise beginning at 4 h post-TBI; mRNA expression of IL-10 peaked within minutes post-injury followed by an acute drop and rebound that progressively declined over the remaining 24 h [98]. These findings demonstrate variability in the degree of the cytokine response in relation to the mechanism of injury.

Clinically, in pediatric TBI patients, IL-10 was detectable in CSF on days 1–3 post-injury [172]. High IL-10 levels were associated with increased mortality and with children under 4 years old [172]. High serum levels of IL-10 in adult patients with severe TBI were also associated with increased mortality and a worse GCS [173]. Csuka et al. monitored CSF and plasma IL-10 levels in severe TBI patients, noting that CSF levels of IL-10 were generally higher than serum levels, with a first peak around days 0–2 post-injury followed by a smaller peak at 7–9 days post-injury with some individual patient variation; these levels did not correlate with BBB dysfunction, but correlated with different cytokines (IL-6, TNF-α) in some patients [70].

3.20. Anti-inflammatory IL-10 in AIS

In AIS, IL-10 can be released by microglia via IL-33/ST2 signaling [174]. IL-10 and IL-10R mRNA levels increase post-AIS with IL-10Rs noted on astrocytes in the infarct zone where astrocytes attempt to wall off the lesion site from viable surrounding tissue [175]. IL-10 plays an important role in neuroprotection post-stroke, as IL-10 knockout mice do not improve histologically with administration of the anti-inflammatory cytokine IL-33 post-AIS (158). Furthermore, IL-10 knockout mice have an exacerbated, delayed inflammatory response with higher mRNA levels of TNF-α, IL-1β, MMP-9, and COX-2 at day 4 post-AIS, whereas wild-type mice express high IL-10 and IL-10R levels at this time point [175]. Studies show that decreased levels of IL-10 are associated with poor stroke outcomes and that administration of IL-10 post-stroke helps to reduce poor histological and behavioral outcomes [17, 175–179]. However, IL-10 knockout mice have been shown to induce a degree of immunosuppression post-AIS with higher levels of T-cell inhibitory CTLA-4 mRNA, phagocytic macrophages, and the M2 microglia marker arginase-1 at day 4 post-stroke [175].

In a clinical study, assessing the presence of IL-10 and IL-4 in AIS in relation to clinical worsening, significantly lower concentrations of IL-10 were found in patients with neurological worsening within the first 48 h after stroke onset versus IL-4 levels which were similar in patients both with and without neurologic deterioration [180]. Lower plasma concentrations

of IL-10 were only independently associated with clinical worsening in patients with subcortical or lacunar strokes [180]. Thus, IL-10 is associated with the acute neuroinflammatory response in AIS, especially in those with cerebral microvascular disease or subcortical infarcts [180].

Another clinical study assessed the relationship between stroke severity and the serum levels of IL-1β, IL-2, and IL-10 in 26 patients with AIS, analyzing neurological outcome and interleukin levels at 72 h post-AIS. In this study, patients with lower IL-10 levels deteriorated neurologically within the first 72 h. Thus, IL-10 may be involved in protective mechanisms during the acute phase of AIS [181]. However, IL-10's role in AIS and post-stroke recovery may be influenced by additional patient characteristics, such as sex. Conway et al. noted that sex may interact with IL-10 levels on stroke outcomes since female patients with higher IL-10 levels at 24 h post-stroke were noted to have a higher incidence of post-stroke urinary tract infections and poorer overall outcomes [182]. However, these levels did not independently predict outcome, suggesting the involvement of other interacting factors such as age, stroke risk, stroke severity and baseline IL-10 levels pre-stroke in addition to sex [182].

Interestingly, pre-clinically, spontaneously hypertensive rats have been shown to have lower baseline IL-10 levels and a decrease in IL-10 levels within the ischemic hemisphere at 24 h post-AIS [183]. This contrasts with the cytokine response in normal rats who have an increase in IL-10 levels at 24 h post-AIS [183]. Spontaneously hypertensive rats have generally poorer outcomes with larger infarcts and degrees of edema [184]. These findings emphasize the influence of baseline cytokine levels and stroke risk factors, such as hypertension, in dictating cytokine responses, as well as stroke severity and recovery.

3.21. Anti-inflammatory cytokine interleukin-33

IL-33, a cytokine belonging to the IL-1 family, is constitutively expressed in oligodendrocytes and astrocytes, as well as endothelial cells [185]. IL-33 undergoes activating cleavage by caspase-1 and interacts with a host of immune cells to shift the neuroinflammatory response towards neuroprotective, anti-inflammatory microglial and Th2 cell phenotypes, increasing the release of anti-inflammatory cytokines IL-4, IL-5, and IL-10, while decreasing the release of pro-inflammatory cytokines like TNF-α [186, 187]. IL-33 binds to suppression of tumorigenicity 2 (ST2) receptor, a receptor that can either be expressed on the membranes of astrocytes and microglia to increase microglia phagocytosis or as a soluble receptor [188].

3.22. Anti-inflammatory IL-33 in TBI

In a study examining the effects of IL-33 in a culture model of the CNS, incubation of cells with IL-33 resulted in microglial proliferation and triggered mRNA expression of pro-inflammatory markers TNF-α and IL-1β, as well as the anti-inflammatory cytokine IL-10 [188]. In IFN-γ stimulated microglia, IL-33 induced iNOS mRNA expression, a demonstrated neuroprotectant in TBI [189], demonstrating the interaction of various cytokines on microglia phenotype [188]. More studies need to be conducted on the role of IL-33 in TBI.

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3.23. Anti-inflammatory IL-33 in AIS

In a recent study by Yang et al., mice deficient in the IL-33 transmembrane ST2 receptor had worse outcomes with a shift in the post-AIS response towards pro-inflammatory microglial behavior accompanied by larger infarcts with higher levels of neuronal cell death and poor behavioral performance at 7 days post-AIS, as well as higher mortality rates [174]. These findings showcase ST2 signaling as an important neuroprotective factor post-AIS. Under normal conditions, transmembrane ST2 receptors are primarily expressed on microglia and astrocytes. However, following AIS, ST2 receptor expression increases in these cells, as well as in macrophages and neutrophils. Post-stroke IL-33 levels increase in parallel, mainly from oligodendrocyte and astrocyte production [174].

Administration of IL-33 in in-vivo and in-vitro models of AIS is associated with improved neurologic scores, smaller infarct volumes, as well as improvements in the level of cerebral edema and neuronal survival with a shift towards protective microglia phenotypes [174, 186, 187]. IL-33's protective effects, mediated via its ST2 transmembrane receptor, are thought to be partially mediated by IL-10, as IL-33 is known to induce IL-10 production by microglia and IL-10 knockout mice did not experience the protective, infarct shrinking effects of IL-33 administration post-AIS [174]. IL-33 administration in AIS also resulted in changes in concentrations of IL-4 post-stroke. Korhonen et al. showed that decreases in lesion size with post-stroke IL-33 administration was associated with increases in IL-4 levels in the penumbra post-AIS and that these improvements diminished with the administration of anti-IL-4 antibody [186]. These observations demonstrate the interaction of IL-4 in the neuroprotective cascade induced by IL-33 [186].

Clinically, Korhonen et al. demonstrated that the soluble ST2 receptor, a decoy receptor that inhibits the actions of IL-33, was higher in the plasma of patients with poorer outcomes as measured by the modified Rankin score at 3 months post-AIS, while lower levels of this IL-33 inhibiting receptor were associated with better outcomes [186]. In agreement with these findings, serum IL-33 levels have also been found to be significantly higher in patients with AIS compared with healthy controls, with higher levels of IL-33 associated with smaller infarct volumes amongst those in the AIS group [190]. Serum IL-33 was also significantly higher in the patients with mild stroke as compared to the patients with severe stroke. Furthermore, serum IL-33 levels in AIS patients were higher in those with better functional outcomes at 3 months [190]. In a smaller study by Liu et al. serum IL-33 levels were also increased in AIS patients in comparison to controls, but IL-33 levels were noted to be positively correlated with infarction volume [191]. These findings suggest a role for IL-33 in the pathophysiology of AIS and a potential use for serum IL-33 levels for diagnostic and prognostic purposes post-stroke.

3.24. Anti-inflammatory cytokine Interleukin-4

IL-4 is generated by eosinophils, mast cells, basophils, and Th2 cells and plays a role in apoptosis, gene expression, the Th2 immune response and cell proliferation [192]. IL-4 produced by T-cells has shown involvement in the formation of memories and learning; mice lacking IL-4 as well as T-cell depleted mice have spatial memory impairments in the MWM task, reversed by transfer of IL-4 producing T-cells [193]. IL-4 knockout mice actually express higher levels of

pro-inflammatory TNF-α mRNA [192]. In a cell culture experiment, astrocytic BDNF production, induced by IL-4, partially ameliorated the pro-inflammatory cytokine induced reduction in astrocytic BDNF [192]. Furthermore, IL-4 receptor complexes (type 1 and type 2) mediate IL-4 signaling through downstream JAK/STAT pathways to induce M2-like microglia, Th2 cell proliferation, and growth factor release, as well as cell growth and survival via the downstream PI3K/Akt and PKB/mTOR pathways, evidencing its neuroprotective role [194].

3.25. Anti-inflammatory IL-4 in TBI

In pre-clinical models of TBI, IL-4 gene and protein expression peaks at 24 h post-TBI in the injured hippocampus of CCI–injured rats, with a largely pro-inflammatory M1 response initiated more acutely within 2 h post-injury and peaking between 2 and 6 h post-injury [195]. IL-4 and IL-13 can activate the M2a polarization state of microglia, an anti-inflammatory microglia subtype and may be an avenue for potential therapy post-TBI [169, 171]. Clinically, in patients undergoing surgery post-severe TBI, IL-4 expression levels were increased in the first 24 h post-injury in brain tissue samples, while lower IL-4 levels were present in patients undergoing surgery on days 3–5 post-injury; in the late group, IL-4 levels were also significantly lower than IL-1β and IFN-γ levels [145].

3.26. Anti-inflammatory IL-4 in AIS

IL-4 is beneficial post-AIS via a variety of mechanisms. It increases the number of astrocytes with BDNF expression, acts on microglia to decrease their release of TNF-α, and increases anti-inflammatory M2 microglia; in conjunction with TGF-β2, IL-4 also activates these microglia [196, 197].

A preclinical study examining the effect of IL-4 on long-term recovery and microglia/macrophage polarization utilized two well-established models of stroke in wild-type and IL-4 knockout mice [198]. In this study, IL-4 deficiency worsened neuronal loss within 5 days post-stroke but had no impact on neuronal tissue loss at 14 or 21 days post-stroke, suggesting a key role for IL-4 in earlier phases of stroke pathophysiology and recovery [198]. Lack of IL-4 promoted the expression of M1 microglia and macrophage markers and dampened the expression of M2 markers at 5 and 14 days post-stroke [198]. Functionally, IL-4 knockout mice exhibited an exacerbation of stroke-induced sensorimotor deficits as early as 5 days post-stroke and impaired long-term cognitive function at 21 days post-stroke [198]. Congruently, a week-long infusion of IL-4 into the cerebral ventricles of wildtype mice post-AIS reversed the effects shown in the deficient IL-4 knockout mice, improving long-term sensorimotor and cognitive recovery [198]. Thus, IL-4 may help to improve long-term neurological outcomes after stroke through anti-inflammatory microglia phenotypes. Additionally, a clinical study by García-Berrocoso et al. demonstrated the utility of the IL-4 receptor as an early biomarker of poor post-stroke outcomes [199].

3.27. Mixed inflammatory and anti-inflammatory cytokine interleukin-6

Interleukin-6 (IL-6) is involved in neuroprotective and neuroinflammatory mechanisms. IL-6 can bind to its membrane-bound IL-6 receptor (IL-6R) or soluble IL-6 receptor (sIL-6R), both

of which can induce transcription through the Janus kinase/signal transducer and activator of transcription (i.e. JAK/STAT) pathway [200–202]. The JAK/STAT pathway is crucial to NMDA receptor triggered-long term depression and could possibly support synapse elimination. Conversely, the cytokine-activated phosphatidylinositol-3-kinase/protein kinase B (i.e. PI3K/Akt) pathway, may support synapse survival through long term potentiation triggered by NMDA receptors [7, 203].

IL-6 also acts as an agonist of VEGF, which modifies tight junction proteins to disturb the integrity of the BBB and interferes with NO production [33, 200, 201]. Concurrently, IL-6 can decrease IL-1 and TNF-α synthesis in activated monocytes and may increase the production of IL-1Ra and soluble TNF receptors to decrease the influence of these largely pro-inflammatory cytokines [204, 205]. IL-6's neuroprotective effects have been shown to be mediated via the upregulation of adenosine A1 receptors on cells [206]. IL-6 also potentially aids in tissue remodeling and recovery through the initiation of astrogliosis and angiogenesis after injury [202, 207, 208].

In a hypoxic environment, such as those created by TBI or AIS, neurons undergo oxidative stress, excitotoxicity, and apoptosis [201]. IL-6 exerts a protective effect during these biochemical processes. As part of the early response to hypoxia, neutrophils, which abundantly express sIL-6R, extravasate to CNS parenchyma [202]. Damaged parenchymal cells' production of cytokines, including IL-6, facilitate leukocyte migration to the hypoxic site. IL-6 inhibits TNF-α, dampening post-injury pro-inflammatory and pro-apoptotic cascades [202]. In the late phase of the hypoxic response, IL-6 inhibits neutrophils and recruits monocytes and T-cells for the initiation of the late inflammatory response [160].

3.28. IL-6 in TBI

IL-6 is upregulated in many models of TBI and demonstrates both protective and inflammatory effects. IL-6 knockout mice show higher levels of oxidative stress, compromised activation of neuroglia, an impaired inflammatory response, diminished recruitment of lymphocytes and restricted healing and recovery rates [66, 209–215]. Corresponding to the results seen in IL-6 knockout mice, GFAP-IL-6 mice, which overexpress IL-6 in the CNS, showed faster recovery and healing after TBI [215, 216]. Transcriptome analyses of IL-6 knockout versus wildtype [217] and GFAP-IL-6 mice [218] post-TBI via cryoinjury, showed that multiple pathways involving inflammation, apoptosis and oxidative stress were affected by IL-6. For example, IL-6 knockouts had lower expression of the gene producing suppressor of cytokine signaling (SOCS), an inhibitory protein transcribed by the JAK/STAT pathway after IL-6 activation [217]. IL-6 knockouts also expressed fewer neurotrophic genes (i.e. brain-derived neurotrophic factor, early growth response 1) post-injury [217]. Injured GFAP-IL-6 mice expressed higher levels of complement component 4 and other inflammatory mediator genes in addition to lower levels of select pro-apoptotic genes and oxidative stress-related genes in comparison to injured wildtype mice [218].

In a study of post-mortem TBI cortical tissue, IL-6 levels were significantly higher in brains from patients with both short survival times of less than 17 min and late survival times ranging from 6 to 122 h post-injury [71]. In biopsies from contused brains in a study by Holmin

and Höjeberg, IL-6 expression was lower than IFN-γ and IL-1 in the late patient group (biopsies taken 3–5 days post-TBI) with higher expression early, at 3–24 h post-injury [145], while a weight drop TBI rat model study by the same authors showed delayed IL-6 expression at 4–6 days post-injury [147]. In a study of TBI patients, plasma IL-6 levels were used to predict infectious complications and patient prognoses; higher IL-6 levels at 1-day post-injury predicted poorer outcomes [219]. McClain et al. observed that plasma IL-6 levels decreased more rapidly in patients with higher GCS scores on admission [220]. However, it must be stated that these correlations do not imply causality, as high IL-6 levels may not have induced poorer healing, but may have been simultaneously elevated in response to the severity of injury or pro-inflammatory response. Additional co-morbidities, such as coronary artery disease can also elevate plasma IL-6 levels, influencing detected levels post-injury [221]. The IL-6 polymorphism (-174C/G) is also associated with fatalities in severe TBI patients [222].

3.29. IL-6 in AIS

IL-6 levels rise within 7 h post-stroke in multiple experimental animal models. This is slightly delayed in comparison to the rapid rise of the pro-inflammatory cytokines, IL-1β and TNF-α [223]. Intracerebroventricular administration of IL-6 in the rat pre- and post-stroke resulted in significantly smaller lesions [224], while IL-6 knockout mice had significantly larger lesions and higher mortality when body temperature was regulated [225], supporting the protective role of IL-6 in stroke.

IL-6 has also demonstrated a role in angiogenesis post-stroke. An experiment utilizing IL-6 knockout mice showed exacerbation of lesion volumes and a reduction in angiogenesis and regional cerebral perfusion at 4 weeks post-stroke [208]. In-vitro models of ischemia after IL-6 administration, resulted in increased IL-6 mRNA expression in neurons, glial cells, and endothelial cells, as well transcription of genes associated with neovascularization [208]. However, a clinical study by Smith et al. correlated peak IL-6 levels in the first week post-AIS with worse outcomes as measured by the modified Rankin score at 3 months and larger ischemic volumes [45]. A study by Acalovschi et al. showed that the post-AIS inflammatory response due to IL-6 expression is influenced by genetic variation and that the induction of the inflammatory response by IL-6 might be enhanced by a transient downregulation of the potential IL-6 antagonist sgp130 [44].

4. Cytokine modulation as therapeutic interventions

Various agents have been used to decrease the pro-inflammatory response and augment the anti-inflammatory response post-TBI and post-AIS. Antibiotics, steroids, anesthetics, immunomodulating therapies, non-steroidal anti-inflammatory agents, as well as hormonal therapies and nutritional supplements have been shown to impact the immune response post-brain injury in pre-clinical and some clinical studies. However, findings in animal models pre-clinically do not always translate clinically. For example, cytokines have been detected in the CSF of TBI patients for up to 1 year post-injury while detected more transiently in animal models of TBI [92].

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Furthermore, cytokine modulation does not always correlate with positive functional outcomes, as treatments may need to be tailored to injury severity and mechanism, patient genotype and sex, immune response time course, and particular cytokine targets, details of which may be obtained through cytokine biomarker identification and monitoring. Currently, there are no acceptable therapeutics for TBI, as management is limited to skull fracture repair, control of increased intracranial pressure and stabilization of the primary injury [10]. The only FDA-approved agent for AIS is intravenous tissue plasminogen activator (tPA) which dissolves clots up to 4.5 h post-stroke symptom onset. FDA-approved retrievable stent devices are also now recommended to physically remove clots in large vessels within an acute time window of up to 6 h post-stroke symptom onset in eligible patients [17, 226].

4.1. The antibiotic minocycline in AIS and TBI

The tetracycline antibiotic minocycline alters inflammatory cytokine production post-TBI and post-AIS, thus improving functional and histological outcomes. Bye et al. examined minocycline in a closed head injury model of TBI in mice and observed that administration of minocycline acutely decreased lesion volume and functional deficits at 1 day post-injury [227]. In a study by Yang et al. examining minocycline administration post-AIS in spontaneously hypertensive rats, infarct size and degree of tissue loss/damage in the ischemic hemispheres were reduced as seen via magnetic resonance imaging and apparent diffusion coefficient mapping at two and 4 weeks post-AIS [228]. Minocycline treatment also reduced AIS-induced levels of TNF-α and IL-1β, and increased levels of TGF-β, IL-10, anti-inflammatory M2 microglia/macrophage markers, as well as cerebral perfusion [228]. Small clinical studies of minocycline in AIS have shown a decrease in IL-6 levels at 24 h post-AIS [229] and have proven safety in AIS patients both with and without tPA administration [230]. Lampl et al. showed improvements in functional recovery as measured by the NIH stroke scale (NIHSS), modified Rankin scale (mRS) and Barthel index(BI), in patients started on 200 mg of minocycline for 5 days within 6–24 h of stroke onset [231]. Srivastava et al., utilizing the same treatment paradigm as Lampl et al., found improved mRS and BI scores at 3 months post-AIS in those treated with minocycline [232]. The same treatment paradigm was also utilized by Amiri-Nikpour et al. who reported improved NIHSS scores at 30, 60 and 90 days post-AIS in male patients receiving minocycline [233]. Kohler et al. utilized lower doses of minocycline (five 100 mg doses) and saw no improvement in NIHSS at 7 days nor in the mRS or BI at 90 days post-AIS [234].

Minocycline administration in multiple adult animal models of TBI has been shown to decrease activation of microglia, improve functional behavioral deficits such as spatial memory deficits and post-TBI anxiety [235], and decrease caspase activation and markers of neuroinflammation and damage [236]. In a study examining the use of minocycline in a blast injury model of TBI in rats, researchers noted a decrease in post-TBI anxiety via the elevated plus maze at 46 days post-injury accompanied by decreased corticosterone levels and improvements in spatial memory via Barnes maze testing post-injury as late as 47 days post-TBI [237]. Minocycline administration also decreased inflammatory and neuron and glial injury-associated markers c-reactive protein, monocyte-chemotactic protein-1, neuron-specific enolase, S100 β, tau, and neurofilament H in this study [237].

In contrast to findings in adult TBI models examining minocycline, in a neonatal model of TBI, minocycline did not improve functional deficits and actually worsened microglial activation and neurodegeneration, highlighting the influence of age on TBI pathophysiology and therapeutic selection [238]. In contrast, in a study of minocycline in neonatal ischemia, microglial activation was not affected, but lesion volume was reduced [239]. Minocycline was also shown to reduce apoptosis and excitotoxicity in neonatal hypoxic-ischemic injury in rats, suggesting a more extensive use for minocycline in ischemic pathologies [240].

To establish the use of minocycline in focal embolic stroke with comorbidities, Type 1 diabetic rats underwent embolic stroke and were given minocycline with or without tPA. It was observed that compared with treatments of saline or tPA alone, minocycline plus tPA combination therapy significantly reduced brain infarction, intracerebral hemorrhage, and hemispheric swelling at 24 h after stroke. The combination also significantly suppressed stroke-induced elevations in plasma levels of MMP-9 and IL-1β up to 24 h after stroke (57).

4.2. Biological response modifiers in TBI and AIS

IL-1R antagonists are currently being examined in AIS and TBI both pre-clinically as discussed above and clinically. A meta-analysis analyzing IL-1R antagonists in rodent models of stroke, reported an overall decrease in infarct volumes and an improvement in functional outcomes [241]. A recent cross-laboratory study of subcutaneous IL-1R antagonist treatment in AIS also found consistent decreases in neurologic deficits and lesion volume across preclinical models of AIS in multiple laboratories [121]. Pradillo et al. demonstrated that post-AIS subcutaneous administration of IL-1Ra in old rats with comorbidities and in young rats increased neurogenesis and functional outcomes in both populations [242]. IL-1R antagonists have also been proven pre-clinically to reach therapeutic levels via intranasal administration, decreasing IL-1β and TNF-α levels post-stroke in a rat model [243]. IL-1R antagonists compete with IL-1β and IL-1α for IL-1R binding, preventing downstream pro-inflammatory cascades from being activated, thus exerting a neuroprotective effect. Emsley et al. showed the safety of a recombinant human IL-1R antagonist administered post-AIS which also incidentally showed lower levels of IL-6 and peripheral inflammation, in addition to improved clinical outcomes [244]. A phase II clinical trial examining the use of subcutaneous IL-1R antagonist, anakinra, initially administered within 6 h post-stroke with repeat dosing every 12 h for a total of 6 injections over 72 h (ISRCTN74236229) has recently been completed. In TBI, as previously stated, Helmy et al. showed the safety of IL-1R antagonists, but actually reported an increase in the pro-inflammatory M1 microglia phenotype, acknowledging the variation in the immune response depending on the mechanism of injury [109]. The off-label perispinal administration of the anti-TNF-α monoclonal antibody, etanercept in post-stroke cognitive dysfunction and TBI have demonstrated benefit as well [245]. Studies by Chio et al. have demonstrated that post-TBI i.p. administration of etanercept in the fluid percussion model of brain injury in the rat improves motor deficits at 7 days and increases markers of neurogenesis [246], decreases acute TBI-induced rises in glutamate, the lactate/pyruvate ratio, and improves injury-induced motor deficits [247], cognitive deficits in the passive avoidance task [248], and the severity of ischemia at 3 days

post-injury [247, 248]. A major barrier to the efficacy of etanercept as a TNF-α inhibitor for AIS and TBI is its poor BBB permeability due to its large size. Therefore, formulations of TNF decoy receptors with better BBB penetrance have been designed and tested in both TBI and AIS. Sumbria et al. show the utility of cTfRMAb-TNFR fusion protein (carboxy terminal transferrin receptor monoclonal antibody-TNF receptor), a genetically engineered monoclonal antibody against the mouse transferrin receptor found in the BBB linked to the TNF receptor to achieve whole molecule transfer into the brain, post-AIS. The administration of intravenous cTfRMAb-TNFR at 45 min post-AIS in mice resulted in significant decreases in subcortical, cortical, and hemispheric stroke volume, in addition to a 54% reduction in neurologic deficits at 24 h and 7 days post-AIS [249]. Clausen et al. examined the use of a dominant-negative inhibitor of soluble TNF called XPro1595 versus etanercept which inhibits both soluble and transmembrane TNF, in a mouse model of AIS. While infarct size was not affected, XPro1595 administration resulted in a decrease in granulocyte influx into the infarct, and improvements in motor and somatosensory function as measured by symmetrical grip strength, rotarod performance, and horizontal rod slip testing [250]. Simultaneously, the acute phase response in the liver was decreased by etanercept administration, indicating the importance of transmembrane TNF-α on the peripheral immune response [250]. TNF-α inhibitors, 3,6′ dithiothalidomide, TNF-α binding protein, and pentoxifylline have also been explored to achieve adequate TNF-α inhibition post-brain injury [69, 72, 251] and post-AIS [252–255], demonstrating improved histologic and behavioral outcomes. More recently, a third generation thalidomide, pomalidomide, was also shown to decrease neuronal cell death and curb neuroinflammation via a large decrease in TNF-α concentration, in addition to improving motor and sensory functional deficits when administered as late as 5 h post-TBI in rats [256]. Additional biologic TNF-α inhibitors, such as infliximab and adalimumab, currently approved for the treatment of inflammatory and autoimmune diseases, such as rheumatoid arthritis, may prove beneficial in AIS and TBI. B-cells have been detected in the brain post-injury due to increased BBB permeability, permitting their infiltration into the brain parenchyma, and have been shown to mediate inflammation in an animal model of stroke resulting in delayed post-stroke cognitive impairment [257]. An analog of the biologic B-cell inhibitor drug, Rituximab, has been shown to decrease B-cell infiltration across the BBB and improve post-stroke cognitive impairment when administered to mice at 5 days post-stroke with biweekly doses for 7 weeks [257].

4.3. Hormonal modulation

Hormonal intervention also impacts post-injury cytokine expression and has been examined in AIS and TBI. Pre-clinically, progesterone administration post-TBI and post-AIS has been shown to decrease edema, lesion size, excitotoxicity, apoptosis, free radical production, microglial activation and pro-inflammatory cytokines like TNF-α and IL-1β, while promoting remyelination and short-term functional preservation [258, 259]. However, these benefits have not been able to translate clinically. While the pilot phase II ProTECT trial (progesterone for traumatic brain injury, experimental clinical treatment) and a randomized controlled trial of progesterone in severe TBI in China showed improved outcomes and decreased mortality in TBI patients, the larger phase III SyNAPSe trial (study of a neuroprotective agent,

progesterone in severe traumatic brain injury) and phase III PROTECT trial showed no benefit [260–263]. Progesterone has not been tested clinically in AIS patients.

4.4. Omega-3 fatty acids

Administration of omega-3 fatty acids in pre-clinical animal studies of TBI and AIS have been demonstrated to decrease post-injury neuronal death, curb increases in pro-inflammatory cytokine IL-1β and caspase-1 and functional deficits post-TBI [264]. In TBI, pre-injury administration of omega-3 fatty acids in rats' diet and via gavage resulted in decreases in neuronal cell death, edema, lesion volume, caspase-1, IL-18, IL-6 and IL-1β, as well as functional deficits in MWM and beam balance; these effects were largely mediated via G-protein coupled receptor 40 (GPR40), as blockage of this receptor reversed these benefits [264]. In patients, fish consumption has been correlated to decreased cerebrovascular disease risk, but omega-3 fatty acid supplementation alone has not been associated with decreased risk [265]. However, in a study of spontaneously hypertensive rats at increased risk for stroke, glucose utilization and cerebral perfusion were improved with omega-3 fatty acid administration [266]. A clinical trial in Japan examining statin therapy in combination with the omega-3 fatty acid, eicosapentaenoic acid, versus statin therapy alone, demonstrated a decreased incidence of recurrent stroke of 20% within 5 years [267].

4.5. N-acetylcysteine

N-acetylcysteine has been shown to have anti-inflammatory and antioxidant actions, increasing glutathione synthesis to scavenge ROS, decreasing IL-1β and TNF-α levels in brain injury, decreasing caspase-3 levels, and shifting microglia towards M2 anti-inflammatory phenotypes [268–271].

A pre-clinical experiment evaluating the modulation of oxidative stress with N-acetylcysteine and selenium treatments in TBI demonstrated that use of these treatments affected the oxidant and antioxidant, pro- and anti-inflammatory cytokines balance in rats by both down-regulating IL-1β, a pro-inflammatory cytokine, and up-regulating IL-4, an anti-inflammatory cytokine [272]. N-acetylcysteine administered 30 min post-TBI in rats resulted in improved MWM performance to near sham-injured levels [273]. When administered 60 min post-weight drop injury in mice, functional deficits in novel object recognition and Y-maze were significantly improved [273]. In combination with minocycline, N-acetylcysteine has been shown to act synergistically to reduce TBI-induced demyelination, augment M2 microglia activation in white matter and modulate TBI-induced neuroinflammation, increasing microglial activation yet decreasing the number of injury-induced phagocytic CD68+ macrophages in the corpus callosum. The combination also improves learning and long-term retention in the active place avoidance task [274]. Clinically, N-acetylcysteine has been tested in a double-blind, placebo controlled study of blast-induced mild TBI; mild TBI symptoms included balance dysfunction, headache, hearing loss, neurocognitive dysfunction and confusion. Patients receiving N-acetylcysteine within 24 h of blast injury had fewer to none of these symptoms on day 7 post-treatment with 86% of treated patients experiencing complete symptom resolution by day 7 versus 42% in those receiving placebo [275].

N-acetylcysteine has also shown efficacy in AIS. In a rat model of AIS, N-acetylcysteine administration reduced infarct volume, apoptosis, as well as TNF-α, IL-1β, and iNOS expression [276]. Functionally, rats receiving N-acetylcysteine had improved motor function [275]. Additional studies showed reduced levels of AIS-induced hippocampal cell death with N-acetylcysteine administration [277] and decreased ischemia evoked levels of Nuclear factor kappaB [271].

4.6. Cannabinoids

In a closed head injury model of TBI in rats, Dexanabinol (HU-211), a synthetic cannabinoid that inhibits TNF-α as well as NMDA receptors and free radical proliferation has been shown to reduce BBB breakdown, edema, and functional deficits [278]. Dexanabinol has also been shown to improve deficits in animal models of AIS [279]. In TBI patients, a phase II clinical trial examining the administration of i.v Dexanabinol within 6 h post-injury was observed to improve rises in intracranial pressure 2–3 days post-injury with non-significant increases in Glasgow outcome scores at 6 months in patients with more severe injuries at presentation [280]. However, in the larger phase III trial of Dexanabinol for severe TBI, no benefits were seen, indicating the need for larger sample sizes and well-defined exclusion criteria and outcome measures to detect true effects [281]. Despite the failure of Dexanabinol, other cytokine-modulating cannabinoids continue to be evaluated and show potential utility in TBI and AIS. The endocannabinoid, 2-arachidonoylglycerol, has been shown to decrease pro-inflammatory cytokine expression, decrease breakdown of the BBB and post-injury edema pre-clinically in a closed head injury model of TBI [282, 283].

5. Brain inflammation and brain injury–conclusions

This chapter has focused on the role of select cytokines in the pathophysiology of TBI and AIS. By reviewing pre-clinical and clinical studies from the TBI and AIS literature, one can identify hypotheses that have been successfully confirmed in patient populations and identify gaps in the translation of pre-clinical observations to the hospital wards. In translating concepts from preclinical studies one must be cognizant of the variability in the patient populations examined. For example, the impact of the TNF-α polymorphisms identified in Caucasians and Asians would likely impact the efficacy of TNF-α targeting treatments in these populations. Common cytokine gene polymorphisms should continue to be studied in detail to understand variations in the AIS and TBI-induced inflammatory responses in different patient groups [284]. Various mechanisms of TBI ranging from blunt injury to blast versus penetrating injury also induce distinct cytokine responses as described above. Therefore pre-clinical trials of therapeutics as well as the spatiotemporal characterization of cytokine expression should examine multiple modalities of injury and ischemic insults (embolic versus thrombotic). Effects noted consistently across multiple models of TBI and AIS are more likely to translate clinically.

The pathophysiology of AIS and TBI have significant overlap with similar cytokine roles post-injury. Using genetic knockout studies, the particular role of cytokines in these conditions has

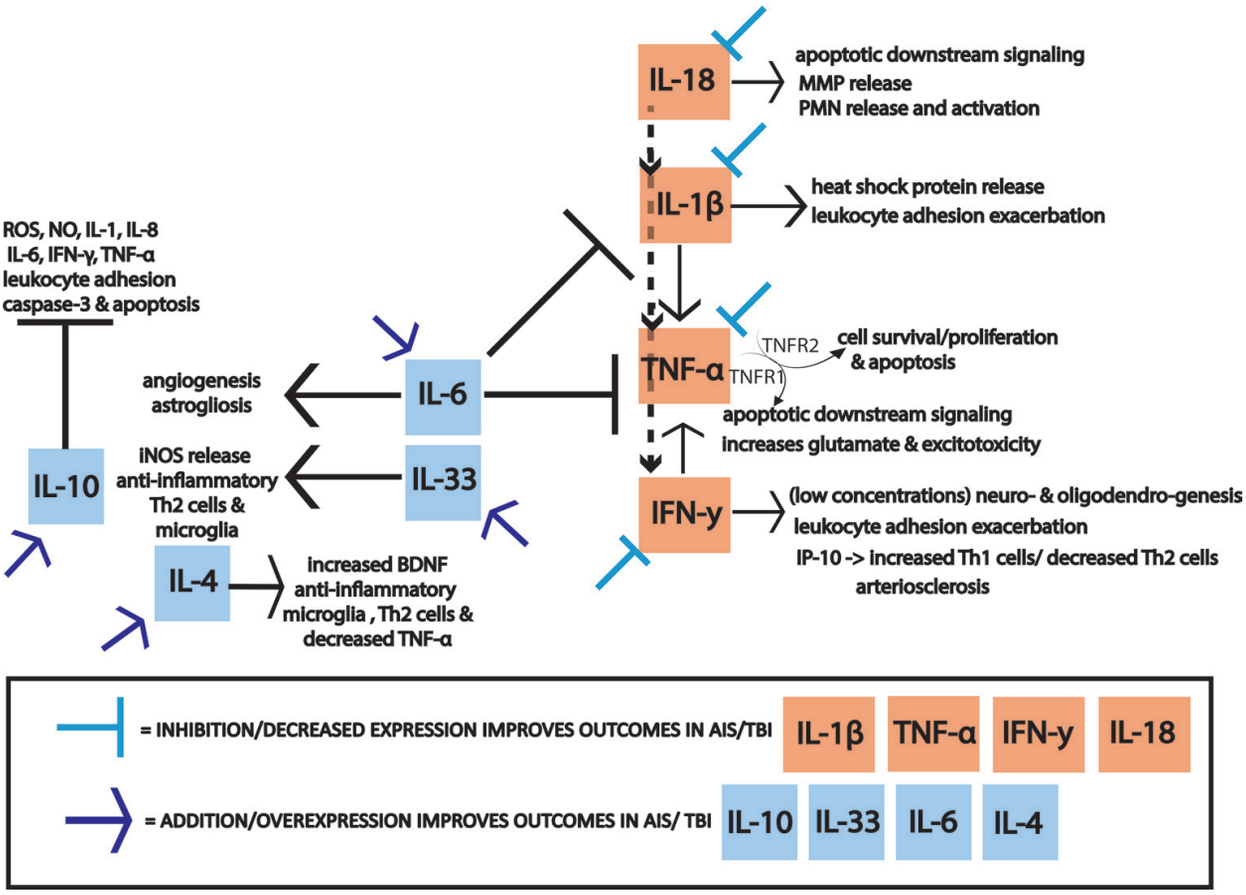

Figure 3. Summary of cytokines reviewed. Knockout or inhibition of TNF-α, IL-1β, IFN-γ, and IL-18 improved outcomes in AIS and TBI. TNF-α can induce cell survival and proliferation, but has been demonstrated to also induce apoptosis via TNFR2 and TNFR1. IFN-γ can induce neuro- and oligodendro-genesis at low concentrations, while higher concentrations cause leukocyte adhesion, arteriosclerosis, and increase the ratio of Th1:Th2 cells via upregulation of IP-10. IFN-γ, IL-18, and IL-1β promote TNF-α expression. IL-18 causes apoptosis, MMP and PMN release and activation, while IL-1β promotes heat shock protein release and leukocyte adhesion. In contrast, addition or overexpression of IL-10, IL-33, IL-6, and IL-4 improves outcomes. IL-6 decreases TNF-α and IL-1β, while promoting angiogenesis and astrogliosis. IL-33 leads to iNOS release and anti-inflammatory Th2 cells and microglia, in addition to the promotion of IL-10 and IL-4. IL-10 decreases brain injury and the cytokines IL-1, IL-8, IL-6, TNF-α, and IFN-γ, while inhibiting nitric oxide, reactive oxygen species, and leukocyte adhesion, as well as apoptosis and caspase-3. IL-4 increases BDNF, anti-inflammatory microglia, and decreases TNF-α expression. Abbreviations: BDNF, brain-derived neurotrophic factor, IP-10, interferon gamma-induced protein 10 (i.e. C-X-C motif chemokine 10), iNOS, inducible nitric oxide synthase, MMP, metalloproteinase, NO, nitric oxide, PMN, polymorphonuclear cells, Th1 cell, T-helper cell type 1, Th2 cell, T-helper cell type 2, TNFR2, TNF-α receptor 2, TNFR1, TNF-α receptor 1, ROS, reactive oxygen species.

been well defined. However, modulation of cytokine expression just prior to injury, rather than from conception is more informative to the molecule's role in AIS and TBI. Knockout or inhibition of TNF-α, IL-1β, IFN-γ, and IL-18 improved outcomes (see **Figure 3**). In contrast, addition or overexpression of IL-10, IL-33, IL-6, and IL-4 improves AIS and TBI outcomes (see **Figure 3**).

Treatments blocking pro-inflammatory and upregulating anti-inflammatory cytokines thr-ough receptor inhibition, synthesis inhibition versus induction, neutralizing antibodies, and

inflammatory response modulators have shown promise. The ratio of pro-versus anti-inflammatory cytokines, as well as baseline cytokine levels may allow one to gauge the overall progression of injury in AIS and TBI to better intervene or establish prognoses. Computational modeling of cytokine patterns and interactions in AIS and TBI of different severities with or without co-morbidities or genetic predispositions may help to further predict how changing one or more variables can impact overall pathophysiology and prognosis.

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