Full-length review

The role of the complement system in traumatic brain injury

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Abstract

A traumatic impact to the brain induces an intracranial inflammatory response, which consequently leads to the development of brain edema and delayed neuronal death. Evidence from experimental, clinical, and in vitro studies highlight an important role for the complement system in contributing to inflammation within the injured brain. The present review summarizes the current understanding of the mechanisms of complement-mediated secondary brain injury after head trauma. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Complement; Traumatic brain injury; Anaphylatoxin; Chemotactic receptor

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1. Introduction

In the United States, approximately 500,000 patients with traumatic brain injury (TBI) require hospitalization every year, and mostly young people with a median age of 25 years are affected [42,67,85,93]. Patients who survive the initial injury still have a mortality rate of over 30% during their intensive care period, and overall survivors are confronted with long-term neurobehavioral and socio-economic consequences [2,67,93]. The high rate of secondary morbidity and mortality after TBI has been attributed to the post-traumatic inflammatory response within the intracranial compartment [3,59,77], leading to the development of cerebral edema, increased intracranial pressure (ICP) and loss of autoregulation of cerebral blood flow [51,76,90,99,121]. Post-traumatic cerebral ischemia and the intracranial release of neurotoxic mediators both contribute to delayed neuronal cell death [90,121,133,161]. However, the inflammatory response of the injured brain seems to induce beneficial effects as well, in terms of
post-traumatic local induction of neurotrophic factors [101]. In clinical and experimental studies, pro-inflammatory cytokines such as interleukin (IL)-1β [20,21], IL-6 [80], and IL-8 [83], have been shown to induce the production of the neurotrophin nerve growth factor (NGF) within the injured CNS. The net balance between detrimental and beneficial aspects of cerebral inflammation after TBI remains to be elucidated [110,125]. The expanding knowledge of the basic cellular and molecular mechanisms responsible for the inflammatory response of the injured brain has evoked new therapeutic approaches for patients with TBI [12,28,81,90,92,98]. However, no efficacious pharmacotherapy has been developed for brain injured patients to date. This lack of success in the clinic keeps the focus of research on the basic pathological mechanisms of secondary neuronal damage following severe TBI [23,29,90].

The complement system, an important effector arm of the immune system in the defence against invading pathogens, has been shown to contribute to intracranial inflammation in a variety of central nervous system (CNS) diseases, including bacterial meningitis [141], multiple sclerosis (MS), and Alzheimer’s disease (AD) [107,136]. In addition, recent studies have begun to investigate the role of complement in the pathophysiology of TBI [8,70,82,137,150], demonstrating that complement contributes to the post-traumatic inflammatory response within the injured CNS.

The present review summarizes the current knowledge on expression and regulation of complement within the traumatically injured brain. Furthermore, hypotheses are provided regarding mechanisms of complement-mediated secondary brain damage after TBI. Therapeutic intervention by blocking intracerebral complement activation may represent a new pharmacologic strategy in the clinical management of brain injury.

2. Mediators of brain damage after trauma

A traumatic impact to the brain initiates metabolic and inflammatory processes which exacerbate the primary traumatic injury to neurons, leading to secondary brain damage [3,77,90,121,133]. The molecular and cellular mechanisms causing secondary cerebral insults after TBI are very complex and not fully understood yet (Fig. 1). The primary effects of head trauma include diffuse and focal brain injuries, such as diffuse axonal injury (DAI), cerebral contusion, and intracranial bleeding [42,51,99]. These initial injury patterns lead to the development of secondary cerebral ischemia, which is aggravated by the additional presence of systemic hypotension and hypoxia due to shock and pulmonary injuries [22].

Post-traumatic cerebral ischemia induces a cascade of secondary events (Fig. 1), leading to cellular energy failure.

![Fig. 1. Cascade of pathophysiological events following traumatic brain injury: mechanisms of elevated complement levels and complement activation within the injured brain. (C, complement; see text for other abbreviations and explanations).](image-url)
by depletion of intracellular adenosine triphosphate (ATP), followed by pathological membrane depolarization and the cellular release of excitatory amino acids (EAA), such as glutamate and aspartate [90,98,133]. In addition to ischemia-induced release of neurotransmitters, DAI can directly cause the release of EAA by damaged neurons, thus inducing a vicious circle [12,90]. Membrane depolarization and localized tissue acidosis due to ischemia-related energy failure, in concert with elevated extracellular EAA levels, contribute to pathological intracellular ion homeostasis by massive influx of Ca\(^{2+}\) and Na\(^{+}\) ions into injured cells [12,90,133]. Elevated intracellular Ca\(^{2+}\) levels induce the activation of enzymes, such as proteases, phospholipases, inducible nitric oxide (NO) synthase and xanthine oxidase, which cause cellular membrane damage by initiation of the arachidonic acid cascade and formation of nitrogen- and oxygen-derived free radicals [12,15,77,90,121,133,158]. Arachidonic acid metabolites and free radicals induce lipid peroxidation [61], thus leading to membrane damage and ultimately to cellular death [121]. Aside from neuronal cell death, damage to endothelial cells and astrocytes can cause a disturbance of the blood-brain barrier (BBB) integrity [55,129,146], consequently leading to passive leakage of serum proteins into the intrathecal compartment [121]. The post-traumatic intracranial release of pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-\(\alpha\) and IL-1\(\beta\) [77,109], further contributes to BBB damage, since these cytokines have been shown to induce BBB dysfunction and intracranial inflammation in a variety of experimental models [118,119,128]. Furthermore, the release of the chemokine IL-8 into the cerebrospinal fluid (CSF) of patients with severe TBI has been associated with BBB damage [83]. As a result of increased BBB permeability, vasogenic brain edema is induced, leading to increased ICP and decreased cerebral perfusion pressure (CPP), thus aggravating cerebral ischemia and the concomitant pathophysiological events [102,121].

In the further course of post-traumatic events, blood-derived leukocytes are attracted across the BBB into the subarachnoid space (SAS) [59,77]. Leukocyte recruitment into the SAS is mediated by up-regulation of endothelial and leukocyte adhesion molecules [16,65,77,116] and by locally released chemotaxant mediators, such as \(\alpha\) and \(\beta\)-chemokines [9,46,49,83], brain-derived chemotactic factor (BDCF) [103], arachidonic acid-derived leukotrienes [121], and activated complement fragments [70], which will be discussed later on. The post-traumatic recruitment of neutrophils into the CNS is particularly important for the inflammatory response of the injured brain, since neutrophils have been shown to significantly contribute to host tissue damage by release of proteolytic enzymes and reactive oxygen intermediates [32,36,160]. In this regard, multiple studies have demonstrated that neutrophil accumulation in the brain is associated with increased secondary brain damage and adverse outcome, based on experimental models of meningitis [127,128,145,147,151], cerebral ischemia [14,169], and TBI [13,16,17]. A recent study on patients with severe head trauma has analyzed the subset of leukocytes invading the perifocal area around brain contusions [59]. This study demonstrated an early intracranial influx of neutrophils within 24 h after injury, which persisted for up to 5 days. Other myeloid cells, such as monocytes and CD4- and CD8-positive lymphocytes, were hardly detected in the injured CNS during the first 24 h, but were abundantly present around the brain contusion sites on days 3–5 after TBI [59]. Neutrophils, blood-derived monocytes as well as resident cells of the CNS, such as astrocytes and microglia, may contribute to intracranial inflammation in TBI by production of pro-inflammatory cytokines [77,84,109], as well as by release of neurotoxic factors [47,48], which induce delayed neuronal cell death. Induction of programmed cell death (PCD; apoptosis) in neurons has been demonstrated in experimental models of TBI [122,164], however, the mediators inducing neuronal PCD have not been identified. One candidate mediator of PCD after head trauma is represented by soluble Fas ligand, which was detected in elevated amounts in the CSF of TBI patients [27].

The complement system seems to be involved in the cascade of intracranial pathophysiological events occurring as a result of TBI. In the following paragraphs, we will present the current understanding of the cellular and molecular mechanisms leading to elevated complement components and to complement activation within the injured CNS. Furthermore, we will discuss the potential mechanisms of complement-mediated brain injury in the course of TBI.

3. Elevated intracranial complement levels in traumatic brain injury

Constitutive complement expression in the normal CNS is low, principally due to the tight separation between the vascular system and the intrathecal compartment by the BBB [129]. However, a number of soluble complement components have been detected in the CSF under physiological conditions. Among these, complement proteins of the classical (C1q, C4) [24,117,165] and alternative activation pathway (C3, factor B) [24,82,138,170], as well as the terminal lytic pathway (C9) [10,54] have been detected in normal CSF in constitutive low levels. The physiological intrathecal presence of complement components has been functionally attributed to immunosurveillance [136]. The source of complement in normal CSF is either derived from passive leakage around the circumventricular organs, as demonstrated for C9 [10], or from intracerebral synthesis by resident cells of the brain [4,106,136]. Among these, astrocytes and microglia have been shown to produce an intact and functional complement system of the classical and alternative pathway, based on data from in vitro...
In CNS trauma (Fig. 1), clinical studies have demonstrated elevated complement levels in serum [120] and CSF [82]. Specifically, Rebhun et al. detected elevated levels of the complement components C3, C4, and C5 in serum of patients with traumatic spinal cord injury. However, CSF complement levels were not assessed in this study [120]. One mechanism of elevated serum complement levels after traumatic CNS injury is induction of the acute-phase response (APR) after trauma, leading to enhanced synthesis of acute-phase proteins, including complement proteins, by hepatocytes [6,136,167]. The pro-inflammatory cytokine IL-6, a potent inducer of the APR [6], has been shown to be released in the brain of TBI patients and to possibly mediate the systemic APR in the liver after leaking across the BBB into the vascular system [79]. Furthermore, some acute-phase proteins are potent activators of the complement cascade, such as C-reactive protein (CRP) and mannose-binding protein (MaBP), leading to systemic complement activation by initiating the classical (by CRP) and the lectin pathway (by MaBP) [136,152]. The activated complement peptide fragment C5α has also been shown to induce the hepatic APR, either by inducing IL-6 expression in monocytes [60,130], or by binding to C5α receptors (C5αR; CD88) expressed on hepatocytes, thus inducing the biosynthesis of acute-phase complement proteins C3 and factor B [95]. Elevated serum complement components might readily leak across a dysfunctional BBB into the injured CNS after trauma. In a recent study from our laboratory, we demonstrated elevated levels of C3 and factor B, the central components of the alternative complement activation pathway, in the CSF of patients with severe TBI [82]. The C3 CSF levels reached values of 69 μg/ml and were significantly higher than in the CSF of control subjects (mean C3 reference levels in CSF: 2.5–3.0 μg/ml) [24,82,138]. Similarly, factor B CSF levels were significantly elevated in TBI patients, reaching maximal values of 6.2 μg/ml (mean factor B reference levels in CSF: 0.3–0.5 μg/ml) [82,138]. The same study on TBI patients suggested that C3 and factor B levels are in large part derived from serum leakage across a dysfunctional BBB, rather than from intracerebral synthesis [82]. This hypothesis was supported by the observation of a dysfunctional BBB in over 50% of the patients analyzed, as determined by calculation of the albumin quotient, combined with the fact that complement levels are several hundred-fold higher in serum (mg/ml range) than in CSF (μg/ml range), thus leading to a massive influx of serum-derived complement proteins into the CNS as soon as the BBB is even mildly damaged [11]. Furthermore, the calculation of C3- and factor B-indices, based on a modification of the ‘IgG-index’ described by Tibbling et al. for determination of intrathecal IgG synthesis in MS patients [149], was not suggestive of intrathecal complement synthesis for most of the timepoints analyzed for 14 days after TBI [82]. However, the post-traumatic biosynthesis of complement components by resident cells of the brain is likely to occur in addition to passive complement leakage across a dysfunctional BBB, since TBI has been shown to induce the release of pro-inflammatory cytokines within the CNS [77,84], mediators which are known to induce the biosynthesis of complement proteins by resident cells of the brain [136]. These include TNF-α, IL-1β, and IL-8, based on in vitro studies with astroglia cell lines and primary astrocytes [136], as well as IL-6, based on studies using transgenic mice with astrocyte-targeted over-expression of IL-6 [5]. Since astrocytes and microglia are capable of synthesizing pro-inflammatory cytokines [109], it is possible that these cell-types induce complement biosynthesis in an autocrine manner following post-traumatic activation [8,57]. Experimental studies, using a weight drop cortical contusion model in rats, have demonstrated intracerebral complement synthesis after head trauma [8,150]. These studies showed induction of C3 gene expression in microglia, as determined by situ hybridization with antisense oligonucleotide probes [8,150]. In summary, these data suggest that TBI induces increased levels of complement components in the intracranial compartment, and that the source of complement is likely from resident cells of the CNS and serum-derived complement leakage across a dysfunctional BBB. Increased levels of complement components within the injured brain provide the substrate required for complement activation following TBI. The additional presence of complement activating proteins in the CNS may lead to initiation of the complement cascade,
thus contributing to the posttraumatic inflammatory response within the intracranial compartment.

4. Intracerebral complement activation following head trauma

It is generally accepted that complement activation occurs in serum after severe systemic trauma [37,56,72]. In head trauma, several studies demonstrated the intracranial presence of activated complement fragments, suggesting local complement activation. As such, intracerebral complement activation has been found in patients with cerebral ischemia, ischemia-reperfusion injury, and subarachnoid hemorrhage (SAH) [19,73,88], conditions which represent a frequent consequence of TBI (Fig. 1). In these studies, activated proteolytic complement fragments C3a and C4a, as well as soluble C5b-9 (sC5b-9), a fluid-phase cytolytic membrane attack complex (MAC) derived from activation of the terminal complement pathway, were detected in the CSF of patients with cerebral ischemia and SAH [19,73,88]. Furthermore, immunohistochemical staining of human brain tissue from stroke patients was positive for C9 and a C5b-9 neo-epitope, demonstrating MAC deposition within necrotic areas of the brain [88]. Immunohistochemical detection of C3d in the same infarcted areas adds further proof of intracerebral complement activation following cerebral ischemia [88]. In addition, the co-incubation of normal human CSF with normal human serum from healthy donors, used as an in vitro model of BBB damage, resulted in complement activation and formation of sC5b-9 [88]. Taken together, these data illustrate that pathophysiological events occurring after TBI, such as ischemic and hemorrhagic cerebral insults, as well as a breach of the BBB, are able to induce complement activation within the CNS.

A clinical study on patients with isolated TBI demonstrated complement activation in serum, whereas CSF from these patients was not analyzed [7]. Definitive evidence of intracerebral complement activation after TBI was seen in animal models, showing immunoreactivity for activated complement fragments [8,150] and complement-mediated neutrophil chemotaxis [70] in the injured rat brain. Mikael Svensson’s group demonstrated enhanced immunoreactivity for the C3-derived complement fragment C3d and for C9, thus indicating complement activation at the C3 level and initiation of the terminal lytic pathway by deposition of C5b-9 [8,150]. Complement C9 represents a fluid-phase protein which is only detected on cells after integration in the C5b-8 complex. Thus, detection of C9 protein in tissue indicates formation of the MAC [8,88]. Immunoreactivity for C3d and C9 in injured rat brains was first detected 48 h post injury, peaked at 96 h, and persisted up to 14 days. These kinetics correlated with intracerebral expression of C3 mRNA in the same model system [8] and with C3 protein release into the CSF of patients with severe TBI, peaking at 2–4 days after trauma [4,82]. Deposition of C3d and C9 in traumatized rat brains was localized in neuronal perikarya around the contusion lesion [8,150], suggesting that the MAC is incorporated in injured neurons, thus possibly leading to neuronal cell death and secondary brain damage.

A study by Patrick Kochanek’s group, using an experimental percussive head trauma model, demonstrated significant inhibition of post-traumatic neutrophil accumulation in the brain of rats that had been systemically pre-treated with soluble complement receptor type 1 (sCR1) before trauma [70]. The fluid-phase complement inhibitor sCR1 is a recombinant soluble form of the membrane inhibitor CR1 (CD35), which acts by binding to C3b and C4b and rendering these opsonins susceptible to cleavage by factor I, thus preventing formation of the classical and the alternative pathway C3 convertases [159]. The demonstration of attenuated cerebral leukocyte infiltration after application of sCR1 in experimental TBI underlines the importance of complement-derived chemotactic factors, such as the anaphylatoxins C3a and C5a, in contributing to the inflammatory response of the injured brain. This finding is supported by recent data from our laboratory, showing expression of the C5aR (CD88) on leukocytes infiltrating the SAS in the course of experimental DAI, thus suggesting a possible chemotactic role of C5a in this model system (Fig. 3) [137]. However, in the study by Kaczorowski et al., intracranial leukocyte recruitment was inhibited only by 41% after application of sCR1, indicating that in the course of TBI other chemoattractants must be released into the CSF as well [70]. Chemotactic mediators expressed in the CNS after traumatic injury, other than complement anaphylatoxins, have been discussed above.

There are distinct pathophysiological mechanisms which may lead to increased local presence of activated complement fragments in the traumatized CNS. First of all, since complement activation in serum occurs as a consequence of systemic trauma [37,56,72] as well as after isolated TBI [7], it seems reasonable to suggest that activated complement fragments might leak across a defective BBB into the CSF, such as already shown for complement proteins (Fig. 1). On the other hand, the tight regulation of complement activation by cellular and fluid-phase complement inhibitors, receptors, and regulatory proteins [86,105] might rapidly inactivate serum-derived bioactive complement fragments before they reach the CSF. Therefore, it is more likely that intracerebral mediators activate the complement system locally in response to TBI. Myelin basic protein (MBP) figures among the potential complement activating proteins in the injured brain, since MBP is released as a degradation product of myelin after axonal injury through traumatic shearing [94] and by post-traumatic activation of Ca$^{2+}$-dependent proteases [155]. A variety of studies have shown that myelin and MBP are able to activate the classical [18,153,154] and the alternative [78,134] pathway of complement. Activation of proteases by elevated intra-
cellular Ca\(^{2+}\) (Fig. 1) might furthermore directly contribute to complement activation by cleavage of complement components. In addition, it is well recognized that thrombin, kallikrein, and plasmin, serum enzymes derived from post-traumatic intracranial bleeding and from systemic activation of the coagulation cascade after trauma, have the ability to cleave and activate complement components [37,143]. β-amyloid peptide (A\(β\)) is a protein which is thought to be involved in the pathogenesis of AD [52,97,136], represents another potent activator of the complement cascade within the brain. A\(β\) has been shown to activate complement through both the classical and the alternative pathway [26,68,124,136,156]. Epidemiologic studies have revealed a significant association between a history of head injury and the subsequent development of AD [43]. Furthermore, extensive A\(β\) deposits have been detected in human head injury brains, in up to 50% of the cases analyzed [52,62,123]. Intracerebral A\(β\) immuno-reactivity after head trauma was found to be associated with enhanced expression of the A\(β\) precursor protein (\(β\)-APP) by pre-alpha neurons of the entorhinal cortex [52,100]. Neuronal \(β\)-APP expression was furthermore shown to be up-regulated by EAA as well as by pro-inflammatory cytokines, such as IL-1\(α\) [52]. A recent study on TBI patients demonstrated that A\(β\)\(_{1-42}\), a β-amyloid peptide of 42 amino acids length, was the predominant form of A\(β\) depositions in the brain [44]. In addition, we have recently found elevated levels of A\(β\)\(_{1-42}\), but not A\(β\)\(_{1-40}\), in the CSF of patients with severe TBI (Raby et al., unpublished observations). Thus, the increased local presence of A\(β\) within the injured brain might lead to intracerebral complement activation following head trauma.

Finally, as already mentioned earlier, the systemic induction of the APR after TBI [79,167], which induces the hepatic synthesis and elevated serum levels of CRP and MaBP, might contribute to local complement activation after passive leakage of these proteins across a compromised BBB (Fig. 1).

Intracerebral complement activation, as part of the inflammatory response in the CNS after traumatic lesion, may add to the deleterious effects of cerebral inflammation by mediating cellular recruitment into the SAS, contributing to BBB damage, and by inducing delayed neuronal cell death.

### 5. Discussion: potential mechanisms of complement-mediated secondary brain injury

The intracranial presence of bioactive complement fragments and complexes resulting from complement activation in the injured brain is likely to induce a potent local inflammatory response, thus possibly contributing to secondary brain damage after TBI. Several distinct mechanisms of complement-mediated brain damage may be postulated (Fig. 2). First, the initiation of the complement cascade by either of the three pathways (i.e. classical, alternative, and lectin pathway) leads to generation of anaphylatoxins C3a and C5a, which are potent mediators of inflammation [63]. Anaphylatoxins have been shown to induce cerebral inflammation following intracranial inoculation in different experimental models [31,38,58,71]. Intraventricular application of C3a-des-Arg in cats [58] and intracisternal application of C5a in rabbits [31,38,71] induced sterile meningitis within 1 to 4 h, characterized by a massive cellular infiltration in the SAS, a disruption of the BBB and the concomitant development of brain edema. Furthermore, local neutrophil extravasation into brain tissue was demonstrated following experimental intracerebral inoculation of C5a in the rat hypothalamus [162].

The main mechanism by which C3a and C5a contribute to intracerebral inflammation is by the induction of BBB damage (Fig. 2), mediated by increased vascular permeability and chemotaxis and activation of blood-derived inflammatory cells [45,63,64]. The anaphylatoxin-dependent activation of neutrophils leads to local tissue damage by release of proteases and free radicals [36,160]. In monocyes and macrophages, both C3a and C5a have been shown to induce the synthesis of pro-inflammatory cytokines, such as TNF-α, IL-1β, IL-6, and IL-8 [25,50,53,60,112,113,144]. The pathological significance of these cytokines in mediating BBB damage and inducing the hepatic APR has been discussed above. Furthermore, C5a can directly induce the APR as well, by binding to the C5aR expressed on hepatocytes [95], as already mentioned earlier.

Anaphylatoxin C5a possesses a wide range of additional functional properties contributing to cerebral inflammation (Fig. 2). Aside from inducing chemotaxis in leukocytes, mediated by the C5aR expressed on these cells [45,63], C5a induces up-regulation of cellular adhesion molecule expression, such as P-selectin expression by endothelial cells and β\(_2\)-integrin (CD11/CD18) expression and shedding of t-selectin on neutrophils [33,34,66]. These mechanisms are important for cellular interaction between endothelial and blood-derived myeloid cells, in order to mediate leukocyte extravasation at the site of inflammation. Combined with the demonstration of C5aR expression by infiltrating myeloid cells in experimental TBI [137], these data highlight the significance of C5a in mediating post-traumatic accumulation of inflammatory cells within the injured CNS. In addition to C5a-mediated chemotactic activity, formation of C5b-9 in sublytic concentrations has been shown to induce synthesis of α- and β-chemokines by endothelial cells [74]. This finding might point to an additional mechanism by which complement attracts blood-derived neutrophils and monocytes into the SAS in the course of TBI.

Activation of microglia by C3a and C5a has been furthermore demonstrated through elevation of intracellular Ca\(^{2+}\) concentrations [104], a phenomenon which might contribute to the pathophysiological sequelae of...
TBI, as already discussed above. In addition, C5a mediates chemotaxis of astrocytes and microglia [1,166], cell-types known to express C5aR mRNA and protein, based on in vitro [39,87] and in vivo evidence [40,111]. Chemotaxis of microglia in response to locally activated C5a might contribute to neurotoxicity at the lesion site by release of neurotoxic mediators, as previously described [35,48,109]. C5a-mediated chemotaxis of astrocytes may induce focal astrogliosis and scar formation [109] and theoretically lead to BBB-damage by shearing of astrocytic end-feet which engulf capillary pericytes at the BBB and contribute to the physiological micro-anatomical barrier between serum and CSF [129]. Recent data demonstrate that neurons also express C5aR mRNA and protein [140]. In experimental TBI, based on a model of DAI in rats, we have detected enhanced C5aR gene expression on cortical neurons and cerebellar Purkinje cells in the course of trauma (Fig. 3) [137]. These findings suggest that C5a mediates neuronal functions, a hypothesis further supported by studies demonstrating the induction of central nervous system functions mediated by C5a [38,162]. In this system, the experimental intracerebral inoculation of C5a into the hypothalamus induced α-adrenergic effects by modulation of eating and drinking behaviour in rats, which were reversible by addition of α-adrenergic antagonists [162]. In another study, intracisternal C5a application in rabbits induced neuronal dysfunction, resulting in pathological central autonomic regulation of heart rate dynamics [38]. In addition, recent in vitro studies demonstrate a direct effect of C5a on neurotoxicity, as demonstrated by the induction of apoptosis/PCD in neuroblastoma cell lines [30]. Secondary neuronal death by apoptosis has already been demonstrated to occur in experimental TBI models [122,164]. Taken together, these findings implicate that the C5aR expressed by neurons [137,140] is functionally active, and might mediate post-traumatic neuronal dysfunction and neuronal cell death. Recent data demonstrate the constitutive expression of the C3a receptor (C3aR) by cultured primary human astrocytes and microglia [41]. Furthermore, up-regulation of C3aR expression by glia was detected in the brain of patients with MS and bacterial meningitis [41]. In addition, unpublished data demonstrate constitutive C3aR expression by neurons in vivo and by neuroblastoma cell-lines in vitro (Davoust et al., unpublished observations). However, the pathophysiological function of ligand binding to the C3aR on neurons and glial cells remains to be determined.

Finally, the intracerebral generation of C5b-9 (MAC) through activation of the terminal complement pathway, may contribute to host cell death by inducing cell lysis. In vitro experiments on rat glial cell cultures have demonstrated the susceptibility of primary rat oligodendrocytes to
Fig. 3. Induction of C5a receptor (C5aR) mRNA expression by cortical neurons and cerebellar Purkinje cells in the rat brain following traumatic diffuse axonal injury. Frozen brain sections were analyzed by in situ hybridization, using digoxigenin-labeled mouse C5aR anti-sense (A, B, D, E) and sense (C) cRNA probes, detected by alkaline phosphatase color reaction, as previously described [137]. In the lateral ventricle of a rat subjected to experimental head trauma, infiltrating cells expressing C5aR mRNA were seen at 24 h after injury (A). Within the brain parenchyma, C5aR transcripts were detected on Purkinje cells in the cerebellum early after trauma (t = 8 h) (B). In the later course of the disease (t = 96 h), strong up-regulation of C5aR mRNA expression was observed on cortical neurons (D, E). No cellular signals were detected by hybridization with C5aR sense riboprobes (C; adjacent section to D). Original magnifications: (A), (B), (E), 50 × ; (C), (D), 20 × .

Homologous complement-mediated lysis [115,163]. In these studies, the glial susceptibility to complement attack was attributed to the lack of CD59 expression by rat oligodendrocytes. CD59 is a complement regulatory molecule belonging to the superfamily of glycosyl-phosphatidylinositol (GPI)-anchored cell surface proteins. These proteins are covalently attached to the glycolipid GPI and lack a transmembrane or cytoplasmatic domain [142]. CD59 regulates complement by preventing the formation of the MAC [86,135]. In contrast to the finding of complement-mediated oligodendrocyte lysis in rats [115,163], another study demonstrated insensitivity of primary human oligodendrocyte cultures to homologous complement attack, and CD59 was shown to be expressed on human oligodendrocytes, contrary to the finding on rat glia [168]. CD59 is furthermore constitutively expressed on human neurons, as demonstrated by analysis of AD brains [96]. In vitro experiments demonstrated protection of human neuroblastoma cells from homologous complement-mediated lysis, by CD59 expression [91,131]. However, whether human neurons and glia express adequate amounts of CD59 to protect from MAC-mediated lysis under inflammatory conditions, has not been explored. Caution should be used in extrapolating results from in vitro studies to in vivo inflammation, such as in a setting of intracranial inflammation in the course of TBI. Nevertheless, the intracerebral activation of phospholipases, induced by elevation of intracellular Ca^{2+} levels following TBI (Fig. 1), will lead to
activated complement fragments contribute to secondary brain damage, the recruitment of inflammatory cells across the BBB and the induction of BBB dysfunction by anaphylatoxins C3a and C5a seem to play important roles in the inflammatory response of the brain to trauma. Furthermore, C5a may also contribute to astrogliosis and scar formation as well as neuronal apoptosis by binding to the C5aR expressed by glial cells and neurons. Finally, the formation of C5b-9 (MAC) by activation of the terminal pathway of complement may induce homologous cell lysis after inactivation of the physiological cellular protection mechanisms against complement attack through the intracerebral inflammatory response.

Strategies of therapeutic inhibition of complement activation have been suggested for a variety of CNS pathologies, such as neurodegenerative and cerebral autoimmune diseases [136]. In TBI, the inhibition of local complement activation might represent a new and promising therapeutic direction. In this regard, we plan to explore the pathological significance of post-traumatic complement activation, based on an experimental model of TBI in newly developed transgenic mice with astrocytic overexpression of Crry, a soluble inhibitor of the murine classical and alternative complement activation pathway (Barnum, personal communication). Furthermore, a variety of inhibitors of complement activation are available for assessment of their impact in the pathophysiology of TBI [75,108,136]. Among these, the application of soluble complement inhibitors, such as recombinant sCR1 [70] and C1-inhibitor (C1-INH), and the administration of blocking antibodies to key proteins of the complement cascade (e.g. C3, C5) and neutralizing antibodies to biologically active complement fragments (e.g. C5a) represent potential future pharmacotherapeutic strategies in TBI. In addition, the blocking of β2-integrin complement receptors (CR3, CR4), which promote adhesive interactions between leukocytes and the BBB endothelium, might attenuate the extent of secondary brain damage by reduction of the post-traumatic cellular infiltration in the SAS. Finally, the administration of recombinant soluble complement regulatory proteins (e.g. sCD46, sCD55, sCD59) might prevent complement-mediated host cell damage within the injured brain. For further insight into therapeutic approaches with regard to inhibition of complement activation in the CNS we refer to an excellent review by Spiegel et al. [136].

Effective pharmacological complement inhibition might provide a future avenue for successful attenuation of secondary brain injury, and might hopefully help reduce the high rate of morbidity and delayed mortality in patients with severe head trauma.

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**References**

1. Becker et al. on TBI patients suggested a correlation between the degree of systemic complement activation and the severity of damaged brain tissue, implying that activated complement fragments contribute to secondary brain damage after TBI. Among the mechanisms of complement-mediated cerebral damage, the recruitment of inflammatory cells across the BBB and the induction of BBB dysfunction by anaphylatoxins C3a and C5a seem to play important roles in the inflammatory response of the brain to trauma. Furthermore, C5a may also contribute to astrogliosis and scar formation as well as neuronal apoptosis by binding to the C5aR expressed by glial cells and neurons. Finally, the formation of C5b-9 (MAC) by activation of the terminal pathway of complement may induce homologous cell lysis after inactivation of the physiological cellular protection mechanisms against complement attack through the intracerebral inflammatory response.

Strategies of therapeutic inhibition of complement activation have been suggested for a variety of CNS pathologies, such as neurodegenerative and cerebral autoimmune diseases [136]. In TBI, the inhibition of local complement activation might represent a new and promising therapeutic direction. In this regard, we plan to explore the pathological significance of post-traumatic complement activation, based on an experimental model of TBI in newly developed transgenic mice with astrocytic overexpression of Crry, a soluble inhibitor of the murine classical and alternative complement activation pathway (Barnum, personal communication). Furthermore, a variety of inhibitors of complement activation are available for assessment of their impact in the pathophysiology of TBI [75,108,136]. Among these, the application of soluble complement inhibitors, such as recombinant sCR1 [70] and C1-inhibitor (C1-INH), and the administration of blocking antibodies to key proteins of the complement cascade (e.g. C3, C5) and neutralizing antibodies to biologically active complement fragments (e.g. C5a) represent potential future pharmacotherapeutic strategies in TBI. In addition, the blocking of β2-integrin complement receptors (CR3, CR4), which promote adhesive interactions between leukocytes and the BBB endothelium, might attenuate the extent of secondary brain damage by reduction of the post-traumatic cellular infiltration in the SAS. Finally, the administration of recombinant soluble complement regulatory proteins (e.g. sCD46, sCD55, sCD59) might prevent complement-mediated host cell damage within the injured brain. For further insight into therapeutic approaches with regard to inhibition of complement activation in the CNS we refer to an excellent review by Spiegel et al. [136].

Effective pharmacological complement inhibition might provide a future avenue for successful attenuation of secondary brain injury, and might hopefully help reduce the high rate of morbidity and delayed mortality in patients with severe head trauma.

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References


