The role of the complement system in CNS inflammatory diseases

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Complement is an important component of both the innate and adaptive immune response that contributes to host defense in a variety of mechanisms, including inflammation, phagocytosis and cell lysis. Complement proteins are produced by all cell types in the CNS, and the same effector functions that protect the host from pathogens can mediate inflammation and tissue destruction in CNS diseases, leading to neurological deficits or even death. In the last 10 years, the development of complement inhibitors and a variety of animal models for CNS diseases has revealed that targeted inhibition of complement offers significant therapeutic potential. This review discusses the subtleties of targeted complement inhibition in CNS disease as an emerging therapeutic strategy.


Although inflammation and immune responses have been appreciated for some time in the CNS, the role of innate immunity, particularly the complement system, has been poorly understood. For the most part, complement has been associated with many CNS disorders and autoimmune diseases as a histological marker of inflammation and tissue destruction. Thus, the presence of C9 or the membrane attack complex (MAC) in tissue sections from brain or spinal cord was frequently interpreted to mean that complement-mediated damage played a critical role in the pathophysiology of the disease. It is now becoming apparent that the role of complement in CNS diseases is more complex than previously appreciated. In disease settings where complement function has been broadly examined, for example in experimental autoimmune encephalomyelitis (EAE), the animal model for multiple sclerosis, it is clear that immunostaining for terminal complement proteins has provided little understanding of complement-mediated disease mechanisms. This review will examine the results of studies that have significantly increased the understanding of complement in CNS disease to a point where complement-specific therapeutics appear to be a promising option in the near future.

Complement biology

The complement system is composed of almost 40 soluble and membrane-bound proteins and plays an important role not only in innate immune responses, but adaptive immune responses as well. Once activated, complement plays a critical role in the elimination of invading pathogens, including bacteria, viruses, fungi and parasites. Complement also contributes substantially to the acute phase response and inflammation, to the trafficking of effector cells in immune responses and to B-cell signaling and development [1–3]. Although traditionally thought of as an efficient neutralizer and killer of microorganisms, there is much evidence implicating complement in a wide range of functions, including cell activation, signaling, growth and differentiation [4]. Complement is activated through three different pathways (classical, lectin and alternative) that share a common terminal pathway, which leads to the formation of the MAC (FIGURE 1). There are a large number of microorganisms and biological molecules that activate complement [5], including CNS-specific activators such as myelin and amyloid peptides [6,7].

Activation of complement leads to the formation of multimolecular enzyme complexes, termed convertases, that cleave C3 and C5, the
central proteins of the complement system. The proteolytic fragments generated by cleavage of C3 and C5 mediate most of the biological activities of complement (FIGURE 1). C3b, and proteolytic fragments generated from C3b, are important opsonins that target pathogens for removal by phagocytic cells via complement receptors specific for these proteins. C3a and C5a are potent anaphylatoxins that chemotact the C3b and granulocytes, and contribute to inflammation through the degranulation of mast cells, basophils and eosinophils and induction of the acute phase response. Generation of C5b by cleavage of C5 initiates the formation of the MAC through the terminal complement pathway. The MAC forms through the self-association of C5b along with C6 through C9 and leads to the formation of a large membranolytic complex capable of lysing prokaryotic and eukaryotic cells [5]. All these biological functions of the complement system contribute in various ways to the inflammation and pathophysiology of the CNS disorders discussed below. However, there is growing evidence that the contribution of complement to these disorders is disease specific. Thus, therapeutic strategies with respect to complement in the CNS may be targeted towards certain complement activation pathways or proteolytic fragments rather than wholesale inhibition (FIGURE 1).

**Complement in demyelinating disease**

Complement has been implicated in the pathology of demyelinating disease for several decades. In early studies, complement was inhibited by treating animals with cobra venom factor (CVF), which transiently depletes serum complement and reduces the severity of EAE [8]. This protective effect was observed when animals were given CVF before or after clinical signs of disease. These findings provided the first evidence that inhibiting complement activation during active demyelinating disease may have therapeutic value. Since this early experimental approach, complement mutant mice (both transgenics and knockouts) have been developed, allowing precise and targeted inhibition of complement functions. This genetic approach overcomes the limitations of transient complement inhibition obtained with CVF or the soluble complement receptor type 1 (sCR1) [9], and allows an assessment of pathway-specific and effector molecule contributions to EAE.

Studies designed to examine the contribution of the classical versus alternative pathways to the pathophysiology of EAE have provided somewhat unexpected results. The prevailing dogma has always suggested that the classical pathway was of greater importance in demyelinating disease, primarily because of an abundance of in vitro data demonstrating ready activation of the classical pathway by myelin-derived components [6,10] and the perceived role of antibody in demyelination [11,12]. The results of EAE studies using C4-deficient guinea pigs and mice demonstrated that the classical pathway is not critical to the development or progression of disease [13,14]. In contrast, deletion of factor B (an essential protein required for the activation of the alternative pathway) provided significant protection in the chronic phase of disease [15]. Factor B-deficient mice had reduced inflammation, cellular infiltration and demyelination compared with control mice. Interestingly, C3-deficient mice and transgenic mice that express a soluble form of the membrane-bound murine complement regulatory molecule, complement receptor-related protein y (sCrry; sCrry/GFAP mice), had a similar level of protection from disease, indicating that the alternative pathway is predominantly activated in EAE [15,16]. A critical observation here is that inhibition of all complement-mediated biological functions through deletion of C3 did not fully prevent EAE. Thus, complete inhibition of complement activity will not provide a stand-alone therapeutic approach to demyelinating disease.

**Figure 1. Schematic representation of the complement pathways.** Activation of the classical, mannose-binding protein (MBP) and alternative pathways leads to the formation of the C3 convertases (C4b2a for the classical and lectin pathways; C3bBb for the alternative pathway). C3 convertases cleave C3 into C3a and C3b, and complement host defense functions are mediated by the binding of C3a to the C3a receptor (C3aR) and C3b. Fragments derived from additional cleavage of C3b bind to the complement receptors 1 through 4 (CR1–4). C5 convertases are formed by the binding of C3b molecules to C3 convertases (derived from either pathway), and these convertases cleave C5 into C5a and C5b. C5a binds to the C5aR and mediates many of the inflammatory functions of the complement system, while C5b starts the formation of the MAC, a multimolecular complex capable of lysiing cells. Listed next to the pathways are many of the functions mediated by complement on activation. Complement complexes or receptors that may be useful therapeutic targets in CNS disease are circled.

**MAC:** Membrane attack complex.
Complete inhibition of complement biological activities at the level of C3 in demyelinating disease, although informative, does not provide a deeper understanding of complement functions, such as those specifically mediated by the anaphylatoxins (C3a and C5a) and their receptors and components of the terminal pathway. To address the roles of complement anaphylatoxins and their receptors in demyelinating disease, transgenic mice that express either C3a or C5a in the CNS under the control of the astrocyte-specific promoter GFAP (C3a/GFAP and C5a/GFAP mice) and receptor-deficient mice have been examined. The results of these studies were surprising, particularly as relating to C5a and the C5aR. The C5aR is expressed on all cell types involved in the development of EAE and expression is elevated on glial cells and infiltrating cells during disease [17]. Despite these observations, the C5aR is not critical to the development and progression of EAE based on studies using C5aR-deficient mice [18]. Similar to control mice, C5aR-deficient mice developed EAE with no difference in clinical disease, cellular infiltration or cytokine production. In addition, ectopic expression of C5a in the CNS did not affect the outcome of EAE as determined in EAE studies using C5a/GFAP mice [19]. From a therapeutic standpoint, the observation that a C5aR antagonist had no effect on the outcome of EAE [20] not only provides additional support for the redundant nature of C5a and its receptor in EAE, but indicates that this receptor/ligand pair is an unlikely therapeutic target in demyelinating disease.

The results of EAE studies using C3aR-deficient and C3a/GFAP mice suggest that this receptor/ligand pair, in contrast to C5a and its receptor, offers a potential therapeutic target. C3aR-deficient and wild-type mice have a similar clinical phenotype during the acute phase of EAE. However, during the chronic phase of disease, C3aR-deficient mice have attenuated disease and a corresponding reduction in the spinal cord histopathology [21]. Perhaps not surprisingly, C3a/GFAP mice have high mortality in the chronic phase of EAE along with massive leukocyte infiltration [21]. In critical control experiments in the same study, C3aR+/− × C3a/GFAP mice presented with EAE similar to that observed in C3aR-deficient mice. Future studies using C3aR inhibitors in animal models will be important in establishing the role of C3aR in the pathogenesis of EAE and in exploring this receptor/ligand pair as a therapeutic option in multiple sclerosis (MS).

EAE studies using animals deficient in complement components and regulatory molecules associated with the terminal complement pathway (C5, C6 and CD59) indicate that the terminal complement pathway is not as critical to complement-mediated pathology as previously believed. Multiple strains of naturally C5-deficient mice develop EAE with onset and severity identical to control mice [22–24]. C6-deficient rats do not present with clinical signs of disease or histopathology identical to controls, but by no means are they significantly protected from EAE [25,26]. In contrast, CD59-deficient mice develop significantly worse EAE than controls [27]. However, it should be noted that noncongenic male mice, which have markedly higher hemolytic activity, were used in this study, possibly skewing the results towards worse disease. Nevertheless, the sum of these studies indicates that understanding of the role of the MAC in EAE remains unclear, and that the MAC may not be a prime therapeutic target [8].

Complement in Alzheimer's disease

The association of complement with Alzheimer's disease (AD) pathophysiology is based primarily on two observations:

- Complement proteins and activation fragments are frequently found in senile plaques and neurofibrillary tangles in AD brain.
- Complement is readily activated by amyloid β-peptides (Aβ) and Tau.

The presence of a limited repertoire of complement proteins in senile plaques was reported over 20 years ago [28,29]. Since these initial observations, a number of investigators have extended the findings to include most components of the classical, alternative and terminal pathways [7,30]. The source of complement proteins in AD brain is probably endogenous, since essentially all complement proteins are produced by glia and neurons [7]. The blood–brain barrier (BBB) does not appear to be significantly compromised in AD, suggesting that serum is not a major source of complement in the AD brain [31,32]. This does not preclude leakage of serum complement across the BBB due to amyloid-mediated disturbances of brain microvasculature or through other mechanisms. The contribution of complement that has leaked across the BBB to the overall complement concentration in the brain is at present difficult to assess and undoubtedly varies widely in the AD population owing to a number of factors including disease heterogeneity and the extent of BBB dysfunction. More importantly, although complement deposition appears to correlate with increases in plaque levels and activated glia, at this time there is no clear relationship of complement levels to disease onset, severity or progression. Studies using Down's syndrome as a model for AD indicated a temporal increase in complement deposition [33,34], although it is unclear if similar increases in complement deposition hold for AD.

The presence of complement proteins and activation fragments on plaques and tangles in AD brain is not by itself sufficient evidence for a significant role for complement in AD pathophysiology. However, a number of studies have demonstrated that complement is activated through both the classical and alternative pathways by Aβ peptides and more recently by Tau, the major component of neurofibrillary tangles [35–40]. In addition, C1q enhances Aβ aggregation in vitro and inhibits resolubilization of Aβ aggregates [41], a function that may exacerbate complement-mediated inflammation should it occur in vivo. The activation of the classical pathway via Aβ peptides is antibody independent, and Aβ residues between four through 11 appear critical for the binding and activation of C1q [38]. Activation of complement by Aβ leads to the covalent attachment of C3 activation fragments, the generation of
C3a/C5a and formation of the MAC [39,42]. Anaphylatoxin generation could recruit activated microglia and augment the production of additional inflammatory mediators that may contribute to neurodegeneration. Together, these data suggest that complement could generate inflammation in the AD brain. However, variable co-expression of other pro-inflammatory mediators and the absence of infiltrating leukocytes indicate that any complement-mediated inflammatory events are tightly regulated and limited to plaques and tangles. Despite a wealth of descriptive data demonstrating complement association with plaques and tangles, real insight into inflammatory disease mechanisms in AD is lacking.

Understanding the role of complement in AD, whether detrimental or protective, requires genetic and therapeutic approaches. Surprisingly, despite the availability of a large number of complement mutant mice, very little has been studied in this regard. To date, only two studies have been performed using complement mutant mice. In the first report, complement activation was inhibited by expressing a soluble form of the complement inhibitor, sCry [43], in the brains of human amyloid precursor protein (hAPP) transgenic mice [44]. sCry inhibits the C3 and C5 convertases of the classical and alternative pathways and, in this transgenic mouse, is expressed in all tissues under the control of a metallothionein promoter [43]. When assessed by immunostaining and Aβ-specific enzyme-linked immunosorbent assay (ELISA), 1-year-old hAPP/sCry mice had two- to threefold higher deposition of Aβ than age-matched hAPP mice [44]. Interestingly, there was reduced microglial activation but greater loss of hippocampal neurons in the hAPP/sCry mice compared with all control groups. The reason for this apparent dichotomy is unclear. The data from this study suggest a protective role for complement in AD, perhaps by aiding in plaque removal through chemoattraction and activation of glial cells surrounding senile plaques. The protective effect is, however, partially offset by neuronal loss.

In contrast to the results of the study using hAPP/sCry mice, mice deficient in C1q, on the genetic background of APP transgenic mice with the Swedish mutation (APPQ-/- mice) [45], had similar levels of Aβ deposition but higher levels of synaptophysin and MAP-2 immunoreactivity compared with control mice. This outcome suggests reduced neurodegeneration in the absence of a functional classical pathway. Furthermore, both APPQ-/- and APPPSQ-/- mice (the latter having both APP and presenilin 1 mutations [46]) had reduced glial cell activation compared with control mice [47]. The conflicting results of these two studies may be due to different genetic backgrounds (C57BL/6 vs C57BL/6 x CD1) and/or confounding effects of blocking only the classical pathway instead of all complement-activation pathways. To better address the role of complement in AD, future studies need to examine a targeted set of mutations that block complement function at multiple points in the cascade, including C3, C5, C3a receptor, C5a receptor and the complement receptor type 3 (CR3). To facilitate interpretation of any future studies, mutant mice should have the same genetic background and gender, and the same APP and/or APP/PS mutations should be used. Without this type of analysis it is difficult to predict the therapeutic future of complement-specific reagents in AD.

Complement & ischemic stroke

Since the first reports in the 1970s, complement has been shown to play a critical role in a whole host of ischemia/reperfusion (I/R) models of tissue damage [48,49]. In light of the fact that complement is intrinsically expressed in the CNS, it appears likely that complement-mediated mechanisms described for I/R injury in peripheral tissues are similar for I/R injury in the CNS [48,50–52]. Numerous studies have demonstrated complement activation and deposition in brain samples from patients who died after ischemic stroke [53-55], as well as in experimental stroke models [56-60]. There is also compelling evidence of complement-mediated brain damage in the setting of acute cerebral I/R injury [53-59,61]. Furthermore, several studies have shown dramatic alterations in complement gene expression in experimental stroke models. For example, mRNA levels for the classical pathway components C1qB and C4 are upregulated in the ischemic cortex within 24 h of experimental middle cerebral artery occlusion (MCAO) [56]. Kato and colleagues reported a spatiotemporal upregulation of CR3 – a receptor which binds the intermediate C3 cleavage product C3bi – in ischemic brain tissue [62]. Increased expression of the receptors for the complement anaphylatoxins, C3a and C5a, in experimental stroke has been shown by two groups of investigators [63,64]. Surprisingly, an initial reduction of intracerebral C3aR expression in focal cerebral ischemia was observed at early time points (3 and 6 h) followed by an increase at later time points (18 and 24 h). Expression of the C5aR increased as much as 23-fold compared with control mice and C5aR expression was, in general, higher than that of the C3aR at most time points [64]. Altogether, these studies have clearly shown that the complement cascade is activated after cerebral ischemia and that complement components and complement receptors are upregulated in the ischemic brain, both in a clinical setting in stroke patients, as well as in experimental model systems.

Despite this information, therapeutic targets with respect to complement have been examined in a very limited fashion in ischemic stroke. Interestingly, C1q, which is dramatically upregulated in the brain in response to transient global cerebral ischemia [56–58], may offer therapeutic potential since newborn C1q-deficient mice show neuroprotection after hypoxic-ischemic brain injury [59]. Perhaps the most exciting studies for assessing the therapeutic potential of complement in stroke are those involving a hybrid molecule termed sCR1sLex, composed of the extracellular domain of sCR1 glycosylated with the sialyl Lewis x moiety. This molecule blocks both complement activation and selectin-mediated adhesion [58]. Both sCR1sLex and unmodified sCR1 localized to ischemic vessels and parenchymal neurons and, most importantly, inhibited neutrophil infiltration and reduced infarct volume. These results open up the potential for early treatment modalities for stroke patients based on complement...
inhibitors due to a window of opportunity within the first few hours after onset of symptoms [65]. This window of opportunity is due to the requirement of gene expression, protein synthesis and cell homing for neuroinflammation to develop. Therefore, its onset is delayed and its progression prolonged compared with other more rapidly occurring injury mechanisms, such as excitotoxicity [66,67]. This is significant because major pathogenic mechanisms in stroke, such as excitotoxicity and peri-infarct depolarizations, occur rapidly after the ischemic insult and require essentially immediate treatment to block their effects [66,68]. Additional studies to determine if a more targeted approach to complement inhibition, such as blocking inflammatory and signaling events mediated by the anaphylatoxins, is warranted.

Complement & traumatic brain injury

The post-traumatic response to head injury is characterized, in part, by activation of the innate immune response, including the complement system. Complement and other immune system components trigger a profound host-mediated inflammatory response within the intracranial compartment [50,69,70]. Clinical and experimental studies in the past decade have revealed important mechanisms of complement-mediated secondary brain tissue damage after head trauma [50,69,71]. These include the recruitment of inflammatory cells into the intrathecal compartment, the induction of BBB dysfunction by the anaphylatoxins C3a and C5a, the induction of neuronal apoptosis through the C5aR expressed on neurons, and complement-mediated homologous cell lysis through the MAC (C5b-9) following inactivation of the physiological cellular protection mechanisms against complement attack [70]. Results from recent experimental studies underline the important role of MAC formation in the brain with regard to induction of secondary neuropathology [72,73]. 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 a few hours [72]. In addition, MAC injection into hippocampus evoked seizures and neurocytotoxicity, thus highlighting the potent detrimental effects of complement activation in the brain [73]. Activated complement fragments were detected in injured human and rodent brains by immunohistochemistry, demonstrating post-traumatic complement activation and deposition of the MAC in homologous tissue [69,74,75]. C3a, for example, is generated to readily detectable levels within hours after experimental traumatic brain injury (TBI) (FIGURE 2). Keeling and colleagues found neutrophil infiltration and concomitant accumulation of complement C3 in cortical and hippocampal brain sections after experimental TBI in rats [76]. In these experiments, C3 accumulation significantly correlated with regions of intracerebral cell death, increased myeloperoxidase activity and neutrophil infiltration [76]. In clinical studies, elevated levels of alternative pathway complement components C3 and factor B [77], and of activated soluble MAC/C5b-9 [71], were detected in the cerebrospinal fluid (CSF) of patients with severe head injuries. Moreover, the extent of intrathecal complement activation was associated with a dysfunction of the BBB in humans [53,71].

Several therapeutic agents that inhibit complement activation have been investigated in experimental models of brain injury [75,78-80]. The first studies used scCR1 in rats after traumatic brain injury and demonstrated a reduced neutrophil accumulation [78]. The use of a C5aR antagonist also reduced neutrophil accumulation and was protective in traumatic cryoinjury [79]. More recent studies investigated the effect of intracranial complement inhibition in TBI using sCrry/GFAP mice [75]. sCrry/GFAP mice showed significantly improved neurological outcome and attenuated post-traumatic BBB dysfunction after closed head injury, compared with wild-type littermates [75]. Based on these insights, the concept of Crry-mediated neuroprotection was extended to a pharmacological study [80]. The use of Crry–immunoglobulin (Ig), a recombinant Crry molecule generated by fusing two Crry molecules to the noncomplement fixing mouse IgG1 Fc region [81], resulted in a significant neurological improvement for up to 1 week, as compared with vehicle-injected control mice [80]. Furthermore, neuronal morphology and integrity of hippocampal neuron structures in the CA3/CA4 sublayers was preserved by post-traumatic Crry–Ig injection, as compared with head-injured vehicle controls [80]. Real-time reverse transcriptase (RT)-PCR analysis revealed that the post-treatment with Crry–Ig resulted in a significant upregulation of candidate neuroprotective genes in the injured hemisphere, such as Bcl-2, 

![Figure 2. Early intracerebral complement activation after experimental traumatic brain injury.](https://www.future-drugs.com)
and the complement regulatory genes for C1-INH, CD55 and CD59 [80]. Altogether, the conclusions of these various studies favor the concept of pharmacological complement inhibition as a promising approach for attenuation of neuroinflammation and secondary neurodegeneration after head injury.

Complement & bacterial meningitis
The pathological role of the inflammatory response to intrathecal bacterial infection is well recognized and provides the basis for current anti-inflammatory treatment concepts for patients with bacterial meningitis [82]. Despite previous controversies on the role of glucocorticoids as adjunctive therapy for patients with bacterial meningitis, there is now evidence which supports a clear benefit if steroids are given as early as possible, preferentially before the start of parenteral antibiotics [82-83]. The rationale for an adjunctive immunosuppressive therapy in bacterial meningitis, such as with dexamethasone, lies in the awareness of the fact that the adverse outcome is mainly due to the overwhelming host-derived neuroinflammatory response to infection, more than to the bacterial pathogen itself [82-84]. Although antibiotic treatment may lead to rapid elimination of the bacterial load, the perpetuated inflammatory cascade in the intrathecal compartment contributes to development of cerebral edema, intracranial hypertension and delayed neuronal cell death [84-86]. These pathophysiological sequelae are in large part mediated by the intrathecal activation of the complement cascade in response to a bacterial challenge [87]. Excessive complement activation through the alternative pathway has been demonstrated to occur in fulminant meningococcal sepsisemia, which represents the most fatal form of bacterial meningitis [88]. Interestingly, membrane-bound complement regulatory molecules, such as membrane cofactor protein (MCP; CD46), have recently been shown to enhance meningococcal disease by enabling the pathogen Neisseria meningitidis to cross the epithelial mucosa into the intrathecal compartment by CD46-facilitated mechanisms [89]. This raises the possibility that a soluble form of CD46 may be a useful therapeutic tool in preventing or reducing meningitis induced by this bacterial species.

The pivotal role of complement in contributing to the inflammatory response in the infected brain has been recognized by clinicians and scientists since the first papers published in the 1930s and 1940s [90,91]. It is now clear that many, if not all, complement components are endogenously produced in the pathogen-infected brain and released into CSF [17,51,87]. The complement anaphylatoxins C3a and C5a mediate potent chemotactic activity in CSF and contribute to the recruitment of blood-derived inflammatory cells into the intrathecal compartment [17,87]. Not surprisingly, intrathecal injection of a recombinant C5a molecule leads to the development of sterile meningitis characterized by a massive intrathecal inflammatory reaction [92]. These observations may be the forerunner of a therapeutic approach for bacterial meningitis, particularly since complement-mediated chemotactic activity for neutrophils in the CSF was inhibited by neutralizing anti-C5 antibodies in pneumococcal meningitis in rabbits [93]. The role(s) of other complement components in bacterial meningitis remains largely unexplored, as does the therapeutic utility of complete inhibition of complement activation.

Although the therapeutic value of modulating complement function in meningitis remains to be established, there is evidence that complement may be of diagnostic value in meningitis. Both C3 and factor B are significantly elevated in the CSF of patients with bacterial meningitis, but not in patients with viral (aseptic) disease [94-96]. The mean C3 and factor B levels in CSF with bacterial infection were 20- and 60-fold higher, respectively, than those in patients with viral meningitis [94]. Intrathecal levels of both C3 and factor B in CSF were highly sensitive (both 100%) and highly specific (95% and 100%, respectively) for establishing the diagnosis of bacterial meningitis [94]. Since C3 and factor B in CSF can be easily and rapidly assayed, they represent a powerful new tool to discriminate patients at risk for the severe form of disease, requiring immediate antibiotic and adjunctive therapies. Translating the knowledge from these clinical and experimental studies into new therapies and new diagnostic tests may provide new strategies for controlling this devastating infectious disease.

Expert commentary
There is little doubt that most, if not all, CNS inflammatory diseases and disorders share common immune-mediated mechanical features. The authors argue that complement is a central contributor to this process for several reasons. First, complement is present, albeit at low levels, in CSF [50]. In addition, glial cells and neurons produce essentially all complement proteins and the production of complement proteins by these cells is increased by many cytokines [97]. These two observations together make it clear that complement is readily available in the CNS to contribute to inflammatory events – a frequently overlooked fact. Activation of complement in the CNS by invading microorganisms (in the case of meningitis) or subcellular components and proteins (in MS, AD, stroke and TBI) generates the complement anaphylatoxins, which can augment cytokine production and inflammation at many levels. This scenario can quickly lead to a vicious circle of increasing inflammation, either locally or throughout the CNS, depending the nature of the initial insult. Complement obviously merits attention as a therapeutic target, if for no reason other than that the successful application of a complement therapeutic in one CNS disease may have broad application to several other diseases.

Despite all this information on complement in the CNS, the question still remains: why hasn’t the complement system been a success story in terms of therapeutics in CNS diseases? Certainly the large number of biologically active fragments generated by complement activation presents a dizzying array of potential therapeutic targets. This alone may discourage many investigators. An additional and central problem is our limited understanding of the involvement of complement biology in many CNS diseases, particularly at various stages of disease.
Complement in CNS disease

development. In MS, for example, complement is presumed to be involved only in the so-called Type II lesion [98]. However, using complement as a biomarker in such a fashion overlooks limitations with immunohistochemical detection methods and the relationship of complement to the immunological life cycle of an MS lesion [99]. Finally, there are animal model issues that complicate the ability to assess the role of complement in CNS disease. In demyelinating disease, EAE remains the model of choice and it has been successfully used to develop immunologically based therapies for MS – the best example being anti-integrin therapy [100]. In contrast, animal models of AD do not recapitulate many of the critical features and have targeted gene mutations that affect only a small fraction of individuals with the disease. Thus, there is the risk that success with complement-based therapies developed using these mice may not translate to the broader population with AD. The development of mutant mice with CNS-targeted expression of a variety of complement inhibitors under the control of inducible promoters would be an important addition to the pool of reagents in this field.

Do these limitations suggest that the effort to develop complement-based therapies for CNS diseases should be abandoned? Clearly the answer to this question is no. The weight of currently available data unequivocally indicates that research is on the right track. There is evidence in all the diseases discussed above that modulating complement activity frequently results in a better outcome (TABLE 1). There is also growing evidence that complement itself may contribute to anti-inflammatory mechanisms in the CNS. For example, C3a protects neurons against glutamate-induced excitotoxicity [101] and induces the production of nerve growth factor and anti-inflammatory hormones [102,103], while targeted expression in the brain protects against endotoxic shock [104]. As promising as these findings may be, C3a appears pro-inflammatory in chronic autoimmune disease in the brain [21], indicating that complement therapeutics may be highly disease specific in practice. Interestingly, one potential complement therapeutic, virus complement control protein, mediates protection against memory deficits rather than neuropathological injury [105,106].

Five-year view

There are a number of complement therapeutic agents, most notably sCR1 and anti-C5 antibodies, and these and many other reagents have been the subject of recent reviews [107–110]. Based on these reviews, it is clear that the future of complement therapeutics in CNS diseases is not limited by the availability of reagents (TABLE 1). Perhaps the greatest limitation of the

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**Table 1. Complement inhibitors and their therapeutic use and potential for CNS disease.**

<table>
<thead>
<tr>
<th>Therapeutic</th>
<th>Effect on complement biology</th>
<th>Therapeutic uses/outcome</th>
<th>Therapeutic potential</th>
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<tbody>
<tr>
<td>CVF</td>
<td>Transient inhibition</td>
<td>EAE/protection</td>
<td>None</td>
</tr>
<tr>
<td>sCR1</td>
<td>Inhibits C3/C5 convertases</td>
<td>EAE/protection Stroke/protection TBI (sCrry, Crry-Ig)* protection AD (sCrry)/protection</td>
<td>Potential BBB limitations</td>
</tr>
<tr>
<td>sCR1sLe⁵</td>
<td>Inhibits C3/C5 convertases Inhibits leukocyte rolling</td>
<td>Stroke/protection</td>
<td>Potential BBB limitations</td>
</tr>
<tr>
<td>Comstatin</td>
<td>Inhibits C3 cleavage</td>
<td>Not tested</td>
<td>Unclear for CNS applications</td>
</tr>
<tr>
<td>CAB-2</td>
<td>Inhibits C3/C5 convertases</td>
<td>Not tested</td>
<td>Potential BBB limitations</td>
</tr>
<tr>
<td>Anti-CR3 Ab</td>
<td>Inhibits CR3 (Mac-1)</td>
<td>EAE/protection</td>
<td>Potential BBB limitations</td>
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<tr>
<td>APT070</td>
<td>Convertase decay acceleration</td>
<td>Not tested</td>
<td>Unclear for CNS applications</td>
</tr>
<tr>
<td>Anti-C5 Ab</td>
<td>Eliminates C5 functions</td>
<td>Not tested</td>
<td>Potential BBB limitations</td>
</tr>
<tr>
<td>C3aR/C5aR antagonists</td>
<td>Inhibit C3a/C5a functions</td>
<td>EAE (C5a)/no effect</td>
<td>Unclear for CNS applications</td>
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<tr>
<td>Protease inhibitors</td>
<td>Inhibit early complement activation steps</td>
<td>Not tested</td>
<td>Poor specificity limits use</td>
</tr>
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<td>VCP</td>
<td>Factor I cofactor activity</td>
<td>TBI/no effect*</td>
<td>Potential immunogenicity</td>
</tr>
</tbody>
</table>

⁵sCry and Crry-Ig are functional analogs of sCR1, see text for details.

*VCP treatment resulted in fewer memory deficits, but provided no neuropathological protection.

Ab: Antibodies; AD: Alzheimer’s disease; BBB: Blood–brain barrier; CNS: Cerebral spinal fluid; CVF: Cobra venom factor; EAE: Experimental allergic encephalomyelitis; sCR1: Soluble complement receptor type 1; TBI: Traumatic brain injury; VCP: Vaccinia virus complement control protein.
currently available reagents is their large size, which limits their accessibility to the brain. This is not necessarily a limitation for all CNS diseases. In acute conditions, such as meningitis, TBI or stroke, where the BBB is breached (at least transiently), many of these larger therapeutic reagents may prove useful in ameliorating complement-mediated pathology without the risk of significantly immunocompromising the patient. In more chronic conditions, such as MS, this group of therapeutics may prove useful in the setting of disease exacerbation, rather than as a primary therapeutic approach. Smaller inhibitors, such as compstatin or those that inhibit the anaphylatoxin receptors, may prove more efficacious from a size perspective. In some clinical settings, direct delivery of complement inhibitors, regardless of size, may also be useful to modulate complement activity.

In the authors’ opinion, the potential of complement inhibitors in the CNS is high and remains unrealized. While there is much to learn about complement biology in the brain, there are tantalizing observations in MS and TBI demonstrating that inhibition at the level of the convertases is most promising. The authors predict that convertase inhibitors will continue to lead the way clinically and that these reagents need to be aggressively moved into other disease models. It is clear from Table 1 that the biggest impediment to the use of complement-specific therapeutics is the lack of assessment of many inhibitors in animal models. For the near future, small molecule inhibitors such as compstatin and inhibitors of the complement anaphylatoxin receptors have high therapeutic potential [111]. C3a and C5a antagonists, in particular, may find utility in acute inflammatory settings such as meningitis and TBI, preventing leukocyte infiltration and downmodulating the acute phase response. Additional future targets include inhibiting the alternative pathway at the level of factor B [109] and blocking the multiple functions of CR3, based on the results of recent studies [112]. While successful application of these reagents may be life saving in some settings (e.g., meningitis), they will most likely be adjunct therapies that contribute to stabilizing or reducing neurological deficits and increasing quality of life in chronic CNS diseases.

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Key issues

- Complement is an ever-present component of the immune system in the CNS and is readily activated by CNS-specific subcellular components and proteins. On activation, complement contributes to inflammation, leukocyte infiltration and cell lysis.
- In a wide spectrum of CNS diseases, including demyelinating disease, Alzheimer’s disease (AD), stroke, brain injury and meningitis, complement activation fragments are deposited on brain tissue and/or found in cerebrospinal fluid.
- In demyelinating disease such as experimental autoimmune encephalomyelitis (EAE), complement appears to be activated primarily through the alternative pathway, and C3-derived biologically active fragments are central to the role of complement in EAE.
- In AD, numerous complement components are found associated with neuritic plaques and neurofibrillary tangles, and complement is activated by β-amyloid peptides.
- Inhibition of complement activation in AD animal models has produced conflicting results, but clearly requires additional investigation.
- In experimental stroke models, inhibition of complement activation, at the level of the classical pathway or globally, has proven therapeutically valuable and resulted in reduced leukocyte infiltration and neuroprotection.
- Several therapeutic approaches to inhibit complement activation in traumatic head injury show improved neurological outcome and reduced neurodegeneration.
- In bacterial meningitis, complement contributes to the host inflammatory response, and elevated cerebrospinal fluid levels of some complement proteins may be diagnostic in discriminating bacterial versus viral meningitis.
- Therapeutic approaches to regulating complement activation in CNS disease require aggressive assessment of available inhibitors in a broader range of animal models.
- New, sophisticated complement transgenic mice and recombinant inhibitors targeting the alternative pathway, the anaphylatoxins and C3b-mediated functions will allow this field to move forward rapidly.
References
Papers of special note have been highlighted as:
• of interest
  •• of considerable interest

•• Highlights novel functions of the complement system.
• Comprehensive review on inflammatory mediators in Alzheimer’s disease (AD).
• Comprehensive review of complement in demyelinating disease.
• Highlights the role of the alternative pathway in demyelinating disease.
• Demonstrates that C5a and its receptor are not required for demyelinating disease.
• Supports the concept that therapeutics directed at C5a are not viable for demyelinating disease.
• Implicates C3a and the C3Ar as important players in demyelinating disease.
• Comprehensive review on complement in AD.

www.future-drugs.com
Highlights mechanism of complement activation by β-amyloid in Alzheimer disease. 

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Wyss-Coray T, Yan F, Lin AH

Quigg RJ, He C, Lim A

Emmerling MR, Spiegel K, Watson MD.

Bradt BM, Kolb WP, Cooper NR.


• Indicates that inhibition of the classical pathway may have therapeutic value.


•• Highlights the therapeutic potential of inhibiting complement activation in stroke.


•• Excellent review on the role of inflammation in ischemic brain injury.


Recent review highlighting the role of inflammation in traumatic brain injury (TBIs).


• Recent review highlighting the role of inflammation in traumatic brain injury (TBI).


• Highlights the utility of inhibiting complement activation in brain injury.


Elevated levels of the complement components C3 and factor B in ventricular cerebrospinal fluid of patients with traumatic brain injury. J. Neuroimmunol. 73, 63–69 (1997.)


• Implications for how complement is hijacked to cause meningitis, perhaps a novel therapeutic as well?

Fohtergill LD. Observations on the presence of complement in the cerebrospinal fluid in various pathologic conditions of the central nervous system. J. Pediatr. 6, 374–381 (1935).

• First documentation of complement in CNS disease.


• Comprehensive review on complement in the normal and diseased brain.

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Complement in CNS disease


Excellent review on complement therapeutics.


The utility of inhibiting the alternative pathway as a therapeutic approach.