

REVIEW ARTICLE

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Is complement good, bad, or both? New functions of the complement factors associated with inflammation mechanisms in the central nervous system

Muriel Tahtouh, Françoise Croq, Christophe Lefebvre, Joël Pestel

Université de Lille 1 (Sciences et Technologies), Laboratoire de neuro-immunologie des annélides, CNRS FRE 2933, IFR 147, Villeneuve d'Ascq, France

Correspondence: J. Pestel, CNRS FRE 2933, IFR 147, Université Lille 1 (Sciences et technologies), SN3, 1^{er} étage, Porte 123, Cité scientifique, 59655 Villeneuve d'Ascq, France
<joel.pestel@univille1.fr>

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ABSTRACT. The complement system is well known as an enzyme cascade that helps to defend against infections. Indeed, this ancestral system bridges innate and adaptive immunity. Its implication in diseases of the central nervous system (CNS), has led to an increased number of studies. Complement activation in the CNS has been generally considered to contribute to tissue damage. However, recent studies suggest that complement may be neuroprotective, and can participate in maintenance and repair of the adult brain. Here, we will review this dual role of complement proteins and some of their functional interactions with part of the chemokine and cytokine network associated with the protection of CNS integrity.

Keywords: complement, CNS, chemokine and cytokine network

The complement system (C) system is a key-component of the innate immune system, playing a central role in host defense against pathogens and in the initiation of inflammation. Complement activation is triggered through three different pathways, the classical, the lectin-dependent and the alternative pathway. The central nervous system (CNS) by itself can design an immune and inflammatory response. Pioneering experiments carried out by Levi-Strauss and Mallat have led to the first report on the ability of brain cells to produce C proteins, which were demonstrated to recognize and kill pathogens while preserving normal cells in the CNS. Numerous additional data have clearly confirmed that expression of C by resident cells is activated and is greatly increased following brain infection or injury [1]. In the CNS, C has been involved in the worsening of many neurodegenerative disorders such as Alzheimer disease (AD), Pick's disease and demyelinating diseases such as MS [2, 3]. However, neuroprotective effects of complement activa-

tion have been recently shown, suggesting that the C might play also an important role in neuroprotection [3, 4]. In this review, we will discuss the data that point to the dual role of complement proteins in the CNS.

THE "BAD SIDE" OF COMPLEMENT IN THE CNS

The primary site of complement protein synthesis is the liver, but CNS complement production in microglia, astrocytes, and neurons has been confirmed [1, 4]. Furthermore, an endogenous synthesis and expression of complement pathway in healthy human peripheral nerves has been recently shown. Epithelial cells, fibroblasts, Schwann cells and macrophages can synthesize complement *in vitro*. Several reports clearly showed that

Abbreviations

| | |
|-----|------------------------|
| AD | Alzheimer's disease |
| BBB | blood-brain barrier |
| C | complement |
| CNS | central nervous system |
| MS | multiple sclerosis |

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increased local C biosynthesis and uncontrolled C activation in the CNS are factors contributing to the pathology of inflammatory and degenerative disorders [4].

The complement system represents an essential, innate immune defense mechanism [5], however, complement activation may contribute to neuronal loss in chronic inflammation reactions. Indeed, bidirectional, molecular communication between the CNS and the periphery contributes to the development of inflammation, which is required to initiate many neuronal disorders. Thus, complement activation leads to the release of various fragments, such as C3a and C5a (obtained by specific cleavage as summarized in *figure 1*) that might exert many immunological activities after binding to specific membrane receptors coupled to G proteins, as demonstrated for C3aR and C5aR, respectively. By inducing the recruitment of immune cells to the inflammation site, and by allowing the production of various chemokines or cytokines, these complement-derived peptides can modulate the immune response. For example, human, immature, plasmacytoid dendritic cells (pDC) (obtained from peripheral blood precursors after culture with IL-3) that express specific receptors for C3a and C5a, might migrate towards a gradient of both these anaphylatoxins. Interestingly, a similar chemotactic effect was described to represent a new pathway to recruit pDC to sites of inflammation [6].

Furthermore, through this anaphylatoxin release, the C system may initiate a cytokine production cascade. The interaction between proinflammatory cytokines and ana-

phylatoxins C3a and C5a was specifically investigated. For example, C3a and C5a enhance monocyte gene expression and protein synthesis of IL-1, this latter increases monocyte expression of receptors for these anaphylatoxins, which amplifies inflammation [7]. Moreover, in the CNS, C5a and C3a have the capacity to modulate synthesis of cytokines such as IL-1 β , tumor necrosis factor α (TNF- α), IL-6 and IL-8 by myeloid cells. In addition, C3a and C5a can play a crucial role in the activation of T cells, to favor the production of proinflammatory cytokines such as IL-17 and INF- γ [8], to which brain cells can respond. Thus, in most CNS-associated diseases that are accompanied by chemokine expression, complement anaphylatoxins play a key role in the initial triggering of neuroinflammation mechanisms [2, 9, 10]. In Alzheimer's disease (AD), the level of C1q mRNA was increased compared to normal brain [1, 11]. Complement activation mediates neuronal injury *via* MAC-induced neurite disintegration and increased levels of reactive oxygen species [4]. In the AD brain, microglial cells are activated and show a dose-dependant increase in pro-inflammatory cytokine production [12, 13]. Activation of microglia by amyloid beta ($A\beta$) is associated with a specific recruitment of microglia cells responding to chemotactic factors and consequently induces $A\beta$ fibril phagocytosis. Evidence for complement involvement in microglial response to $A\beta$ has been reported [14, 15]. Indeed, the presence of C1q, locally produced by activated microglia in close proximity to the amyloid plaques, suggests that C1q may interact with the $A\beta$ or

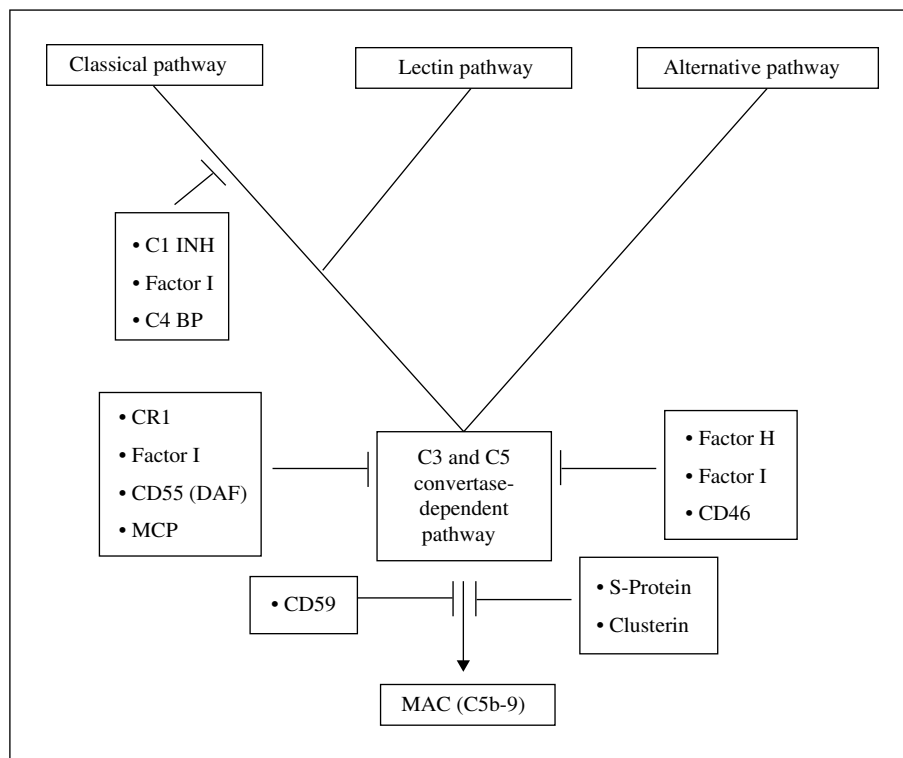


Figure 1

C activation pathways. Activation of the classical, alternative and lectin pathways leads to the generation of C3 and C5 component fragments following regulated activation of specific C3 and C5 convertases. The cleavage of C5 leads to the initiation of membrane attack complex (MAC) formation. These pathways are regulated by several inhibitors, either cell-surface complement inhibitors: MCP (membrane cofactor protein), CD46, CD55 (or DAF, decay accelerating factor), CR1 (also known as CD35, C3b/C4b receptor) CD59 and/or serum complement inhibitors: C1 inhibitor, Factor I (C1 INH), Factor H, C4bp, clusterin and S-protein (vitronectin).

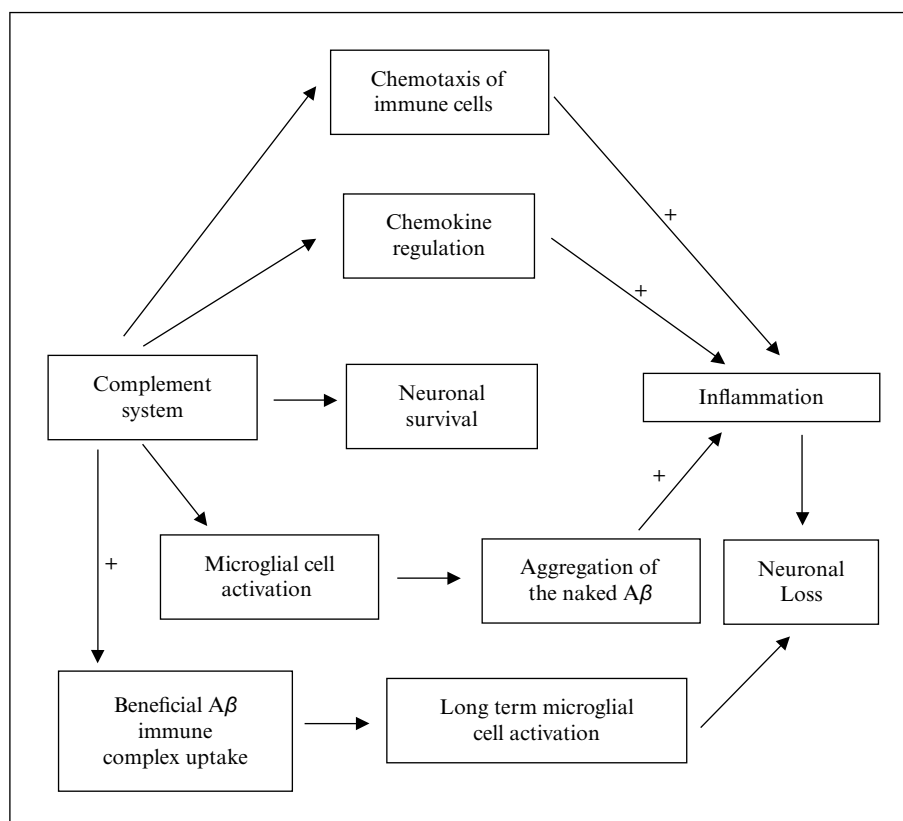


Figure 2

Diagram showing the dual role of C in the CNS as observed in various neuronal disorders (for example in Alzheimer's disease). Complement activation can be detrimental, causing neuronal loss, and neuroprotective, leading to neuron survival. Designing drugs that modulate rather than totally inhibit the activation of complement may represent the perfect therapy.

other components of the amyloid plaques, such as serum amyloid P. Interaction of C1q with A β can result in activation of complement when C1q is complexed with the serine proteases C1r and C1s, or in the induction of A β aggregation in the absence of C1r, C1s, and other complement proteins (as summarized in *figure 2*). In this second case, C1q can block access of microglia to A β and may contribute to the accumulation of the peptides in plaques, which is considered to be lethal [16, 17].

To avoid this self-destructive tendency, host cells are protected by a battery of regulatory molecules (C inhibitors), as recently underlined in some reviews (*figure 1*) [1, 4, 5], which mainly act at the level of the C3- or C5-cleaving enzymes and under the control of the final, membrane attack complex (MAC) formation. C1 inhibitor (C1-INH), C4b binding protein (C4 BP), Factor H, Factor I, S-protein (also called vitronectin) and clusterin are all soluble C inhibitors, which may be secreted and present in the fluid phase. The other C inhibitors, including membrane cofactor protein (MCP, CD46), decay accelerating factor (DAF, CD55) and CD59 are expressed on the cell membrane surface. Indeed, in brains with chronic, but not acute experimental autoimmune encephalomyelitis, the decay-accelerating factor (CD55) neuronal expression was highly associated with C3b opsonization of neuronal cell bodies and axons. In contrast, in both conditions, the CD35, CD46 and CD59 levels were not affected in neurons and glial cells. However, in another trauma situation, focal closed head injury, CD59, which is considered to be

a major regulator of MAC formation, was demonstrated as an essential protector from homologous cell injury after complement activation in the injured brain. Thus, brain cells can generate a C system to kill pathogens, while they are relatively well protected from direct C lysis by expressing soluble and membrane C inhibitors. However, there is now considerable evidence that increased local C biosynthesis and uncontrolled C activation in the CNS are contributing factors in the pathology of degenerative disorders leading to neuronal loss and local inflammation, especially during brain infections where C inhibitors are downregulated compared to C proteins [1, 11].

In ischemia, local expression of complement proteins by resident cells is increased following brain infection. Cerebral ischemia leads to an increased expression of receptors for the complement-derived anaphylatoxic peptides C3a and C5a (C3aR and C5aR) in the ischemic cortex in mice. Complement activation exacerbated the inflammation process leading to secondary tissue damage [18]. In the experimental autoimmune encephalomyelitis (EAE) model of MS, a pathological role of complement factors has been suggested in the selective cell lysis of neurons and oligodendrocytes [4, 19]. This deleterious effect was also suggested for C1q in adult glaucomatous retinas, where it tags synapses for elimination even at early stages of disease. This early complement-dependent synapse loss could drive axon degeneration [20].

C has been also implicated in neurological diseases associated with pain although its role in neuropathic pain has

not yet been clearly defined. Disorders of the immune system, involving the activation of complement, play an important role in several neurological diseases in which chronic pain is significant. Complement activation in the peripheral nerve contributes to recruitment of immune cells and to neuropathic pain due to nerve injury. As complement activation following nerve injury has multiple consequences, further studies are needed to investigate the importance of these factors in neuropathic pain [21]. Gene expression microarray analysis performed on the rat spinal nerve ligation (SNL) model of neuropathic pain revealed that several complement components were upregulated in microglia of the dorsal horn (CNS), while inhibitors such as DAF (decay accelerating factor) were downregulated. The complement pathway might be a novel target for the development of neuropathic pain treatments [22].

THE “GOOD SIDE” OF COMPLEMENT IN THE CNS

However, the same system responsible for deleterious effects can be, in some circumstances, beneficial to the host [23-25]. Complement is of special importance in the brain, where the entrance of elements of the adaptive immune system is restricted by the blood-brain barrier (BBB). Local production of complement factors in the peripheral nerve could protect the healthy nerve from infections, and could facilitate regeneration of injured axons by the clearance of myelin debris, thought to be inhibitory to axon growth. Indeed, complement activation accelerates the vacuolated myelin elimination by macrophages [3]. In addition, C1q has an important role in the clearance of apoptotic cells. It can bind directly to the surface blebs of ultraviolet light-induced apoptotic cells [26]. In AD pathology, some authors have suggested a potential neuroprotective role for complement by reducing the accumulation of degenerating neurons and brain A β deposits and by promoting their clearance [27]. Indeed complement is known to be actively involved in the removal of targeted molecules by phagocytes, which supports the notion that complement components are upregulated in A β -rich areas of the AD brain [16]. Incidentally, microglial cells that express C1q receptors behave in this immune-privileged site as efficient macrophages, and the increase in C1q receptor expression is linked to the enhancement of the phagocytosis capacity observed. Thus, C1q may have opposing effects on the development of the disease, either by accelerating its aggregation as previously indicated, or by enhancing A β immune complex uptake [17]. Indeed, in Alzheimer's disease, the specific role of C1q in the ingestion of the amyloid β peptide, might be used as a potential therapeutic target (figure 2).

C1q was also shown to be expressed transiently by postnatal neurons and to mediate synapse elimination during development. Several reports have underlined the crucial activity of C1q and/or C3 in synapse elimination mechanisms [20, 28]. Mice deficient in C1q or C3 complement components exhibit large defects in CNS synapse elimi-

nation. It is suggested that C1q-dependent mechanisms are involved in the elimination of weaker synapses, while C1q-independent pathways are used to strengthen appropriate synapses. In addition, the binding of C3b fragments to its specific receptors expressed on activated microglia, might induce increased phagocytosis and contribute to efficient synapse elimination. Interestingly, C1q was shown to be expressed by postnatal neurons in response to immature astrocytes and localized to synapses throughout the postnatal CNS and retina. Thus, unwanted synapses can be tagged by complement for elimination, and this complement-dependent mechanism may become aberrantly reactivated in neurodegenerative disease. As neuronal C1q was characterized at the nerve level, it can be assumed that C1q family proteins can act as new, transneuronal cytokines and regulate normal or abnormal brain functions.

Complement anaphylatoxins C3a and C5a were also described as having multiple effects relevant to neuronal survival. C5a can have a protective effect against glutamate-induced neurotoxicity, and apoptosis in neurons through MAPK-mediated regulation of caspase activation. C3a was reported to have a positive impact against NMDA-induced neuronal cell death. In mice, C3a and C5a were demonstrated to be critical for liver regeneration by promoting hepatocyte proliferation and, in parallel experiments, similar observations have led to evidence of the impact of C3a and C5a complement components in the modulation of neuronal survival [2, 3]. The key role of these factors in nerve integrity preservation is reinforced by the fact that the specific receptors, C3aR and C5aR, can be detected, *in vitro*, at the surface of neural stem cells and, *in vivo*, on neuroblasts. Another interesting point is that complement factors might be involved in the production of energy sources required for nerve activity, mainly for neurite outgrowth. Indeed, following cleavage by carboxypeptidase B (CBP), present in the endoneurial compartment of the nerve, C3a components release C3adesArg fragments (called acylation stimulating protein [ASP]) that might stimulate triglyceride synthesis in adipocytes, and consequently might be an energy source for peripheral axons. Other complement factors have been described as beneficial for nerve systems. The final C5b-9 complement complex was reported to have neuroprotective effects in MS and in experimental autoimmune encephalomyelitis [3]. Thus, complement appears to function as a positive regulator of both basal and ischemia-induced adult mammalian neurogenesis [29]. Indeed, it can participate in the clearance of toxic debris and apoptotic cells, as well as promoting tissue repair, mainly through the anti-inflammatory activities of C3a.

Until now, the complement system, in the brain, was mainly described as involved in the basic mechanisms of defence against various pathogenic microorganisms. Its activation can be associated with bad or good effects. For example, C appears to mediate partial killing, but not clearance of *S. pneumoniae* from the CSF, both the classical and the alternative pathways are vital for host defense against *S. pneumoniae* infection in the CNS. Inflammation is harmful when it causes tissue damage as in meningitis, but useful when it helps in the preven-

tion or clearance of harmful pathogen deposits. C plays a critical role in host defense, in this removal of bacteria. Brain cells can generate C to kill pathogens while they are relatively well protected from direct complement factors lysis by expressing soluble and membrane inhibitors [30]. Thus, this new data reported for the neuroinflammatory disorders, seem to indicate that complement factors might have similar, dual effects at the level of the CNS. Finally, it might be hypothesized that their involvement in neuronal systems is part of an ancestral mechanism. Indeed, in the *Hirudo medicinalis* invertebrate CNS, we have identified, for the first time, a molecule homologous to mammalian C1q. Its chemoattractant activity on microglia highlights a new field of investigation, which might lead to a better understand of leech nerve cord repair, during which complement seems to be well regulated [31].

CONCLUSION

C is one of the most critical defence mechanisms of innate immunity against cerebral infection by viruses, bacteria and fungi, with different molecular pathways contributing to the clearance of the invading pathogens. C is now described as having special importance in the CNS. Complement activation can be neurotoxic as well as neuroprotective, and can lead to nerve recovery and repair [23, 25]. Therefore, a tight balance between complement activation and regulation is essential to protect and maintain healthy tissues [5]. With the aim of proposing alternative therapeutic agents, it is necessary to take into account the fact that, in neurodegenerative disorders, a more severe and chronic inflammation can ultimately result in a worsening of these diseases. An ideal treatment for reducing many CNS disorders would boost the clean-up function of complement, but suppress any excessive inflammatory effect. It should harness the “good side of complement activation” and suppress their “bad side”. Up to now, the results obtained in the leech nerve CNS seem to indicate that complement-like factors are involved in the efficient nerve cord repair seen following a nervous system injury. In this ancient invertebrate, complement factors seem to preserve its bright side of protection, preventing harmful inflammation caused by long lasting complement activation.

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