The Role of Neuroinflammation in Traumatic Brain Injury

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Abstract

In industrialized countries, traumatic brain injury (TBI) still represents the leading cause of death and persisting neurologic impairment among young individuals < 45 years of age. Patients who survive the initial injury are susceptible to sustaining secondary cerebral insults which are initiated by the release of neurotoxic and inflammatory endogenous mediators by resident cells of the central nervous system (CNS). The presence of hypoxia and hypotension in the early resuscitative period further aggravates the inflammatory response due to ischemia/reperfusion-mediated injuries. These are induced by the intrathecal generation of free radicals and activation of the complement cascade. Posttraumatic neuroinflammation is further exacerbated by the subsequent intracranial recruitment of blood-derived immunocompetent cells, leading to secondary cerebral edema and increased intracranial pressure. The profound endogenous neuroinflammatory response after TBI, which is phylogenetically aimed at defending the CNS from invading pathogens and repairing lesioned tissue, is, in large part, responsible for the development of secondary brain damage and adverse outcome. However, aside from these deleterious effects, posttraumatic inflammation mediates neuroreparative mechanisms after TBI as well. This "dual effect" of neuroinflammation has been the focus of extensive experimental and clinical research in the past years and has led to an expanded basic knowledge on the cellular and molecular mechanisms which regulate the intracranial inflammatory response after trauma. The present article provides an up-to-date overview on the pathophysiological mechanisms of neuroinflammation after TBI. New potential therapeutic strategies for reducing the extent of secondary brain damage after neurotrauma are discussed.

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Key Words

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Introduction

Despite significant advances in the development of therapeutic concepts for patients suffering from traumatic brain injury (TBI) in recent years, the incidence of delayed mortality and persistent neurologic morbidity after severe TBI remains unacceptably high. In Germany, the incidence amounts to 350 per 100,000, and approximately 280,000 patients are hospitalized for TBI each year [1]. Of these, about 75% can be classified as mild and 25% as moderate to severe, and the mortality of severely head-injured patients remains as high as 35–50% [1–4]. A similar incidence pattern has been reported for other Central-European countries as well [4–7].

The extent of residual brain damage is determined by primary and secondary injuries (Figure 1). The *primary injury* results from mechanical forces applied to skull and brain at the time of impact, leading to either focal or diffuse brain injury patterns. Focal brain injury is due to direct concussion/compression forces, while diffuse axonal shearing injuries are usually caused by indirect trauma mechanisms, such as sudden deceleration or rotational acceleration [3, 8].

Secondary brain injury occurs after the initial trauma and is a consequence of complicating processes initi-

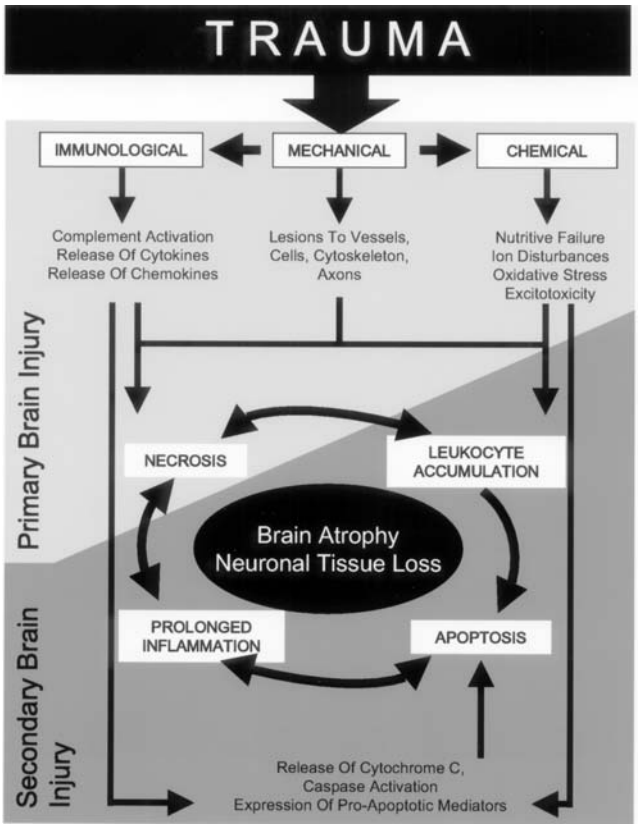

Figure 1. Pathophysiological mechanisms of primary and secondary brain injury after severe head trauma. See text for details and explanations.

ated by the primary injury, whereby the main risk factors are constituted by early hypoxia and hypotension during the resuscitative period [3, 8–13]. The most frequent patterns of secondary brain damage include ischemia/reperfusion injuries, cerebral edema, intracranial hemorrhage, and intracranial hypertension [3, 8, 10–12, 14]. Evidence of secondary brain injury has been found at autopsy in 70–90% of all fatally head-injured patients.

The cascade of events which contribute to the development of secondary brain damage after TBI is very complex and not yet fully understood. This is mainly due to the variety of endogenous mediators released in the intracranial compartment after trauma and the complexity of their interactions as well as time-dependent regulation of agonistic and antagonistic functions. The role of neurotoxic mediators released within the injured brain after trauma in contributing to delayed neuronal cell death has been well documented. Among these, the massive extracellular release of neurotransmitters, such as the excitatory amino acids (EAAs) glutamate and aspartate, has been shown to induce a posttraumatic imbalance of intra- and extracellular ion homeostasis and to contribute to secondary neuronal death [14–16]. Since the mechanisms of secondary neuronal cell death, such as excitotoxicity and apoptosis, are not part of the scope of the present article, the reader is referred to excellent review articles which have been recently published by other authors [14, 17–19].

In addition to the excitotoxic and apoptotic cascades initiated after TBI, a potent inflammatory response is evoked within the injured brain after trauma [20–24]. The neuroinflammatory cascade is triggered by different stimuli after TBI, such as the traumatic impact itself in the initial phase, as well as ischemia/reperfusion-mediated mechanisms and tissue necrosis-induced inflammation (Figure 1). The inflammatory response is characterized by a cascade of events which induce the activation of glial cells, the intrathecal release of pro-inflammatory cytokines and chemokines, upregulation of endothelial adhesion molecules, and the intracranial activation of the complement system with formation of potent inflammatory anaphylatoxins [24–29]. These events mediate the recruitment and activation of blood-derived leukocytes across the *blood-brain barrier* (BBB) and a perpetuation of intracranial inflammation by the "oxidative burst" of polymorphonuclear leukocytes (PMNLs) and release of cytotoxic proteases, leading to a breakdown of the BBB and development of cerebral edema [12, 21, 22, 30–35].

Although the central nervous system (CNS) has been historically defined as an "immunologically privileged organ" due to its tight separation from peripheral circulation by the BBB, research efforts in recent years have revealed that the CNS is a rich source of inflammatory mediators. Resident cells of the brain, such as neurons, astrocytes and microglia, have been shown to be capable of synthesizing essentially all immune mediators of the "peripheral" immune system, including cytokines, chemokines, and complement activation proteins, and to express the receptors for these immune mediators [26, 28, 36–39]. It is nowadays generally accepted that a physiological immune surveillance is present in the CNS and that a potent immune response can be induced within the injured brain. The controversial concept of a "dual role" of neuroinflammation emerged in recent years, based on experimental studies demonstrating a neurotoxic as well as neuroprotective function of inflammatory mediators, depending on the kinetics of regulation and expression in the time course after trauma [38, 40–44].

Since the mechanisms involved in the evolution of secondary brain damage after TBI are highly complex and multifactorial, and since many single factors have been attributed a "dual function" in the pathophysiology of head trauma, targeted specific therapies for TBI have not yet been developed and current treatment concepts remain largely supportive and symptomatic [45–47]. The disadvantages of most studies are that research protocols have focused on single mechanisms only and that results from experimental studies in rodents cannot be directly extrapolated to the clinical setting, as has been demonstrated by the failure of success in several clinical studies on TBI patients [45–50]. Clinical trials on TBI patients have been difficult to design and conduct because of the heterogeneity of the individual injury pattern and additional concomitant factors which affect the outcome, such as associated extracerebral injuries. Thus, the "golden bullet" for specific therapies in TBI has not yet been developed [45–47].

The present review will outline the current understanding on the mechanisms of posttraumatic neuroinflammation after TBI and discuss potential new targets for therapeutic intervention, based on new insights from recently published experimental and clinical studies in the field.

Cytokine-Mediated Neuroinflammation

In the past decade, the important role of pro-inflammatory cytokines released within the injured brain in contributing to the pathophysiological sequelae and adverse outcome after TBI has been well documented in various experimental models and clinical studies [20, 29, 40, 51–54]. Among the cytokines which have received increased attention regarding their role in neurotrauma are tumor necrosis factor (TNF), interleukin-(IL-)1β, IL-6, as well as IL-12 and IL-18, two interferon-(IFN-)γ-modulating cytokines [20, 29, 40, 52–56].

The important role of *TNF* as a mediator of intracerebral inflammation has been highlighted by evidence from studies in the early 1990s. These studies demonstrated that the local administration of recombinant TNF can induce cerebral inflammation, a breakdown of the BBB, and intracranial leukocyte recruitment in various experimental settings [57–60]. These deleterious effects were shown to be abrogated by experimental TNF inhibition, e.g., by the use of specific neutralizing antibodies [61]. Experimental studies on different TBI models have shown that TNF is upregulated in the intracranial compartment within a few hours after trauma [62–67]. Clinical investigations have supported these findings by the detection of elevated TNF levels in human serum and cerebrospinal fluid (CSF) after TBI [53, 68–70]. The potent detrimental role of TNF with regard to cellular neurotoxicity in the CNS and to adverse outcome after TBI has been documented in various experimental studies [64, 71]. These studies have also provided evidence that the pharmacological inhibition of TNF after head injury mediates neuroprotective effects [29, 72–75].

Aside from its neurotoxic effects, TNF was also shown to induce adhesion molecule expression on astrocytes and to regulate leukocyte movement and chemokine expression in the injured CNS [58, 76–78]. Interestingly, recent experimental TBI studies using gene-knockout mice [79] defined a new role for TNF after brain injury, since the deficiency of TNF was shown to be beneficial only in the early period after trauma, but detrimental in the later posttraumatic course [41, 80]. This newly defined "*dual role*" of TNF [40] will be discussed later on.

A potent mediator of intracranial inflammation and BBB damage is also represented by the pro-inflammatory cytokine *IL-1* [54, 58, 81–83]. While IL-1α represents the mainly membrane-bound form, IL-1β is the secreted molecule which is largely responsible for IL-1-induced neurotoxicity [54, 82]. Both isoforms have been shown to be upregulated after experimental brain injury [66, 67, 84–87]. Cellular localization of IL-1α immunoreactivity was detected on injured striatal neurons after experimental hippocampal injury [66]. The potent neurotoxic effects of IL-1 have been shown to be synergistically enhanced in the presence of TNF, suggesting that these crucial cytokines mediate posttraumatic inflammation and secondary brain injury "in concert" [52, 88]. As reported for TNF, the experimental inhibition of IL-1 by administration of IL-1 receptor antagonist (IL-1RA) also resulted in a reduced extent of neuronal damage after TBI [89]. These findings were recently corroborated by studies on transgenic mice with CNS-specific overexpression of soluble IL-1RA [90]. Those transgenic mice showed a delayed intracerebral expression of IL-1β and an improved neurologic recovery after experimental closed head injury, as compared to wild-type littermates [90]. These data underline the important role of IL-1 in the induction of neuroinflammation after traumatic injury.

Another member of the IL-1 family is *IL-18*, a potent IFN-γ-inducing factor, which was found to be expressed and upregulated in the CNS under various

inflammatory conditions, such as infectious, ischemic, and autoimmune-mediated neurologic diseases [52, 91–93]. Recent studies demonstrated posttraumatic induction of IL-18 in the intracranial compartment, both in experimental brain injury models as well as in clinical studies on patients with severe TBI [94, 95]. Yatsiv et al. [94] demonstrated significantly elevated IL-18 levels in murine brains 1 week after closed head injury. In these studies, the posttraumatic upregulation of IL-18 was effectively blocked by systemic administration of a specific recombinant IL-18 inhibitor, *IL-18 binding protein* (IL-18BP). In addition, the posttreatment with IL-18BP 1 h after trauma resulted in improved neurologic recovery during the 1st week after trauma [94]. Since, in the clinical setting, elevated IL-18 levels in human CSF were also detected for > 1 week after trauma [94], these data suggest that IL-18 may represent a potential target for pharmacological modulation of the neuroinflammatory response after TBI.

Similar to IL-18, *IL-12* also represents a pro-inflammatory cytokine with IFN-γ-modulating properties [96]. In the CNS, this heterodimeric cytokine can be synthesized by astrocytes and microglia upon stimulation with different inflammatory mediators [97]. Elevated intracranial IL-12 levels were detected in various neuropathologic states in humans, such as bacterial meningitis [98], multiple sclerosis [99], and head injury [100]. In TBI patients, intrathecal IL-12 levels were significantly elevated for up to 14 days after trauma, compared to daily matched serum samples and control CSF [100]. Regarding the potential role of T-cell-activating cytokines like IL-12 within the injured brain, Holmin et al. [101] recently provided first evidence of the presence of infiltrating $CD4^+$ T-cells in human brain contusions, suggesting that cellular immune responses may also play a role in the immunologic events following head injury. However, the exact pathophysiological role of T-cell-mediated immunity in brain injury remains to be further investigated in experimental models.

In contrast to the mainly pro-inflammatory effects mediated by the aforementioned cytokines in brain injury, *IL-6* is a cytokine with pleiotropic functions in the CNS. While IL-6 was originally characterized as a neuroprotective cytokine and a regulator of intracerebral homeostasis [102], these premises were recently rejected by experimental studies which demonstrated that IL-6 contributes to adverse outcome in autoimmune neuropathology [103]. Elevated intracranial IL-6 levels have been reported in experimental models of TBI [62, 65, 86] as well as in serum and cerebrospinal fluid of head-injured patients [104–107]. Clinical studies have demonstrated that IL-6 released in the intracranial compartment after TBI induces the hepatic acute-phase response after leaking into the peripheral circulation across a defective BBB [108]. In addition, the ex vivo co-incubation of human CSF from TBI patients with primary astrocyte cultures induced the protein production of the neurotrophin nerve growth factor (NGF). This neuroreparative effect of IL-6 could be partially inhibited by co-incubation with neutralizing anti-IL-6 antibodies [106]. These clinical data support the conventional opinion of IL-6 being a mainly neuroreparative cytokine in the pathophysiology of head injury [102]. This notion is further supported by experimental studies on axotomy and cryogenic brain injury models, which provided evidence of strongly comprised inflammatory reactions in the brains of IL-6 gene-deficient mice [109, 110]. Astrocyte-targeted overexpression of IL-6 in the intracranial compartment was previously reported to induce neurotoxicity by induction of a neurodegenerative inflammatory encephalopathy [111]. However, recently published studies on these transgenic mice also reported beneficial brain-repairing effects of IL-6 overexpression in the CNS by induction of antioxidants, such as metallothioneins [112, 113]. Furthermore, the notion of neuroreparative effects of IL-6 was recently supported by demonstration of IL-6-dependent induction of angiogenesis and gliosis in a model of experimental neurotrauma [114]. Thus, a "*dual role*" with predominant neuroprotective properties may be attributed to IL-6 in the pathophysiology of brain injury [43, 53, 102], as for other pro-inflammatory mediators, to be discussed later on.

Intracranial Complement Activation

The complement system plays a key role in innate immunity aimed at protecting against tissue injury or infection [26, 115]. The generation of proteolytic complement fragments leads to pleiotropic inflammatory effects, such as opsonization of invading pathogens for phagocytosis, induction of increased vascular permeability, recruitment of phagocytic cells, augmentation of the acute-phase response, B-cell activation, and cytolysis of pathogens by membrane pore formation through the terminal complement pathway [26, 115]. Studies in recent years have shown that resident cells in the CNS, such as neurons and astroglia, can produce all activation proteins of the complement system and express essentially all complement receptors [36, 115, 116]. It is now

broadly accepted that the activation of the complement system contributes to a variety of CNS pathologies, such as neurodegenerative, autoimmune, infectious, and prion disease [26, 116–121]. Evidence from clinical and experimental studies has, furthermore, revealed pathophysiological mechanisms of complement-mediated secondary brain injury after TBI [25]. These include the recruitment of inflammatory cells into the intrathecal compartment [122, 123], the induction of BBB dysfunction by the *anaphylatoxins* C3a and C5a, the induction of neuronal apoptosis through the C5a receptor (C5aR) expressed on neurons, and complement-mediated homologous cell lysis through the *membrane attack complex* (MAC/C5b-9), following inactivation of the physiological cellular protection mechanisms against complement attack [117, 124–126]. Results from recent experimental studies underline the important role of MAC formation in the brain with regard to induction of secondary neuropathologic events [127, 128]. In these studies, the intracerebroventricular injection of MAC induced a marked upregulation of adhesion molecule expression and leukocyte infiltration in the subarachnoid space with emigration into cerebral parenchyma within 6 h [127]. In addition, MAC injection into the hippocampus evoked seizures and neurocytotoxicity, thus underlying the potent detrimental effects of complement activation in the brain [128]. Activated complement fragments were detected in injured human and rodent brains by immunohistochemistry, demonstrating posttraumatic complement activation and deposition of the MAC in homologous tissue. This suggests that complement may contribute to posttraumatic destruction of brain tissue [129, 130]. Keeling et al. [123] detected neutrophil infiltration and concomitant accumulation of complement C3 in cortical and hippocampal brain sections after experimental TBI in rats. In these studies, a potent role of complement-mediated secondary brain injury was suggested, as C3 accumulation was significantly related to places of intracerebral cell death, increased myeloperoxidase activity, and neutrophil infiltration [123].

In clinical studies, elevated levels of alternative pathway complement components C3 and factor B [131] as well as activated soluble C5b-9 [125] were detected in the CSF of severely head-injured patients. Moreover, the extent of intrathecal complement activation was associated with a dysfunction of the BBB [125, 132]. *Complement C3* represents the most abundant complement component with high constitutive levels in serum (mg/ml), and it plays a central role in the complement activation cascade, since all three activation pathways merge at the C3 activation step (Figure 2). Cleavage of C3 by C3 convertases of either activation pathway leads to formation of C3b, an activation product which acts as opsonin by covalent binding of pathogen surfaces, and to formation of a small peptide fragment, *anaphylatoxin C3a*, a potent inflammatory mediator implicated in cell activation and chemotaxis. Biological effects of C3a are mediated via binding of the C3a receptor (C3aR), a member of a large superfamily of seven transmembrane domain-spanning receptors that are G-protein-coupled [124]. Resident cells of the brain, such as astrocytes, microglia, and neurons, have been shown to express the C3aR constitutively, and upregulation of cellular C3aR expression was detected within the brain under various inflammatory conditions [124, 133, 134]. Aside from the C3a-mediated inflammatory effects, recent in vitro studies have highlighted a potential neuroprotective role for C3a by demonstrating that recombinant C3a protects neurons in a dose-dependent fashion against N-methyl-

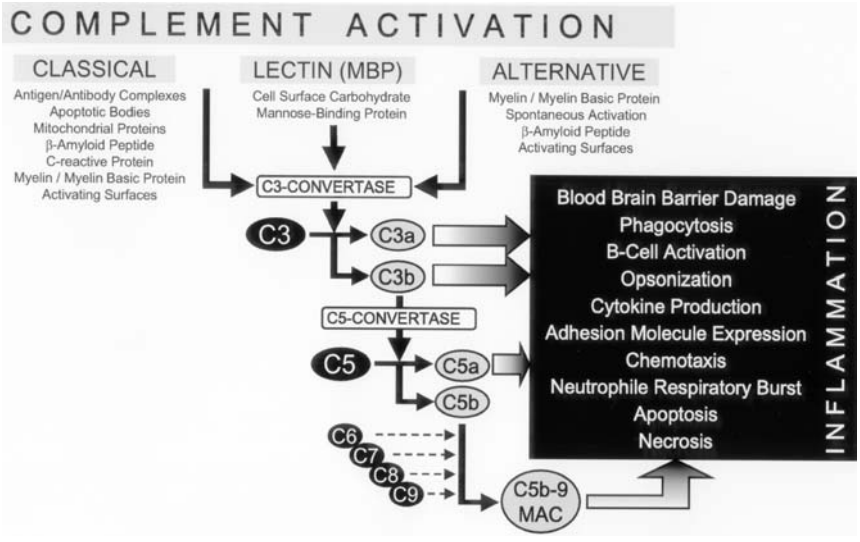

Figure 2. Complement activation pathways and complement-mediated mechanisms of neuroinflammation and secondary brain damage. See text for details and explanations. MAC: membrane attack complex.

D-aspartate-(NMDA-)induced excitotoxicity [135]. In addition, a study by Heese et al. revealed that C3a can induce the production of NGF by microglia, thus supporting a "dual role" for complement anaphylatoxins in mediating cerebral inflammation as well as neuroprotective and neuroregenerative mechanisms after brain injury [135, 136].

The most potent inflammatory mediator derived from activation of the complement cascade is the *anaphylatoxin C5a*, a small peptide fragment of 74 amino acids generated by proteolytic cleavage of the amino terminus of the α-chain in the fifth complement component (C5) by C5 convertases. In peripheral tissues, the inflammatory functions mediated by C5a include the degranulation of mast cells and basophils, leading to increased vascular permeability and edema, the activation of neutrophils and macrophages, neutrophil chemotaxis and induction of the respiratory burst, as well as the enhancement of the hepatic acute-phase response [137, 138]. Aside from those effects in blood and peripheral tissues, recent studies have provided evidence of a wide range of C5a-mediated responses also in the CNS. These include recruitment of neutrophils across the BBB, glial cell chemotaxis, modulation of neuronal functions in the hypothalamus, and activation of signal transduction pathways in astrocytes and neurons [124]. A recent study has provided evidence of C5a-mediated neuronal apoptosis in vitro [139]. Contrary to these findings, C5a-mediated protection from apoptotic neuronal death was reported in a model of intraventricular kainic acid injection in mice [140], suggesting that C5a may also mediate neuroprotective effects, as shown in a model of glutamate-induced neurotoxicity in vivo [140] and recently also in an in vitro model of β-amyloid-induced neurotoxicity [141]. The functional responses to C5a are mediated by binding to the C5aR (CD88), a member of the rhodopsin family of G-protein-coupled receptors with seven transmembrane segments [124]. Low constitutive expression of the C5aR by resident cells of the brain has been demonstrated in astrocytes, microglia, oligodendrocytes, and neurons [124, 142–144]. Upregulation of C5aR expression on these cell types occurs under various pathologic conditions, such as excitotoxic neurodegeneration, autoimmune neuropathology, and meningoencephalitis [117, 119, 124]. In experimental models of TBI, induction of C5aR gene and protein expression was detected on cortical neurons, cerebellar Purkinje cells, and infiltrating leukocytes in the intrathecal compartment [145, 146]. Interestingly, the neuronal C5aR expression was attenuated in mice double-deficient in genes for TNF and lymphotoxin-α by 7 days after closed head injury, suggesting a regulation of the intracerebral C5aR expression through TNF receptor-dependent pathways [146].

The functional role of complement activation in the injured brain was recently investigated in experimental complement inhibition strategies in TBI models [147, 148]. Hicks et al. [147] used a recombinant vaccinia virus complement control protein (VCP) in a fluid percussion model of head injury. They could demonstrate that intracranial VCP administration had protective effects against impairment in spatial memory but not against neuropathologic damage. However, the fact that the truncated form of VCP (VCPt), which lacks complement inhibitory activity, also provided protection against spatial memory impairment, indicates that VCP-mediated protective effects are independent of complement inhibition [147]. Rancan et al. [148] have provided first evidence of a potent functional role of intracranial complement activation in TBI based on studies in transgenic mice with CNS-restricted, astrocyte-targeted expression of the soluble complement inhibitor sCrry. In a model of closed head injury, the transgenic mice showed a significantly reduced neurologic impairment and an improved BBB function, both on a quantitative and qualitative level, compared to wild-type C57BL/6 littermates [148]. These results further implicate the complement system as a participant in secondary progression of brain damage after TBI and provide a strong rationale for future studies of posttraumatic pharmacological complement inhibition.

Chemokines, Adhesion Molecules, and Leukocyte Recruitment

The intracranial infiltration of blood-derived leukocytes represents a crucial event which significantly contributes to the development of secondary brain damage after TBI [32, 55, 149]. Most importantly, the recruitment of neutrophils (PMNLs) across the BBB is detrimental for the intracerebral cellular homeostasis due to the release of proteases and free oxygen radicals by PMNLs which mediate neurotoxicity and contribute to the development of cerebral edema and BBB breakdown [32]. The accumulation and activation of leukocytes in the injured brain are mediated by chemoattractant factors, such as chemokines and com-

plement anaphylatoxins, and adhesion molecules [33, 150].

Chemokines are a large family of structurally related small proteins of 8–10 kDa sharing the ability to induce chemotaxis and tissue extravasation and to modulate various functions of leukocytes [38]. The chemokine subfamilies are distinguished by the position of the first two conserved cysteines, which are either separated by one to three amino acids (CXC and CX_3C chemokines) or adjacent (CC chemokines). While *CXC chemokines* act primarily on neutrophils, the *CC chemokines* exert their functions in monocytes, lymphocytes, mast cells, and eosinophils. Resident cells in the CNS, including neurons, astrocytes, and microglia, have the ability to produce chemokines in response to inflammatory stimuli [28, 38, 151]. As for the complement anaphylatoxins, the biological activities of chemokines are mediated through G-protein-coupled serpentine receptors. In the CNS, chemokine receptors are constitutively expressed on various neural cells [28, 38, 151].

Clinical studies reported significantly elevated intrathecal levels of the CXC chemokine IL-8 (CXCL8) in severely head-injured patients [107, 152, 153]. A detrimental role of this chemokine in TBI patients was suggested by the finding of a significant correlation between intrathecal IL-8 levels and the extent of posttraumatic BBB dysfunction [152] and posttraumatic mortality [153]. These findings were corroborated by experimental studies showing that the hippocampal injection of recombinant IL-8 or macrophage inflammatory protein-(MIP-)2, the rodent homologue for human IL-8, into rodent brains induced a dramatic accumulation of neutrophils and an increased BBB permeability [154]. These detrimental effects could be reduced by prior depletion of circulating leukocytes [154]. Contrary to these pro-inflammatory effects, IL-8 was also shown to mediate neuroreparative mechanisms after brain injury [152]. This was shown by ex vivo stimulation of cultured primary astrocytes with human CSF from TBI patients containing high levels of IL-8. These CSF samples were shown to induce NGF production in astrocytes, an effect which could be reproduced in a dose-dependent manner by stimulation with recombinant IL-8 alone and could be inhibited by co-incubation of human CSF with neutralizing anti-IL-8 antibodies [152]. Otto et al. [155] further investigated the regulation of intracranial *MIP-2* (CXCL2) expression, the murine homologue for IL-8, and its receptor *CXCR2* in a mouse model of closed head injury. Significantly elevated MIP-2 levels were found in the injured murine brain hemisphere by 4 h after trauma, compared to the contralateral hemisphere and to sham-operated mice. In addition, within 24 h after head injury a dramatic upregulation of the MIP-2 receptor CXCR2 was found on astrocytes. Intracranial MIP-2 expression was also found to be regulated by a TNF receptor-dependent pathway, since chemokine levels were significantly attenuated in knockout mice deficient in genes for TNF and lymphotoxin-α [155]. In the same model, increased intracranial levels of the CC chemokine *MIP-1α* (CCL3) and its receptor CCR5 were found; however, the TNF receptor-dependent regulation of expression was not evident for the CC chemokine [155]. These findings are in contrast to recently published data demonstrating that the stereotactic intracerebral injection of recombinant TNF in mice induces expression of several CC chemokines, such as RANTES (CCL5) or MIP-1α (CCL3), and their corresponding receptors CCR1, CCR2, and CCR5 [156]. Upregulation of CXC and CC chemokines and chemokine receptors was also found in various other models of brain injury by other groups [38, 39, 55, 157, 158]. Otto et al. further investigated the molecular interdependence of TNF with IL-8 and the *intercellular adhesion molecule-(ICAM-)1* (CD54), an adhesion molecule which was previously found elevated in injured brains [159, 160] as well as in soluble form in the CSF of brain-injured patients [161]. Based on in vitro experiments on cultured murine microvascular endothelial cells and primary astrocytes, sICAM-1 was found to be a strong inducer of MIP-2 protein in a dose-dependent manner [78]. In addition, a synergistic effect of MIP-2 production by these cell types was observed upon concomitant stimulation with sICAM-1 and recombinant murine TNF, suggesting that both mediators represent potent regulators of chemokine expression and regulation of posttraumatic leukocyte trafficking into the injured brain [78].

Although the intracerebral upregulation of ICAM-1 seems pivotal in mediating the extravasation of leukocytes across the BBB, studies in ICAM-1 gene-deficient mice did not confirm this notion, based on experiments demonstrating irrelevance of ICAM-1 absence in knockout mice after TBI [162]. However, in the absence of both ICAM-1 and P-selectin a significant reduction of posttraumatic brain edema was found, implying that concomitant adhesion molecule expression and upregulation are required for modulation of the intracranial leukocyte accumulation [34]. In this regard, the important role of selectins in mediating intracranial neu-

trophil infiltration after TBI was further supported by studies by Grady et al. [163] who demonstrated that the P-selectin blockade with neutralizing monoclonal antibodies resulted in significantly reduced myeloperoxidase activity in injured rat brains. Carlos et al. [33] investigated the temporal pattern of intracerebral *E-selectin* and *ICAM-1* expression after experimental TBI. They found a significant increase of both adhesion molecules in the injured hemispheres by 4 h after trauma [33]. Similar to the finding in ICAM-1 knockout mice [162], the experimental blocking of ICAM-1 by administration of neutralizing antibodies did not prevent neutrophil accumulation in the injured brain, thus supporting the crucial requirement for selectins for posttraumatic leukocyte trafficking after brain injury [33].

Posttraumatic Ischemia/Reperfusion Injury and Oxidative Stress

An important contribution to intracerebral inflammation and delayed secondary brain damage after TBI is due to ischemia/reperfusion-mediated injuries and oxidative stress (Figure 3) [14, 164, 165]. Pathophysiologically, contused brain areas are surrounded by a penumbra zone which is characterized by hypoperfusion and local ischemia following vascular damage, loss of cerebrovascular autoregulation, and systemic hypotension. After resuscitation, these hypoperfused and ischemic brain areas are reperfused, which leads to activation of the complement cascade and of reactive oxygen intermediates by activation of the xanthine oxidase [166, 167]. After activation of these pathways, potent biologically active mediators are generated which promote leukocyte extravasation and secondary brain tissue damage. Free oxygen radicals, such as hydroxyl ions, hydrogen peroxide, and superoxide anion, induce lipid peroxidation, cell membrane disintegration, and delayed neuronal cell death. In addition, activation of the complement cascade by ischemia/reperfusion-mediated mechanisms results in formation of potent inflammatory anaphylatoxins and terminal complement complex-induced cell death, leading to the pathophysiological sequelae which have already been outlined earlier. In recent years, models of experimental cerebral ischemia have confirmed the potent role of complement activation in ischemia/reperfusion injury [138, 166, 168].

Data derived from experimental head injury models emphasize the important role of *oxidative stress* for the development of secondary brain injury [31, 35, 165, 169].

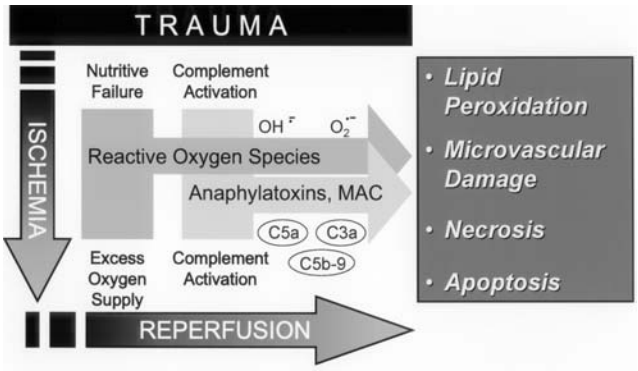

Figure 3. Role of ischemia/reperfusion injury on secondary neuropathologic events after head trauma. The traumatic insult to the brain leads to local ischemic conditions in the contused brain tissue and to local complement activation. In addition, systemic hypotension and hypoxemia due to associated extracranial injuries can contribute to temporary global cerebral ischemia after breakdown of the cerebrovascular autoregulation. Ischemia-induced nutritive failure and adenosine triphosphate (ATP) degradation lead to generation of hypoxanthine. During the reperfusion phase, xanthine oxidase generates reactive oxygen species with neurotoxic properties, such as superoxide anion and hydroxyl anion. Furthermore, the reperfusion contributes to an inflow of complement-activating mediators, thus generating the potent inflammatory anaphylatoxins C3a and C5a and the terminal pathway membrane attack complex (MAC/C5b-9). The local generation of oxygen radicals and activated complement fragments contributes to the ischemia/reperfusion-mediated secondary brain injuries by inducing lipid peroxidation, microvascular damage, cellular necrosis, and apoptosis.

Lipid peroxidation by generation of oxygen radicals is facilitated in the brain due to its genuine vulnerability to oxidative stress based on specific morphological characteristics, such as a high "membrane-to-cytoplasm" ratio and high levels of polyunsaturated fatty acids in the CNS [165]. The brain's *endogenous antioxidants* include superoxide dismutase and other low molecular weight antioxidants, such as α-tocopherol and others. Shohami et al. have thoroughly investigated the brain antioxidant capacity and the overall reducing antioxidant profile in a model of closed head injury in the rat [21, 35]. Tyurin et al. [31] examined the role of oxidative stress in experimental neuro-trauma and showed significant posttraumatic decrease and delayed restoration of intracerebral antioxidant levels, as a sign of posttraumatic consumption. In these studies, α-tocopherol was detected as a highly efficient antioxidant agent in the injured brain [31]. These data were recently supported by clinical studies which detected a marked and sustained decrease of total antioxidant reserve in human CSF following head injury [30]. In addition to reactive oxygen intermediates, the generation of *nitric oxide* (NO) by NO synthase upregulation also

occurs in the injured brain and contributes to posttraumatic neuropathology [170, 171]. Most importantly, additional metabolites emerging from the interaction between superoxide anion and NO, such as the highly reactive oxidant peroxynitrite, have been shown to mediate neurotoxicity and secondary neuronal cell death [172]. The importance of oxygen- and NO-derived free radicals in brain injury has been elucidated by experimental studies using pharmacological approaches with free radical scavengers, demonstrating improved functional and morphological outcome after blocking of free radicals [173, 174]. Altogether, posttraumatic ischemia/reperfusion injury seems to represent an important mechanism of neuroinflammation contributing to secondary brain damage by complement activation and formation of oxygen- and NO-derived free radicals.

Dual Role of the Inflammatory Response

Based on manifold experimental and clinical studies published in recent years, there is conflicting evidence on the role of the neuroinflammatory response in the injured brain. Many of the formerly designated "pro-inflammatory" mediators have shown to possess potential effects in mediating deleterious as well as repair processes in the CNS. Thus, the concept of a "*dual effect*" of inflammatory mediators regarding their role in the pathophysiology of brain injury has arisen in recent years [40, 42–44, 53]. "Classic" inflammatory cytokines like TNF have been historically determined as harmful mediators based on in vitro data of neurotoxicity and in vivo data showing neuroprotection by pharmacological inhibition of TNF in various models of neuroinflammation and neurodegeneration [40]. This notion was rejected in recent years by data on brain injury models in genetically engineered mice. These studies provided evidence that mice with a genetic deficiency of TNF and members of the TNF family, such as lymphotoxin (formerly designated as TNF-β), as well as the deficiency in TNF receptors have a worse outcome and a higher mortality than their wild-type littermates in the later period after injury [40, 41, 80, 175]. These findings were corroborated by Sullivan et al. [176], who subjected mice to experimental brain injury. They reported an enhanced BBB damage and increased cerebral lesion volume in knockout mice lacking p55 and p75 TNF receptors compared to wild-type animals. Other studies on neuropathologic models nicely demonstrated that the pretreatment with TNF was capable of preconditioning rodents to tolerate ischemia, that TNF promotes the accumulation of proliferating oligodendrocyte progenitors required for remyelination in demyelinating disease [177], and that TNF knockout mice have a greater cerebral infarction area in experimental stroke [40].

Many pro-inflammatory mediators have been found to induce neurotrophin production after brain injury, as demonstrated for cytokines (e.g., IL-1β, IL-6), chemokines (e.g., IL-8), and inflammatory complement activation fragments (e.g., C3a) [106, 136, 152, 178–180]. In addition to neurotrophin induction, the neuropoietic cytokines like IL-6 have been shown to possess additional mechanisms of neuroprotection in TBI models, by mediating the generation of antioxidants, such as metallothioneins [113, 181]. Furthermore, the intracranial activation of the complement cascade has been shown to mediate neuroprotective effects aside from the previously known neuroinflammatory and deleterious functions [135, 140, 166]. Even for C5a, the most potent pro-inflammatory complement fragment, neuroprotective mechanisms have been described by achieving neuroprotection in glutamate-mediated excitotoxicity [140]. In addition, C5a was shown to inhibit caspase-3 activity and neuronal apoptosis in glutamate-mediated neurodegeneration [182]. Altogether, in order to reconcile the apparently conflicting reports of beneficial and deleterious effects of various pro-inflammatory mediators, the exact timing and extent of mediator production and activation must be taken into account, as well as the presence of additional factors which may take over redundant functions, e.g., in neuropathology models with use of genetically engineered mice. Thus, appropriate context of concomitant factors and the kinetics and localization of inflammatory mediator expression and activation will determine the harmful or protective properties in the context of neuroinflammation.

Anti-Inflammatory Mechanisms and Therapeutic Strategies

Despite thorough insights into pathophysiological mechanisms of neuroinflammation after TBI, multiple prospective randomized clinical neuroprotection trials performed in the past decades have failed to provide a benefit of anti-inflammatory pharmacological strategies with regard to the outcome after head trauma [9, 45, 47, 183, 184]. The disappointing awareness of this failure in the clinical setting indicates that the complex processes of neuroinflammation cannot be efficiently interrupted

by targeting just one single mediator of inflammation [47]. In addition, the characteristic "dual effect" of most pro-inflammatory agents implies that the irreversible blocking of any inflammatory mediator will inevitably lead to concomitant adverse effects associated with the pharmacological intervention. This notion was dramatically confirmed by the report of an unexpected increased mortality of septic patients treated with a recombinant soluble TNF-neutralizing fusion protein compared to placebo-treated patients in a randomized prospective double-blind multicenter trial on 141 patients with septic shock [185].

Endogenous anti-inflammatory mediators released intracranially after brain injury, such as transforming growth factor-(TGF-)β or *IL-10*, provide a rationale for potential therapeutic interventions aimed at reducing the extent of neuroinflammation-associated adverse events after TBI, such as secondary cerebral edema [70, 105, 186]. The known anti-inflammatory properties of IL-10 include the inhibition of IL-1 and TNF expression and of cytokine-mediated glial activation as well as the inhibition of leukocyte adhesion [187, 188]. In experimental neurotrauma, the administration of recombinant IL-10 was shown to inhibit intracranial TNF synthesis and glial cell activation and to induce an improved neurologic recovery [186, 188]. Further studies are required for assessment of the kinetics of BBB penetration following intravenous administration of IL-10 after brain injury and for determination of intracranial concentrations and the therapeutic window of opportunity. The central role of pro-inflammatory cytokines, such as TNF, IL-1, or IL-18, in the pathophysiology of TBI has been discussed previously in this article. Inhibition of these mediators at determined time points after head injury may represent a means for pharmacological reduction of neuroinflammatory events after trauma. This hypothesis has been supported by recent experimental studies which provided evidence of neuroprotection by inhibition of TNF, IL-1, and IL-18 in rodent models of closed head injury [54, 72, 90, 94].

Corticosteroids are potent immunosuppressive agents which inhibit pro-inflammatory cytokine synthesis; however, their application in the setting of neurotrauma has been controversially discussed [184]. Extensive meta-analyses of prospective randomized clinical trials conducted on the role of corticosteroids in brain injury failed to provide an overall benefit for TBI patients [184]. Due to the relatively small number of patients included in the single studies and due to the heterogeneity of head trauma with the bias of interference by associated systemic injuries, neither moderate benefits nor moderate harmful effects of corticosteroids could be demonstrated with statistical significance [184]. By contrast, the use of methylprednisolone has been established as a therapy for patients with acute spinal cord injury, based on the positive results from the prospective randomized NASCIS II and III trials [196]. This success in the clinical setting of spinal cord injury has renewed the interest in a possible role of methylprednisolone for neuroprotection in TBI patients. The currently ongoing prospective randomized, placebo-controlled *"CRASH"* trial (see: *http://www.crash.lshtm.ac.uk*) has been designed as the largest ever conducted clinical trial on head injury [189]. The study is aimed at elucidating the effects of a 48 h infusion of high-dose methylprednisolone on the outcome after TBI with the aim of recruiting a number of 20,000 patients by the year 2005. This large cohort has been required in order to avoid a statistical type-2 error regarding the statistical significance of a potential benefit in a small percentage range, which could overall have a positive effect on several thousand patients each year based on the high incidence of TBI [189].

In addition to corticosteroids, *endocannabinoids*, such as 2-arachidonoyl glycerol (2-AG), have received increased attention in recent years with regard to their strong potential of neuroprotection after head injury [73, 190–193]. Endocannabinoids have been shown to inhibit the release of pro-inflammatory cytokines, reactive oxygen intermediates, and glutamate after brain injury [192, 193]. As a pharmacological agent, *dexanabinol (HU-211)* has emerged as a nonpsychotropic, synthetic cannabinoid which exerts beneficial effects in terms of TNF inhibition and radical scavenging associated with reduction of brain edema in experimental brain injury models [9, 73, 190, 192, 194]. The role of dexanabinol has been investigated in phase II clinical trials and is currently under investigation in phase III trials on patients with severe closed head injury [9, 195]. In the phase II studies, the post-injury intravenous therapy within 6 h has proven effective, safe, and tolerable. In addition, preliminary data imply that the systemic administration of dexanabinol leads to improvement of posttraumatic cerebral perfusion pressure and neurologic outcome [195]. The observed reduction of intracranial hypertension combined with the anti-excitotoxic, antioxidant and anti-inflammatory properties of dexanabinol render this agent a "key candidate" for future therapeutic strategies aimed at amelioration of brain damage after TBI [9].

Future studies will have to determine whether dexanabinol represents the long-sought "golden bullet" for reduction of secondary brain damage and induction of improved outcome after head injury. It seems reasonable to suggest that a combination of dexanabinol with other potent anti-inflammatory therapeutic agents, e.g., complement inhibitors [116, 122, 147, 148], using a determined kinetic regimen of administration after trauma, should represent an efficient new avenue for success in pharmacological therapies in neurotrauma.

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