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Roles of the Complement System in Human Neurodegenerative Disorders

Pro-Inflammatory and Tissue Remodeling Activities

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Abstract

Complement is an important component of the innate immune response with the capacity to recognize and clear infectious challenges that invade the CNS through a damaged blood brain barrier. For instance, the membrane attack complex is involved in cytotoxic and cytolytic activities while other smaller fragments lead to cell activation (chemotaxis) and phagocytosis of the intruders. It is noteworthy that there is a growing body of evidence that uncontrolled complement biosynthesis and activation in the CNS can contribute to exacerbate the neuronal loss in several neurodegenerative disorders. We provide here an insightful review of the double-edged sword activities of the local innate complement system in the CNS and discuss further the potential therapeutic avenues of delivering complement inhibitors to control brain inflammation.

Index Entries: Complement; innate immunology; inflammation; neurodegeneration; CNS infection; therapy.

Introduction

The complement (C) system is a key component of the innate immune system, playing a central role in defense against pathogens *(1)*. It is also a powerful drive to initiate inflammation and can, if unregulated, cause pathology leading to severe tissue damage. C has been implicated in diverse human neurodegenerative disorders such as Alzheimer's disease (AD), Huntington's disease (HD), and Pick's disease (PiD) (for review, *see* ref. 2). We herein discuss the evidence that has demonstrated a significant

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role of C in the pathogenesis of these diseases and highlight the role of cells of the nervous system as sources of C and as targets for activation and/or damage by C. Although the C system is primarily involved in cytotoxic, cytolytic, and apoptotic activities against neurones, there is now compelling evidence indicating that C may also be involved in brain-tissue remodeling. Recent data support the view that C may participate in the clearance of deposits such as amyloid fibrils present in neuritic plaques in AD, as well as mediating phagocytosis of necrosed/apoptotic neurons by microglia while at the same time preserving normal cells. It is important that debris is cleared very efficiently from the brain to prevent further elicitation of the local inflammation. Moreover, it has been shown that microglia (the resident macrophage in the CNS) exposed in vitro to C3a anaphylatoxin express nerve growth factor (NGF), a molecule involved in early processes of neuronal regeneration. Therefore, it is timely to review and attempt to integrate the diverse roles of C in the CNS and particularly in human neurodegeneration.

The C System

The C system consists of some 30 fluid-phase and cell-membrane proteins (*see* Table 1) and is important in innate immunity to recognize and kill pathogens such as bacteria, virus-infected cells, and parasites but preserving normal "self" cells (for review, *see* ref. *1*). The liver (hepatocyte) is the major source of C proteins, but many cell types, including monocytes, fibroblasts, epithelial and endothelial cells, can also synthesize most of the C components *(48).* C can be activated by two distinct routes, the classical and the alternative pathway (CP and AP, respectively) (*see* Fig. 1). The CP (involving C1q, C1r, C1s, C4, C2, and C3 components) is activated primarily by the interaction of C1q with immune complexes (antibody-antigen) but activation can also be achieved after interaction of the C1q with nonimmune molecules such as polyanions (bacterial lipopolysaccharides, DNA, and RNA), certain small polysaccharides, viral membranes, C reactive protein (CRP), serum amyloid P (SAP); and so on (for review, *see* ref. *49*). The initiation of the AP (involving C3, factor B [fB], factor D [fD], and properdin [P]) does not depend on the presence of immune complexes and leads to the deposition of C3 fragments on the target cells. The target cell coated with C opsonins (C1q and C3 fragments: C3b, iC3b) will be specifically recognized and phagocytosed by macrophages bearing C receptors (C1qRp, CR1, CR3, CR4; *see* Table 2) *(1,77).* C anaphylatoxins (C3a and C5a) released in the fluid phase during C activation after enzymatic cleavage of C3 and C5, respectively, are important proinflammatory molecules involved in the stimulation and chemotaxis of myeloid cells bearing specific anaphylatoxin receptors (C3aR and C5aR; *see* Table 2) *(78).* The ultimate goal for the activation of the C system is the formation through the C terminal pathway, TP (involving C5, C6, C7, C8, and C9 components), of a membrane attack complex (MAC, also called the C5b-9 complex), which disrupts and forms a pore (hole) in the phospholipid bilayer to lyse the target cell. However, the C system, when activated at an inappropriate site and/or to an inappropriate extent, is remarkably effective at damaging host tissues and causing pathology as seen in degenarative disorders of the CNS (*see* below). To avoid this self-destructive tendency, host cells are protected by a battery of regulatory molecules (C inhibitors) that inhibit assembly of either the C3-cleaving enzymes or the formation of the MAC (*see* Table 1 and Fig. 1) *(79,80).* C1 inhibitor (C1-INH), C4b binding protein (C4bp), factor H (fH), factor I (fI), S protein (Sp), and clusterin are all soluble C inhibitors secreted and released in the fluid phase. The other C inhibitors are expressed on the cell membrane and include membrane cofactor protein (MCP, CD46), decay-accelerating factor (DAF, CD55), and CD59.

Table 1
Expression of Complement Proteins by Human Brain Cells

Brain tissue (RT-PCR analysis, ISH results, and immunohistochemistry)		
CP	(C1q, C1r, C1s, C4, C2, C3) *(3–6)*	*(C1-INH) (6,18)*
TP	(C5, C6, C7, C8, C9) *(4,7)*	*(Sp, clusterin) (6,18,33,34)*
CR	(CR1, CR2, C3aR, C5aR, C1qRp) *(8–18)*	
CI		*(MCP, DAF, CD59, CR1) (35,36)*
Astrocytes and cell lines		
CP	(C1q, C1r, C1s, C4, C2, C3) *(19–24)*	*(C1-INH not C4bp) (5,20,22)*
AP	(C3, fB, fD) *(19,25–27)*	*(fH, fI) (25)*
TP	(C5, C6, C7, C8, C9) *(23,28)*	*(Sp, clusterin) (28)*
CR	(CR1, CR2, C3aR, C5aR) *(9,10,12,15–17,29)*	
CI		*(DAF, MCP, CD59, CR1) (32,37–43)*
Microglia and cell lines		
CP	(C1q, C4, C2, C3) *(30)*	*(C1-INH) (44)*
CR	(CR1, CR3, CR4, C3aR, C5aR, C1qRp) *(15,16)*	
Neurones and neuroblastoma		
CP	(C4, C3, no C1q) *(24)*	*(C1-INH) (5,44,45)*
AP		*(fH) (23)*
TP		*(Sp, clusterin) (45,46)*
CR	(C3aR) *(31)*	
CI		*(CD59, MCP) (35,45)*
Oligodendrocytes and oligodendroglioma		
CP	(C3) *(32)*	*(C1-INH, C4bp) (32)*
AP	(C3) *(32)*	*(fH) (32)*
TP		*(Sp, clusterin) (32)*
CI		*(CD59, MCP, DAF) (32,47)*

Abbreviations: CP, Classical pathway; AP, Alternative pathway; TP, Terminal pathway; CR, C receptors; CI, C inhibitors.

Local Biosynthesis of a C System in the CNS: Recognition and Clearance of Pathogens

The CNS is separated from the plasma by the blood-brain barrier (BBB), formed by the endothelial cells of microvessels; the smooth-muscle cells (also called pericytes); and astrocytes (the major glial cell in the CNS). The BBB acts as a molecular sieve and restricts passage of large molecules from the plasma as well peripheral immunocompetent lymphocytes, macrophages, and natural killer (NK) cells *(81).* Thus, absent or restricted immunosurveillance by peripheral immunocompetent cells is assured in the CNS, highlighting the necessity for the brain to have its own local defense immune system in order to generate a set of molecular weapons against infiltrating pathogens. The CNS by itself can shape a immune and inflammatory response and we and others have proposed that brain cells can synthesize C proteins to recognize and kill pathogens while preserving normal cells in the CNS *(82,83).* Levi-Strauss and Mallat in 1987 *(84),* were the first to describe that brain cells were able to produce C. They showed that cul-

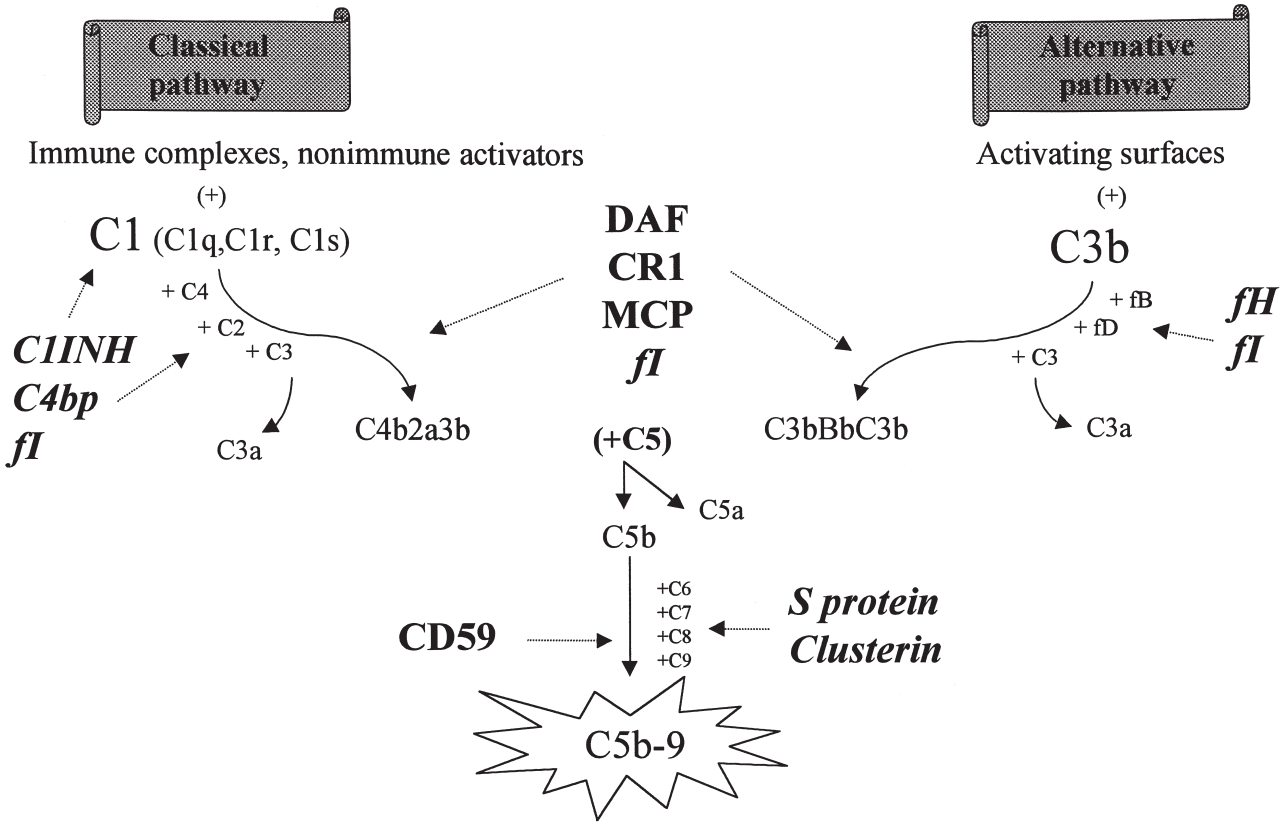

Fig. 1. Activation and regulation of the complement system. The C system is tightly regulated by a number of soluble (in bold italics) and membrane associated (in bold) proteins. For abbreviations, *see* text.

tured rodent astrocyte cells lines and primary murine astrocytes produced C3 and factor B and that the expression of C was increased after stimulation with lipopolysaccharides (LPS). The astrocyte is the most abundant glial cell type and was at that time thought to have a predominantly structural role, making this an unexpected finding. However, in the last 10 years these findings have been extended to include astrocytes, microglia, neurones, and oligodendrocytes (*see* Table 1). Primary cultures and cell lines of human origin were used to show that glial cells and neurones in vitro were capable of producing almost all C proteins, particularly after stimulation with cytokines. IFN-γ (as low as 20IU/mL) was the most effective cytokine to upregulate the expression of almost all C proteins by glial and neuronal cells *(19–21,25,27,28,32,45,83,85–87).* In contrast, tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) were shown to upregulate mainly C3, C2, and fB synthesis *(19–21,25,27,28,32,45,83,85–87).* From these studies, it was proposed that brain cells appropriately stimulated with cytokines could generate a full C system to assemble a toxic and lytic activity against pathogens. C mRNAs were also found to be expressed, albeit at a low level, in human brain tissues by reverse transcriptase-polymerase chain reaction (RT-PCR), Northern blot, and *in situ* hybridisation (ISH) analysis *(34,69,70,88).* Moreover, there is now considerable evidence that local expression of C by resident cells can be dramatically

Table 2
Evidence Implicating Complement in Human Neurodegenerative Disorders

AD	C activation in and around neuritic plaques and NFTs in AD brains *(8,50–58)*
	C1q can bind to fibrillar but not soluble β amyloid resulting in the activation of the CP (in vitro) *(59–63)*
	C1q can bind to CRP and SAP present in neuritic amyloid plaques (in vitro evidence) *(49,64)*
	The level of C mRNAs is increased in AD brains (RT-PCR analysis) *(6,65–70)*
	Pyramidal neurones and reactive microglia in AD brain express C mRNAs (ISH results) *(3,4,71,72)*
	The expression of C inhibitors (CD59, Sp, C1-INH) is slightly increased in AD brains *(6,33–35,44)*
	Increased expression of C receptors by reactive glial cells in AD brains (C5aR, C3aR) *(12,13)*
HD	Antibody-independent C activation occurs on neurones in the caudate area *(18)*
	C activation products (C1q, C4, C3, iC3b, and C5b-9 neo) are also detected on myelin and astrocytes *(18)*
	Myelin is a strong activator of the CP in vitro *(73,74)*
	C biosynthesis by microglia is increased in HD caudate comapared to normal brain (ISH results) *(18)*
	Expression of C receptors is increased in HD brains (C5aR, C3aR, C1qRp) *(12,13,16)*
PiD	Strong C staining (C1q, C4, C2, C3, C5, C6, C8 but not C9 or C5b-9neo) on neurones with Pick's bodies (ballooned neurons) *(75,76)*
	Increased expression of C inhibitors (Sp, clusterin, CD59) by neurons and astrocytes in PiD *(75,76)*
	Membrane C inhibitors (MCP, CR1, DAF) are not expressed in PiD brains *(75)*
Age-matched controls	
	C activation products are not detected in normal aged brains *(18,53)*
	C biosynthesis is minimal *(51,87)* and C inhibitors are expressed in normal brain (CD59»MCP, DAF, not CR1) (clusterin) *(36)*
	C receptors are weakly expressed in normal brain *(9–15)*

increased following brain infection. The level of C mRNAs was found to be significantly increased in inflamed human brains and in experimental model of brain infection (meningitis, scrapie, encephalitis) in rodents *(89–92).*

Susceptibility of Brain Cells to C and Expression of C Inhibitors in the Normal CNS

Most nucleated cells can express various C inhibitors to control C activation on their membranes (see Table 1 and Fig. 1). The first observation that brain cells were extremely susceptible to C lysis was made by Scolding and collaborators in 1989 *(93).* It was demonstrated that antibody-independent C activation occurs in vitro at the oligodendrocyte cell membrane while O-2A progenitors, astrocyte type I and II, remained unaffected *(93).* C activation was taking place through the CP and further studies have demonstrated that rat oligodendrocytes were lacking the major inhibitor of C lysis, CD59 *(94).* The situation in man appears to be different. Human oligodendrocytes and human oligodendroglioma cell lines have been

shown to express abundant levels of C inhibitors (particularly CD59) and failed to spontaneously activate the C system *(32,47,95).* Human astrocytes as well as microglia, from primary cultures and cell lines, express several membrane (CD59>MCP>>DAF) and soluble (fH, fI, C1-INH, Sp, clusterin but not C4bp) C inhibitors and are well-protected against C killing. In addition, there is no evidence that astrocytes and microglia activate spontaneously the C system. This is in sharp contrast to neurones, which are extremely susceptible to killing by homologous C. We and others have shown that human neurones (fetal neurones and neuroblastoma) cultured in the presence of human serum (source or C) are rapidly lysed owing to the formation of the MAC on their membranes *(45,96,96a)*. C1q binds specifically to the membrane of neurones and leads to the activation of the CP in an antibody-independent manner. C1q may bind to a neuronal 'C1q receptor' as yet uncharacterized. Furthermore, neurones and neuroblastoma cell lines seem to be extremely susceptible to C-mediated lysis since they express low level of C inhibitors (CD59, MCP, C1-INH, fH) and lack DAF *(45,96a)*. With the exception of neurones, it seems that brain cells are relatively well-protected from C-mediated lysis by expressing membrane and soluble C inhibitors. Most recent investigations using immunohistochemistry, ISH, and RT-PCR confirmed that C inhibitors (membrane and soluble forms) are constitutively expressed in vivo by glial, neuronal, and endothelial cells in the central nervous system (CNS) albeit at a low level compared for example to the level in placenta or the kidney *(36,47,97)* (*see* Table 1). The immunostaining for CD59, MCP, and DAF was stronger on microglia compared to astrocytes while neurones were found to weakly express CD59 and MCP and lack DAF. The expression of soluble C inhibitors (e.g., C1-INH clusterin mRNAs) was also demonstrated by RT-PCR analysis of normal brain tissues (*see* Table 1). Overall, it is clear that brain cells can generate a C system to kill pathogens while they are relatively well-protected from direct or bystander 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 (*see* below). It should be stressed that the participation of C in neuronal loss and brain inflammation is nonspecific and must be regarded as a consequence and not as the primary cause of the neuropathology.

Increased Local C Biosynthesis by Activated Glial Cells and Neurones in Neurodegeneration

The liver is regarded as the principal source of C proteins to be released in the serum. There is clear indication that the BBB, for instance in AD *(20),* is intact excluding the possibility of transudation of serum from the plasma as a potential source of C. The hypothesis that the brain itself acts as a source of C arose from the in vitro work showing that glial cells as well as neuronal cells can synthesise C components (*see* above). Early studies used RT-PCR analysis to measure the level of C mRNAs in AD, HD, and normal age-matched brains. Although the level of C mRNAs was found to be weak in normal brains, diseased brains showed markedly upregulated C mRNA expression particularly in areas of primary pathology (entorhinal cortex, hippocampus, midtemporal gyrus in AD, and caudate in HD) *(18,70)* (*see* Table 2). In AD, the level of C1q mRNA was increased from 11- to 80-fold when compared to normal brain. The levels of C3, C4 and C9 mRNAs were also found to be upregulated in AD (*see* Table 2). The levels of C3 and C4 mRNAs were also increased in HD caudate compared to the temporal lobe. Surprisingly, immunohistochemical and ISH analysis indicated that not only reactive glial cells (microglia>>astrocytes) but also neurones, were abundant sources of C (*see* Table 2). It has been postulated that cytokines expressed in

neurodegenerative disorders constitute a driving force to stimulate local C biosynthesis by resident cells *(34).* In contrast, RT-PCR analysis, ISH and immunohistochemistry have indicated that the levels of C inhibitors are barely increased in neurodegenerative disorders (*see* Table 2). Moreover, there is now considerable evidence that C is activated in neurodegenerative diseases. Therefore, given the increased local C biosynthesis together with a strong propensity of C to be activated locally, it would be surprising if C was not a contributing factor in the pathology of AD, HD and other neurodegenerative disorders.

C Activation in Degenerative Disorders of the CNS: Role of C1q Binding Molecules

AD is the commonest cause of pre-senile dementia and is a multifactorial syndrome rather than a single disease. Senile (neuritic) plaques and neurofibrillary tangles (NFT) comprise the major neuropathological lesions particularly in limbic and association cortices (for review, *see* ref. *98*). Neuritic plaques contain extracellular deposists of amyloid-β protein (Aβ) that include abundant amyloid fibrils intermixed with nonfibrillar forms of this peptide and also contain degenerating axons and dendrites (neurites). Such plaques can be specifically stained with thioflavine, which labels only β-amyloid in a β-sheet conformation. They contain variable numbers of activated microglia as well as reactive astrocytes surrounding the core. Immunohistochemistry using antibodies against Aβ reveals an even larger number of deposits in AD brains (thioflavine-negative plaques), which seem to lack microglia, astrocytes, and dystrophic neurites. These are referred to as diffuse plaques and are exclusively composed of the highly amyloidogenic 42-amino-acid-residue form of the peptide. Eikelenboom and Stam in 1982 *(8)* were the first to demonstrate the presence of C proteins in senile amyloid plaques and NFTs in AD brains using immunohistochemical techniques and subsequently numerous groups have also reported the association of C proteins of the CP in AD pathology (*see* Table 2). In contrast, immunohistochemical staining for two proteins of the AP, fB, and P, has not been observed in the AD brain. Interestingly, C1q immunostaining was co-localized to nearly all neuritic plaques, while no staining was detected in β-amyloid-immunopositive diffuse plaques *(99).* It has since been shown in vitro that C1q can bind directly to fibrillar but not soluble Aβ resulting in the activation of the CP as seen in AD brains. Thus, it appears that the conversion from the nonfibrillar diffuse Aβ to the fibrillar plaques correlates with the initiation of C activation. There is now some debate as to whether C1q binds to βA4 through its collagen stalk (involving Asp 7) *(59,62)* or by its globular head as recently demonstrated *(61).* Other molecules associated with AD lesions such as serum amyloid P (SAP) and C reactive protein (CRP) are known to interact with the collagen part of C1q and could also contribute to activation of the C cascade. NFTs are intraneuronal cytoplasmic lesions consisting of paired helically wound filaments (PHF) and containing hyperphosphorylated insoluble forms of a microtubule-associated protein, tau. NFT were also immunopositive for C1q, C3, C4, but not for fB and P (*see* Table 2). The mechanism involved in the CP activation on NFT remains unknown. It is possible that NFT express a 'C1q receptor' allowing C1q to bind to their membranes with the initiation of the CP activation.

C activation has also been detected in other human neurodegenerative disorders, such as HD and PiD *(18,75,76).* HD is an autosomal, dominant, inherited neurodegenerative disease and the gene associated with the disease encodes a mutant protein named huntingtin with expanded polyglutamine repeats *(100).* The neuropathologival hallmark of HD is atrophy of the caudate nucleus with a profound loss of neurones in the putamen accompanied by reactive gliosis (astrocytes and microglia). We have found that neurones as well as myelin and astrocytes in HD brains were

strongly stained with antibodies to C1q, C4, C3, iC3b-neoepitope, and C9 neoepitope. C activation takes place through the CP on neurones and astrocytes where, both the wild type and the mutant huntingtin are expressed. By analogy with the role of β-amyloid fibrils to initiate the CP activation in AD, we have proposed that mutant huntingtin with a long glutamine stretch could be involved in C activation in HD caudate. Moreover, since mutant huntingtin is involved in apoptosis of neurones (for review, *see* ref. *100*) and since C1q can bind directly to the surface of apoptotic cells *(101),* we propose that C activation in HD caudate could occur primarily on apoptotic neurons. Although the activation of the C system would initially be restricted to very few apoptotic cells, it is possible that C would cause damage to surrounding cells by bystander lysis with the capacity of soluble C5b6/C5b7 to diffuse and bind nonspecifically to cell membranes to form a lytic MAC. These attractive hypotheses can now be tested, for instance using an in vitro model of neurones hyperexpressing mutant huntingtin. C is also involved in non-Alzheimer's dementias such as PiD. The histological hallmark of PiD is the neuronal Pick body, which is strongly stained for tau protein and ubiquitin. Neuronal loss and gliosis occurs in the areas of disease that appear to be restricted to frontal and temporal lobes. It has been reported that Pick bodies stain for MAC *(76).* Our data demonstrate strong staining for components of the CP with little or no evidence for MAC *(75).* The mechanism behind the activation of the CP in PiD is uncharacterized. Nevertheless, the possibility of C1q binding to a specific component of the Pick body or even to apoptotic cells or necrosed cells must be considered. It is well-known that C can be activated by a variety of intracellular components released by necrosed cells (nucleic acids, intermediate filaments, mitochondrial membranes) (for review, *see* ref. *49*). It should also be stressed that neurones, at least in culture, seem to have the natural propensity to activate spontaneously the C system (*see* above). The hypothesis that the putative neuronal 'C1q receptor' is enough to mediate C activation on neurones, Pick bodies, and NFT is attractive and worthy of consideration.

Roles of Receptors for C1q and Other Opsonins in Neurodegeneration: Phagocytosis

The obvious and well-defined role of C1q is to bind to immune complexes and "non-self" membranes (pathogens) to initiate the activation of the CP leading to lysis of the cell by the MAC. However, C1q is also an important opsonin labeling specifically a target cell so that it can be recognized by macrophages bearing C1q receptors (or C1q binding protein): CR1 (complement receptor type 1) and the phagocytic C1q receptor (C1qRp) *(77).* CR1 is mainly expressed by macrophages but was not identified on microglia *(36).* Fetal astrocytes and some astrocyte cell lines were found to express CR1 in vitro *(10).* In contrast, only microglia but not astrocytes expressed the C1qRp *(16).* Interestingly, the immunoreactivity of microglia for C1qRp was particularly prominent in HD and PiD compared to age-matched normal brains *(16),* indicating that this receptor may be involved in brain inflammation. However, the function of CR1 on astrocytes and the C1qRp on microglia in neurodegeneration remains to be identified. It is possible that both cell types in concert are involved in the clearance by phagocytosis of C1q-opsonized cells (neurones, NFT, Pick's body) as well as C1q-opsonized βA4 amyloid fibrils residing in the neuritic plaques. The possibility that glial cells could clear necrosed or apoptotic cells as well as amyloid deposits in the CNS is fascinating and future works along this promising line is warranted. The role of other C receptors (CR3 and CR4) to mediate phagocytosis of C-opsonized target cells should not be underestimated. Macrophages and microglia express both receptors and will phagocytose C3-opsonized targets *(11).* All these data would suggest that C activation,

at least in the early stages of these neurological diseases, could play an important and beneficial role in phagocytosis and clearance of otherwise toxic molecules. It is important that cell debris is removed efficiently to prevent further elicitation of the local inflammation. An interesting and elegant study has recently shown that the vaccination with Aβ1–42 peptide of a transgenic mouse hyperexpressing mutant human APP accelerated clearance of neuritic amvloid plaques from the brain and reduced the extent and progression of the AD-like pathology *(102).* It was proposed that microglia expressing high level of immunoglobulin (Ig) Fc receptors were able to phagocytose the Ig-βA4 complex. The role of C was not investigated in this model although from data discussed earlier, it is likely that increased C biosynthesis and CP activation were taking place. Therefore the possibility of glial cells expressing C receptors to phagocytose the opsonized antibody-amyloid complex coated with C is an interesting and attractive hypothesis that remains to be tested.

Roles of the Anaphylatoxins C3a and C5a in Neurodegeneration

Anaphylatoxins are small polypeptides (less than 12kDa) that are cleaved from the large and abundant C components C3 and C5 during C activation and released in the fluid phase. They are known to be important inflammatory mediators by binding and stimulating cells bearing specific anaphylatoxin receptors : C5aR and C3aR *(78).* C5aR is a seven transmembrane (7TM) spanning receptor coupled to a G protein involved in cell signaling. It was thought that only myeloid cells (including microglia) expressed C5aR, but we and others have shown that astrocytes and neurones also expressed the C5aR (*see* Table 3). C5a is an important chemoattractant molecule and stimulates cells to express increased level of cytokines, chemokines, adhesion molecules, and C components (for review *see* ref. *(78)*). Therefore, it is possible that C5a released during C activation, for example in AD, could induce chemotaxis and stimulate glial cells to produce pro-inflammatory cytokines contributing to exacerbate the pathology (*see* Table 3). Interestingly, It has recently been shown that human astrocyte cell lines stimulated with C5a produced increased level of IL-6 while the level of IL-1, TNF-α, and transforming growth factor-β (TGF-β) remained unaffected *(104).* Unexpectedly, C5a has also been reported to induce apoptosis of neurones in vitro. Farras and colleagues have shown that a human neuroblastoma cultured in the presence of a C5a peptide analogue underwent programmed cell death as judged by DNA fragmentation *(105).* This experiment has yet not been confirmed using primary neurones but nevertheless indicates that C5a could contribute directly to neuronal damage. The human C3aR was only recently cloned, allowing the production of specific reagents for immunohistochemistry and ISH *(78).* The distribution of C3aR in the CNS is similar to that of C5aR. C3aR is also a 7TM spanning receptor expressed by macrophages, mast cells, microglia, astrocytes, and neurones (*see* Table 1). The role of C3a in tissue inflammation is less certain *(78).* In contrast to the broad pro-inflammatory effects of C5a, the effects of C3a appear to be much more selective and rather anti-inflammatory. C3a is chemoattractant but only for mast cells and eosinophils and not for either macrophages or microglia. Recent experiments have indicated that C3a decreased the production of pro-inflammatory cytokines by LPS-stimulated macrophages and on the other hand induced the production of immunosuppressive cytokines such as IL-10 (for review see ref. *78*). Recently, Heese and collaborators have shown that a human microglia cell line stimulated with C3a expressed de novo NGF, a molecule involved in neuronal growth *(106).* These types of study need to be extended to other glial and neuronal cell cultures for instance looking at the expression of anti-inflammatory cytokines and growth factors following C3a stimulation. These studies could also profit from the recent development of the micro-array technology to

Table 3
Role of Opsonins (C1q, C3b, iC3b), Anaphylatoxins (C3a, C5a), and C5b-9 Complex on Brain Cells

(C ligand)	Receptor (binding molecule)	(Target)	(Roles)
C1q	'C1q receptor'	Neuron	Activation of the CP (*see* text)
	Amyloid fibrils	Plaques.	Activation of the CP
	CRP, SAP, Myelin	Plaques (myelin)	Activation of the CP
	C1qRp (100kDa) *(77)*	Microglia	Clearance of amyloid fibrils?
			Clearance of C1q-opsonised cells?
			Clearance of apoptotic cells?
C1q, C3b	CR1 (CD35)	Astrocytes	Phagocytosis of C1q-, C3-opsonised target
iC3b	CR3, CR4, (CD11b,c/CD 18)	Microglia	Phagocytosis of C3-opsonised target
C5a	C5aR (CD88) (45kDa)	Microglia	Chemotaxis, ↑cytokines?, ↑chemokines? *(103)*
	(coupled to Gp)	Astrocytes	Chemotaxis, ↑cytokines, ↑chemokines? *(104)*
		Neurones	Apoptosis *(105)*
C3a	C3aR (65–85 kDa)	Microglia	↑ or ↓cytokines/chemokines?
	(coupled to Gp)		↑ growth factors (NGF …) *(106)*
		Astrocytes	↑ or ↓ cytokines/chemokines? *(104,107)*
			↑ growth factors?
		Neurones	Apoptosis?
C5b-9	Lytic	Neurones	Cytotoxicity, Cytolysis
	Sublytic (low level of C5b-9)	Neurones	↑ C inhibitors? *(108,109)*
		Glial cells	↑ C inhibitors? *(108,109)*
			↑ release AA, LTB4 *(110)*
			↑ mitotic signalling *(111–113)*
			↑ cytokines?, chemokines?, adhesion molecules *(103,108,109,114)*

Abbreviation: C, complement; Gp, G protein; ↑, increased production; ↓ decreased production. The ? indicates that the effect has been described on a nonbrain cell type and remains to be tested on glial or neuronal cells.

identify rapidly the expression of multiple genes up- or downregulated after cell treatment, for instance with anaphylatoxin. Whether C3a, like C5a, has a role in apoptosis of neurones remains untested.

Roles of C5b-9 (Lytic and Sublytic Effects)

The MAC is by definition involved in cytotoxic and cytolytic activities. Aside from these functions, the MAC at sublytic level is also involved in cell stimulation (for review *see* refs. *108,109*) (Table 3). It has been shown that cultured glial cells release phospholipid and generate arachidonic acid (AA) and AA-derived inflammatory mediators (leukotriene B_4) in response to sublytic level of C5b-9 *(115).* The effects were observed on cell lines and it remains unproven as to whether the same effects can be reproduced on primary cultures of either glial cells or neurones. The MAC at sublytic level has also been shown to stimulate endothelial cells to express increased level of C regulators and to protect against secondary C attack *(116).* These data suggest that the MAC at sublytic level could act as a stress signal to stimulate cells to express increased level of C inhibitors. It will be interesting to test whether the same effects are observed on glial and neuronal cultures. Along the same lines, it would be interesting to test whether brain cells stimulated with sublytic MAC alter their expression of pro-inflammatory or immunosuppresive molecules.

Control of C in Diseases: Therapeutic Intervention

The aforementioned studies implicate C activation in the initiation and/or exacerbation of inflammation and tissue injury in diseases of the CNS. Effective inhibition of C might be of potential therapeutic value. Several C inhibitors have been developed, some of which are recombinant forms of the naturally occuring C inhibitors *(79).* Some of these molecules (e.g., soluble CR1) have been used successfully to control C activation in animal models of CNS disorders *(118,119),* but the use of this molecule in chronic disorders such as AD would not be possible, since it is too expensive, must be administered systemically, and has a short half-life in vivo. In order to be used in CNS diseases, the next generation of C inhibitors will have to be designed so that they can either be specifically delivered to the brain (by the use of specific targeting moieties) or expressed in the brain (expression by gene therapy). In addition, it will be interesting to search for new drugs which might be able to control the pro-inflammatory activities of C-derived fragments such as C3a and C5a. The use of specific receptor antagonists (peptides or chemical drugs) could prove to be useful for this purpose. Combination of these aforementioned therapeutic approaches with anti-inflammatory drugs (e.g., indomethacin) might be of clinical utility in the treatment of neurodegeneration *(120).*

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