Innate immunity and brain inflammation: the key role of complement

Karen Francis, Johan van Beek, Cecile Canova, Jim W. Neal and Philippe Gasque

The complement inflammatory cascade is an essential component of the phylogenetically ancient innate immune response and is crucial to our natural ability to ward off infection. Complement is involved in host defence by triggering the generation of a membranolytic complex (the C5b-9 complex) at the surface of the pathogen. Complement fragments (opsonins; C1q, C3b and iC3b) interact with complement cell-surface receptors (C1qRp, CR1, CR3 and CR4) to promote phagocytosis and a local pro-inflammatory response that, ultimately, contributes to the protection and healing of the host. Complement is of special importance in the brain, where entrance of elements of the adaptive immune system is restricted by a blood–brain barrier. There is now compelling evidence that complement is produced locally in response to an infectious challenge. Moreover, complement biosynthesis and activation also occurs in

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neurodegenerative disorders such as Alzheimer's, Huntington's and Pick's diseases, and the cytolytic/cytotoxic activities of complement are thought to contribute to neuronal loss and brain tissue damage. However, recent data suggest that at least some of the complement components have the ability to contribute to neuroprotective pathways. The emerging paradigm is that complement is involved in the clearance of toxic cell debris (e.g. amyloid fibrils) and apoptotic cells, as well as in promoting tissue repair through the anti-inflammatory activities of C3a. Knowledge of the unique molecular and cellular innate immunological interactions that occur in the development and resolution of pathology in the brain should facilitate the design of effective therapeutic strategies.

In tissues other than the central nervous system (CNS), immune responses involving both the innate (nonspecific phagocytosis) and adaptive (antibody-mediated) immune systems have pivotal roles in the efficient clearance of pathogens (Ref. 1). However, the brain is 'immunoprivileged' in that it is relatively isolated from peripheral immunosurveillance by neutrophils/monocytes and lymphocytes (Ref. 2) (Fig. 1). The limitation of an adaptive immune response in the CNS has been attributed to the intricate nature of susceptible neuronal networks and is thought to derive from an evolutionary adaptation (Ref. 3). The most prominent element preventing the infiltration of intruders (immune cells or pathogens) in the CNS is the blood–brain barrier (BBB). In addition, there is an immunological barrier, manifested by: (1) the reduced expression of adhesion molecules, major histocompatibility complex (MHC) HLA molecules and costimulatory molecules; and (2) an immunosuppressive microenvironment mediated by astrocytes and microglia, which suppress infiltration of peripheral immunocompetent lymphocytes. For instance, there is compelling evidence that astrocytes and microglia can express abundant levels of tumour necrosis factor (TNF)-related death ligands that mediate apoptosis of infiltrating cells (T cells and neutrophils) (reviewed in Ref. 4). Overall, these observations have lead to the conclusion that all forms of CNS inflammation would potentially do more harm than good and, hence, control of immune activation is required to prevent extensive damage.

Despite the general effectiveness of the BBB, pathogens infiltrate the CNS on rare occasions. When this happens, it is generally accepted that two principle defensive strategies developed by the resident cells come into play. Stimulated glial cells produce several soluble cytotoxic and cytolytic innate immune molecules, such as complement (C) proteins and perforin, which have a destructive effect on invading pathogens. In addition, specialised professional and amateur phagocytes engulf (phagocytose) and kill the intruders. The cells that shoulder the main burden of CNS-specific phagocytic defence are the microglia (Ref. 5) (Fig. 2). These cells are thought to derive from the monocyte/macrophage population that have infiltrated the CNS during embryogenesis; they express only low levels of MHC molecules, which makes them inefficient stimulators of T cells, thereby suppressing any immune response. Moreover, there is mounting evidence that astrocytes, oligodendrocytes, endothelial cells and perhaps a subset of neurons also have nonprofessional phagocytic properties (Ref. 6).

This review will examine the role of the local innate immune response in host defence and inflammation associated with neurodegenerative diseases such as Alzheimer's disease (AD) and Huntington's disease (HD). Given the multiple functions of many innate immune molecules, it is important to pinpoint the roles of the local innate immune response in specific pathophysiological situations and to highlight the 'double-edged sword' that it creates, in that sustained expression of innate immune proteins can either promote or counteract neurodegenerative processes. Finally, the concept of directing and instructing the brain immune response for therapeutic purposes will be discussed.

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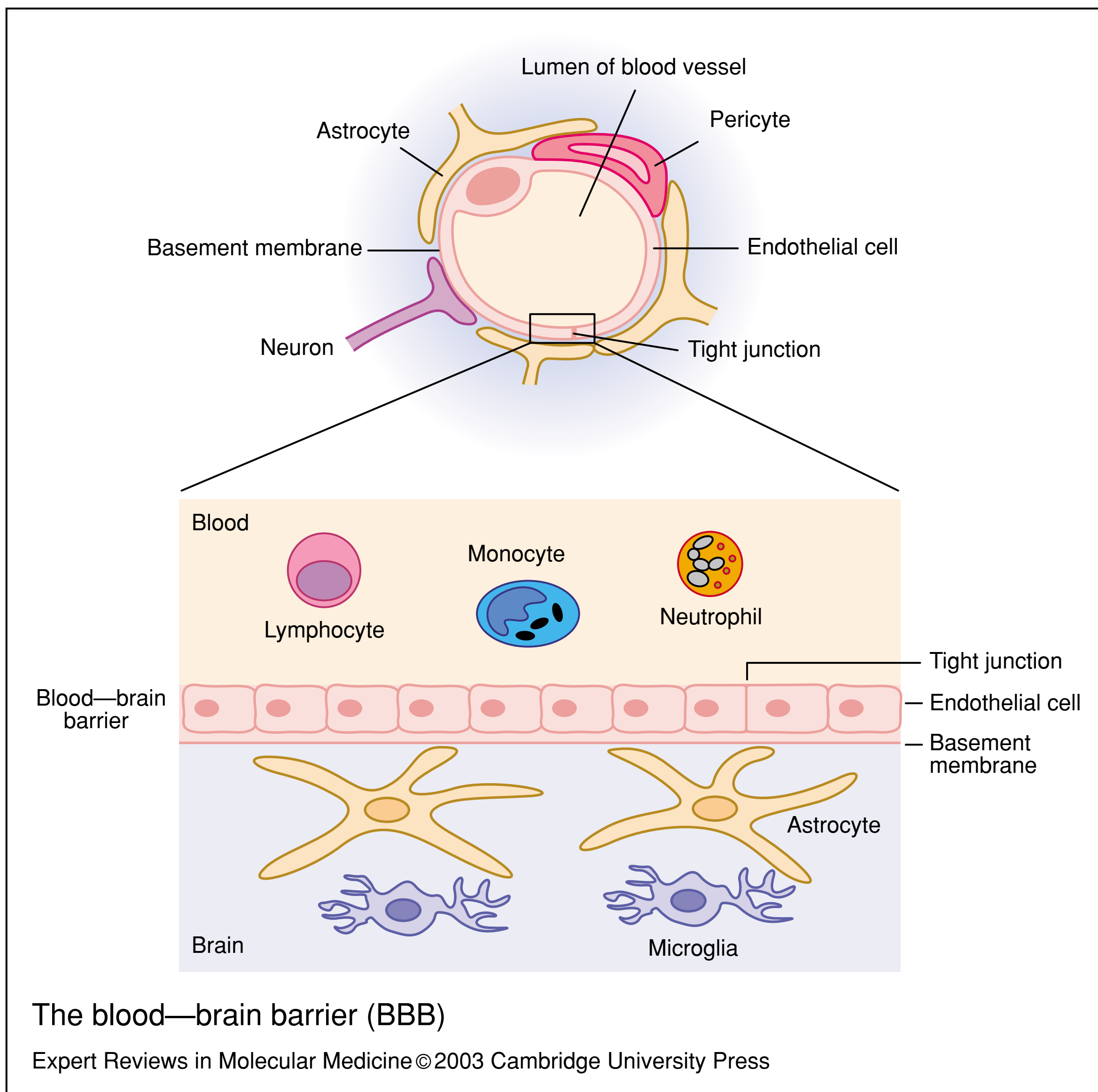

Figure 1. The blood–brain barrier (BBB). The BBB is created by the tight apposition of endothelial cells lining blood vessels in the brain, forming a barrier between the circulation and the brain parenchyma (astrocytes, microglia). Blood-borne immune cells such as lymphocytes, monocytes and neutrophils cannot penetrate this barrier. A thin basement membrane, comprising lamin, fibronectin and other proteins, surrounds the endothelial cells and associated pericytes, and provides mechanical support and a barrier function. Thus, the BBB is crucial for preventing infiltration of pathogens and restricting antibody-mediated immune responses in the central nervous system, as well as for preventing disorganisation of the fragile neural network. This, together with a generally muted immune environment within the brain itself, protects the fragile neuronal network from the risk of damage that could ensue from a full-blown immune response. On rare occasions, pathogens (e.g. viruses, fungi and prions) and autoreactive T cells breach the endothelial barrier and enter the brain. A local innate immune response is mounted in order to limit the infectious challenge, and pathogens are destroyed and cell debris is removed, a vital process that must precede tissue repair **(fig001pgc)**.

The innate immune response in the CNS

Recognition of PAMPs

Extrapolating from the concept originally presented by Medzhitov and Janeway (Ref. 7), we have proposed that soluble and membrane defence molecules of the innate immune system expressed by activated glial cells in the CNS are able to recognise pathogen-associated molecular

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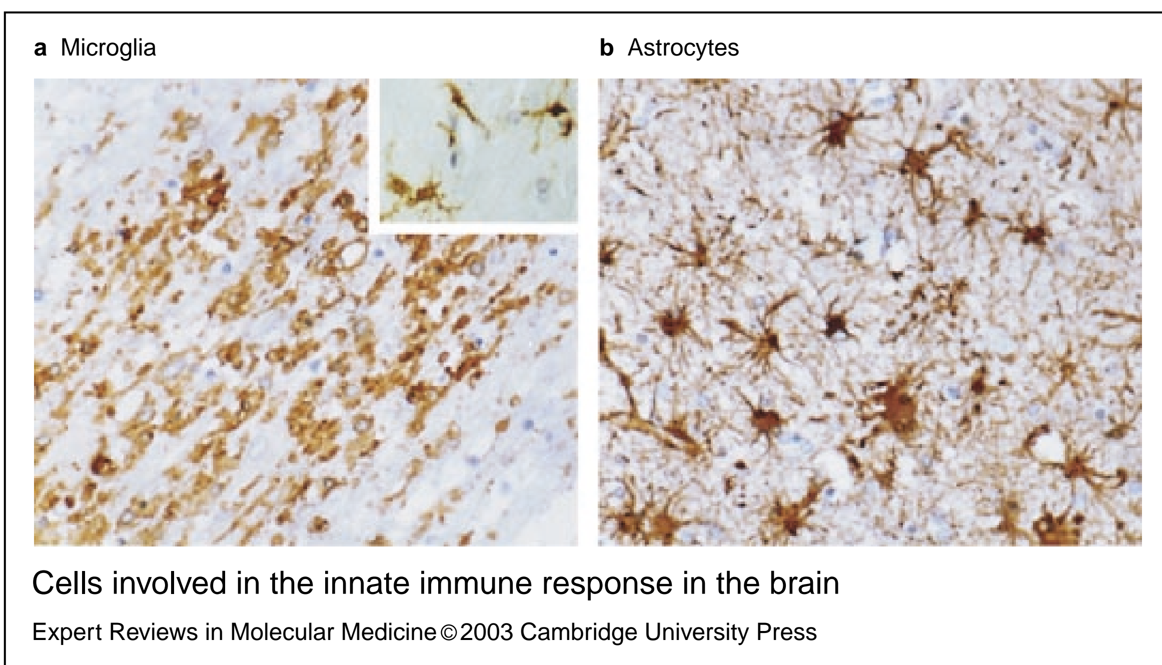

Figure 2. Cells involved in the innate immune response in the brain. Most phagocytosis occurring within the central nervous system is undertaken by microglia (a; insert shows migroglia stained for HLA-DR in a section of inflamed human brain). However, other cell types such as astrocytes (b), oligodendrocytes, endothelia and even neurons may act as nonprofessional phagocytes. For details of the staining procedure used, see Refs 54 and 101. Magnification = ×200 **(fig002pgc)**.

patterns (PAMPs) (Ref. 4). PAMPs are conserved microbe structures shared by large groups of pathogens that, upon interaction with elements of the host innate immune system, trigger the initiation of host protective responses, resulting in the clearance of the pathogen by phagocytic cells (Ref. 7).

The innate immune system is also involved in the clearance of foreign, potentially dangerous and toxic entities such as apoptotic cells (Ref. 8). By analogy to PAMPs, we and others have proposed that CNS-derived innate immune molecules recognise apoptotic-cell-associated molecular patterns (ACAMPs) expressed de novo by cells undergoing programmed cell death (Refs 4, 9). It is suggested that glial cells, endothelial cells and neurons recognise PAMPs and ACAMPs through specific pattern-recognition receptors (PRRs; e.g. phagocytic receptors such as CD14 and macrophage mannose receptor), which lead to clearance of the different target cells (Refs 8, 9). Another group of molecules that recognise PAMPs and ACAMPs has been collectively named the 'defence collagens' (Ref. 10). Usually, the globular C-terminal domain of these proteins recognises relatively broad categories of molecules and the collagen-like N-terminal domain links the invading organism to powerful effectors of the immune system (i.e. PRRs) expressed by macrophages. Soluble members of the defence collagens include C1q (the recognition C component of the classical pathway) and the collectins mannan-binding lectin (MBL) and pulmonary surfactant protein A (SPA) (Ref. 10). However, although there is a strong body of evidence that C1q is expressed in the CNS (Ref. 4), particularly in disease conditions, the expression of MBL and SPA by brain cells has not been reported.

Routes of C activation: pathogens, apoptotic cells and toxic cell debris

The C system consists of some 30 fluid-phase and cell-membrane proteins and is important in innate immunity to recognise and kill pathogens such as bacteria, virus-infected cells and parasites yet preserving normal 'self' cells (reviewed in Ref. 11). Recent studies have indicated a marked conservation of the C system between invertebrates and mammals, which points to a common ancestry of this system in host

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defence and raises the paradigm of a critical role of C in tissue homeostasis (Refs 12, 13, 14). In invertebrates (e.g. insects), the C system is very simple, comprising only a small number of components that, surprisingly, are not produced in the fat body (the functional equivalent of the mammalian liver) but are expressed instead by phagocytes (Refs 15, 16). In mammals, hepatocytes in the liver are the major source of most C proteins, with the exception of C1q, factor D (fD) and C7 (Ref. 17). Many cell types including monocytes, fibroblasts, epithelial cells and endothelial cells can also synthesise most of the C components (Ref. 17).

C can be activated by three distinct routes: the classical, alternative and lectin pathways (Fig. 3). The classical pathway (involving C1q, C1r, C1s, C4, C2 and C3 components) is activated primarily by the interaction of C1q with immune complexes (i.e. antibody–antigen). However, activation can also be achieved after interaction of C1q with nonimmune molecules such as polyanions [bacterial lipopolysaccharides (LPS), DNA and RNA], certain small polysaccharides, viral membranes, C-reactive protein (CRP), serum amyloid P (SAP) and, more importantly, some bacterial, fungal and viral membranes (reviewed in Ref. 18). Initiation of the alternative pathway [involving C3, factor B (fB), fD and properdin] does not depend upon the presence of immune complexes and leads to the deposition of C3 fragments on the target cells (Ref. 18). The lectin pathway shares several molecules with the classical pathway and is activated by binding of MBL to carbohydrates expressed on pathogens but not generally found on 'self' cells. Two serine proteases, the MBL-associated serine proteinases (MASP-1 and MASP-2) are activated upon binding of MBL and subsequently cleave C4 and C2 (Ref. 4).

Recently, several lines of evidence have suggested that C1q has an important role in the clearance of apoptotic cells. Three independent studies have shown that C1q can bind directly and specifically to surface blebs of ultraviolet light-induced apoptotic cells (keratinocytes and T cells), leading to the activation of the classical pathway (Refs 19, 20, 21). Moreover, it has been reported by Botto and colleagues that C1q-knockout mice show a profound impairment in the clearance of apoptotic cells, which then accumulate in the kidney and lead to glomerulonephritis with immune deposits (Ref. 22). C1q-deficient mice that also lack C2 and fB develop glomerulonephritis without glomerular C3 deposition (Ref. 23). However, C1q-sufficient mice lacking C2 and fB do not develop either glomerulonephritis or autoantibodies. These observations support the hypothesis that C1q serves as an opsonin in the efficient recognition and physiological clearance of apoptotic cells. Interestingly, C1q mRNA is not detectable in the liver and the lung but is expressed in spleen, thymus and heart, as well as by tissue macrophages (Ref. 24). In the brain, microglia and astrocytes express C1q, albeit at a lower level (Refs 25, 26, 27). C1q can also bind spontaneously to apoptotic neurons, amyloid-β protein (βA4), myelin basic protein (MBP) and myelin oligodendrocyte glycoprotein (MOG) (reviewed in Ref. 4). This suggests that C1q may be involved in the clearance of cell debris and toxic components from the CNS.

Role of C receptors in phagocytosis and signalling events

Coating of the target cell with C opsonins (i.e. opsonisation with C1q, C3 and C4 fragments: C3b, iC3b) results in specific recognition and phagocytosis by macrophages bearing C receptors (C1qRp, CR1, CR3, CR4; see Table 1) (Refs 28, 29, 30, 31, 32, 33, 34, 35, 36).

In recent years, it has emerged that a cell-surface molecule, designated the C1q receptor (C1qRp), functions as a defence collagen receptor for C1q, MBL and SPA. Indeed, monocytes that have adhered to surfaces coated with C1q (or MBL or SPA) display a 4–10-fold enhancement of ingestion of targets opsonised with IgG or C (Ref. 37). Monoclonal antibodies selected for their ability to inhibit this C1q-mediated enhancement of phagocytosis were used to clone the C1qRp cell-surface transmembrane glycoprotein (Ref. 38). C1qRp has been shown to be the analog of the rodent foetal stem cell marker AA4, which is involved in cell–cell interactions during haematopoietic and vascular development (Refs 39, 40, 41, 42). AA4 is abundantly expressed by endothelial cells and microglia, and recent studies in humans further support the concept that C1qRp/AA4 is involved in cell signalling to promote phagocytosis and adhesion (Ref. 43). Interestingly, C1qRp is the antigen recognised by a pro-adhesive monoclonal antibody, mNI-11, and several antibodies against CD93 (Ref. 44). Understanding of the cellular and molecular properties of this receptor is still in

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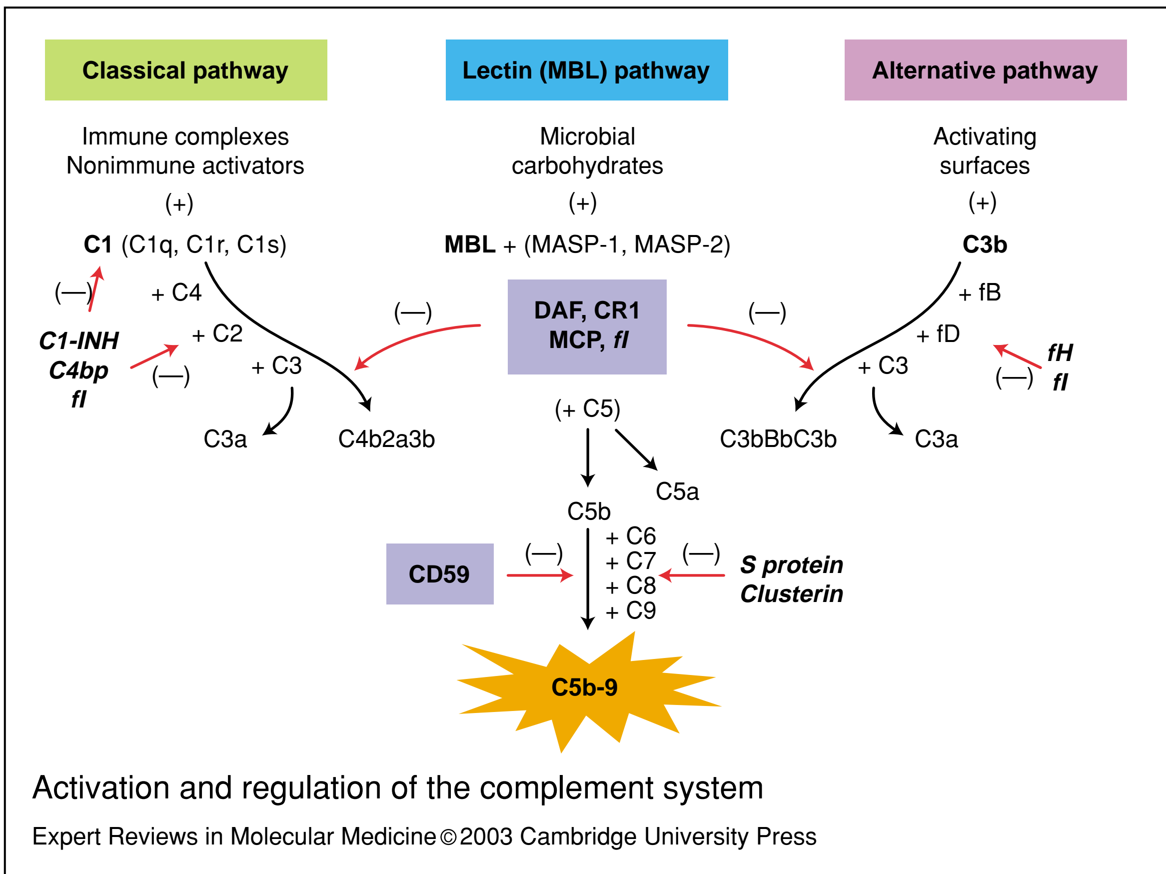

Figure 3. Activation and regulation of the complement system. Complement is a highly conserved innate immune cascade of 30 or so proteins that interact to recognise and kill pathogens. Activation is triggered by one of three pathways – classical, alternative and lectin – depending on the nature of the foreign molecule and therefore the activating surface. The classical pathway is activated primarily by the interaction of C1q with immune complexes of antibody with antigen, but can also be achieved after interaction of C1q with nonimmune molecules. The alternative pathway does not depend upon the presence of immune complexes and leads to the deposition of C3 fragments on the target cells. The lectin pathway shares several molecules with the classical pathway and is activated by binding of MBL to carbohydrates expressed on pathogens but not generally found on 'self' cells. The end result of all three pathways is either the opsonisation or the destruction (through formation of the lytic molecule C5b-9) of nonself cells and target organisms. The system is regulated by proteins such as C1-INH, C4bp, fI, fH, DAF, CR1, MCP, CD59, S protein and clusterin, which help to protect the host from immune attack. Some of these inhibitors are soluble (in bold, italics) and some are membrane associated (in bold, boxed). Abbreviations: C, complement component; C1-INH, C1 inhibitor; C4bp, C4b-binding protein; DAF, decay accelerating factor; f, factor; MASP, MBL-associated serine proteinase; MBL, mannan-binding lectin; MCP, membrane cofactor protein **(fig003pgc)**.

its infancy and certainly warrants further investigation.

When activated on a cell surface, C3 becomes covalently bound (opsonised) as C3b, which is subsequently cleaved to yield a very stable fragment, iC3b. There is well-documented evidence that CR3 (CD11b/CD18) and CR4 (CD11c/CD18; also known as p150,95) are involved in the phagocytosis of targets opsonised with C3b and iC3b fragments (reviewed in Refs 33, 35). Perhaps more importantly, the binding of phagocytes by way of CR3 recognition, either of natural microbial surface components, such as β-glucan, LPS, lipophosphoglycan and other as-yet-undefined structures, or by way of iC3b, is the crucial event leading to the elimination of pathogens, toxic debris and apoptotic cells. A CR3-like molecule has recently been described in invertebrates, and CR3 is now considered a key receptor in innate immunity and legitimately joins

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Table 1. Role of opsonins, anaphylatoxins and C5b-9 in the brain[a] (tab001pgc)

Ligand[b]	Receptor or binding molecules	Target	Established and proposed roles[c]
Opsonins			
C1q	Uncharacterised 'C1q receptor'	Neurons	Activation of CP
	Amyloid fibrils	Plaques	Activation of CP
	CRP, SAP, myelin	Plaques	Activation of CP
	C1qRp (renamed CD93)	Microglia	Clearance of amyloid fibrils, C1q-opsonised cells and apoptotic cells?
		Endothelium	Cell adhesion, PECAM-like?
iC3b	CR3 (CD11b/CD18)	Microglia	Phagocytosis of C3-opsonised target
	CR4 (CD11c/CD18)		Role in adhesion/phagocytosis?
C1q, C3b, C4b	CR1 (CD35)	Astrocytes	Phagocytosis of C1q/C3-opsonised target?
Anaphylatoxins			
C5a	C5aR (CD88) (coupled to Gp)	Microglia	Chemotaxis, ↑ cytokines and chemokines?
		Astrocytes	Chemotaxis, ↑ cytokines and chemokines?
		Neurons	Apoptosis
C3a	C3aR (coupled to Gp)	Microglia	↑ or ↓ cytokines/chemokines? ↑ growth factors (NGF and neurotrophins)
		Astrocytes	↑ or ↓ cytokines/chemokines? ↑ growth factors?
		Neurons	Apoptosis?
Lytic complex			
C5b-9	Lytic	Neurons	Cytotoxicity, cytolysis
	Sublytic (low level of C5b-9)	Neurons	↑ C inhibitors?
		Glial cells	↑ C inhibitors? ↑ release AA, LTB_4 ↑ mitotic signalling ↑ cytokines/chemokine adhesion molecules?

[a] Table adapted with permission from Elsevier © 2000 (Ref. 4), which provides further references.
[b] C ligands: activation of the C cascade leads to the opsonisation (coating) of the target by C opsonins (C1q, C3b, iC3b and C4b), which are then recognised by professional (microglia) or amateur (astrocyte) phagocytes bearing CRs. C anaphylatoxins (C3a and C5a) are soluble polypeptides cleaved from the parent C molecules (i.e. C3 and C5, respectively) to attract by chemotaxis cells expressing anaphylatoxin receptors. Finally, although high levels of C5b-9 will cause lysis of the target cells, there is emerging evidence that sublytic doses of C5b-9 can induce signalling.
[c] ↑, indicates increased production; ↓, indicates decreased production; ?, indicates the effect has been described on a non-brain-cell type and remains to be tested on glial or neuronal cells.
Abbreviations: AA, arachidonic acid; C, complement; CR, complement receptor; CRP, C-reactive protein; CP, classical pathway; Gp, G protein; LTB_4: leukotriene B_4; NGF, nerve growth factor; PECAM, platelet/endothelial cell adhesion molecule; SAP, serum amyloid protein.

the ranks of the host PRRs such as CD14 and the macrophage mannose receptor (Refs 45, 46). CR3 and CR4 belong to the β2 subgroup of the integrin superfamily mainly expressed by phagocytes and natural killer (NK) cells, and perhaps binding of CR3 to other ligands on the endothelium [e.g. intercellular cell adhesion molecule (ICAM-1)] might be a necessary step in the migration of leukocytes into the brain parenchymal tissue. Microglia within the parenchyma and Kolmer cells of the choroid plexus express abundant levels of CR3 and CR4 (Ref. 47). The interactions of

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CR3/CR4 with different extracellular matrix molecules (such as fibronectin, laminin and collagen) might contribute further to tissue invasion.

CR1 (CD35) is also a multifunctional receptor both in its ligand specificity and in its C-regulatory activities (Ref. 34). CR1 binds to C4b, C3b, iC3b and C1q, and is involved in phagocytic activities. CR1 is broadly expressed and has been found on CNS macrophages (Ref. 48).

Role of C anaphylatoxins

One aspect of the C system that has received consistent attention is the functions and mechanisms of action of C anaphylatoxins C3a and C5a, which are biologically active fragments derived from C molecules. These molecules are small polypeptides (less than 12 kDa) that are cleaved from large and abundant C components C3 and C5 during C activation and released into the fluid phase (Refs 4, 11). They are important pro-inflammatory molecules involved in the stimulation and chemotaxis of myeloid cells bearing specific anaphylatoxin receptors (C3aR and C5aR), and they mediate pro-inflammatory activities such as the release of lysosomal enzymes from leukocytes and the secretion of histamine from mast cells, as well as smooth muscle contraction and the chemoattraction of eosinophils and mast cells (Ref. 11).

C3a has been shown to regulate inflammatory functions by interacting with C3aR, which belongs to the rhodopsin family of seven-transmembrane, G-protein-coupled receptors (Refs 49, 50). C3aR was thought to be present only on myeloid cells such as macrophages, eosinophils and mast cells (Refs 51, 52, 53). However, the demonstration that C3aR mRNA is expressed throughout the body, and particularly in the adrenal gland, pituitary and CNS, is consistent with C3aR having a much broader role in the pathogenesis of inflammatory and autoimmune diseases than was previously suspected (Refs 50, 54, 55).

Several investigators have found a close relationship between elevated plasma levels of C3a and inactivated C3a (known as C3adesArg) in patients with septic shock and the risk of developing either adult respiratory distress syndrome or multiorgan failure (Refs 56, 57, 58). Although the accepted wisdom has been that C3a participates positively in inflammatory reactions (Ref. 59), recent reports have strongly suggested that C3a can also exhibit anti-inflammatory properties by suppressing LPS-induced secretion of TNF-α, interleukin 1β (IL-1β) and IL-6 from isolated peripheral blood mononuclear cells (PBMCs), and can attenuate TNF-α and IL-6 secretion from lymphocytes (Refs 60, 61, 62, 63). Furthermore, in an elegant and pioneering study, the genetic deletion of C3aR in mice demonstrated an important protective role for C3aR in endotoxin shock, notably by attenuating LPS-induced production of pro-inflammatory cytokines (Ref. 64). The role of C3a and C5a in neurodegeneration is discussed below.

Role of the membranolytic terminal complex and C inhibitors

The end-point of activation of the classical, alternative and lectin pathways is the formation of a membrane attack complex (MAC; also called the C5b-9 complex as it involves C5, C6, C7, C8 and C9 components) (Fig. 3). This disrupts and forms a lytic pore (hole) in the phospholipid bilayer of the target cell, through which the cell contents leak, leading to cell death. Activation of this terminal pathway of the C system at an inappropriate site and/or to an inappropriate extent is remarkably effective at damaging host tissues and causing pathology, as seen in degenerative disorders of the CNS such as multiple sclerosis. To avoid this self-destructive tendency, host cells are protected by a battery of regulatory molecules (C inhibitors), which inhibit assembly of either the C3-cleaving enzymes or the formation of the MAC (Fig. 3). C1 inhibitor (C1-INH), C4b-binding protein (C4bp), factor H (fH), factor I (fI), S protein (Sp) and clusterin are all soluble C inhibitors that are secreted and released in the fluid phase. The other C inhibitors are expressed on the cell membrane and include CR1, membrane cofactor protein (MCP; CD46), decay accelerating factor (DAF; CD55) and CD59 (Ref. 65).

Expression of the innate C system by glial and neuronal cells

In 1987, Levi-Strauss and Mallat were the first to demonstrate that brain cells were capable of producing C (Ref. 66). They showed that cultured rodent astrocyte cell lines and primary murine astrocytes produced C3 and fB and that the expression of C was increased after stimulation with LPS. The astrocyte is the most abundant glial cell type and, at that time, was thought to have a predominantly structural role, making this an

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unexpected finding. However, in the past decade, these reports have been extended to include astrocytes, microglia, neurons and oligodendrocytes (reviewed in Ref. 4). Primary cultures and cell lines of human origin were used to show that glial cells and neurons in vitro were capable of producing almost all C proteins, particularly after stimulation with cytokines. Interferon γ (IFN-γ) was the most effective cytokine at upregulating the expression of almost all C proteins by glial and neuronal cells. By contrast, TNF-α and IL-1β were shown to upregulate mainly C3, C2 and fB synthesis.

From these studies, it was proposed that brain cells, after appropriate stimulation 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 (Refs 67, 68, 69). Moreover, there is now considerable evidence that local expression of C by resident cells can be dramatically increased following brain infection. The level of C mRNAs was found to be significantly increased in human brains following meningitis (Ref. 70) and in experimental models of brain infection and inflammation such as scrapie and encephalitis (Refs 25, 71, 72).

Role of the C system in the CNS: clinical implications

Susceptibility of brain cells to C and the protective role of C inhibitors

Most nucleated cells can express various C inhibitors (see above) to control C activation on their membranes. In 1989, Scolding and colleagues (Refs 73, 74) made the first observation that brain cells were extremely susceptible to C lysis. It was demonstrated that antibody-independent C activation occurs in vitro at the oligodendrocyte cell membrane, whereas O-2A oligodendrocyte progenitors and type I and II astrocytes remained unaffected (Refs 73, 74). C activation was taking place through the classical pathway and further studies have demonstrated that rat oligodendrocytes lack the major inhibitor of C lysis, CD59 (Ref. 75). The situation in humans appears to be different with respect to the C susceptibility of brain cells. Human oligodendrocytes and human oligodendroglioma cell lines have been shown to express abundant levels of C inhibitors (particularly CD59) and fail to activate the C system spontaneously (Refs 76, 77, 78). Human astrocytes and microglia, from either primary cultures or cell lines, express several membrane (CD59>MCP$\gg$DAF) and soluble (fH, fI, C1-INH, Sp and clusterin, but not C4bp) C inhibitors and are well protected against C killing (reviewed in Ref. 79). In addition, there is no evidence that astrocytes and microglia activate the C system.

In contrast to oligodendrocytes, human neurons are extremely susceptible to killing by homologous C. Indeed, human foetal neurons and neuroblastoma cultured in the presence of human serum as a source of C are rapidly lysed via MAC formation on their membranes (Refs 80, 70, 81, 82). C1q binds specifically to the membrane of neurons and leads to activation of the classical pathway in an antibody-independent manner. C1q might bind to an as-yet-uncharacterised neuronal ‘C1q receptor’. Furthermore, neurons and neuroblastoma cell lines seem to be particularly susceptible to C-mediated lysis because they express low levels of C inhibitors (CD59, MCP, C1-INH, fH) and lack DAF (Refs 81, 83).

Thus, with the exception of neurons, it seems that human brain cells are relatively well protected from C-mediated lysis by expressing membrane-bound and soluble C inhibitors. Other recent investigations (Refs 84, 85) using immunohistochemistry, ISH and RT-PCR have confirmed that C inhibitors (membrane and soluble forms) are constitutively expressed in vivo by glial, neuronal and endothelial cells in the CNS, albeit at a low level compared, for example, with the level in the placenta or kidney. The immunostaining for CD59, MCP and DAF was stronger on microglia compared with astrocytes, whereas neurons were found to express CD59 and MCP weakly and to lack DAF. The expression of soluble C inhibitors (e.g. C1-INH and clusterin mRNAs) was also demonstrated by RT-PCR analysis of normal brain tissues (Refs 84, 85).

Overall, it is clear that brain cells can generate a C system to kill pathogens and yet be relatively well protected from direct or bystander C lysis through expression of 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

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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 in the brain

As mentioned above, the liver is regarded as the principal source of C proteins to be released in the serum. In neurodegenerative disorders such as AD, there is clear evidence that the BBB 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 in vitro work showing that glial cells and neuronal cells can synthesise C components (Ref. 4). Early studies used RT-PCR analysis to measure the level of C mRNAs in AD, HD and normal age-matched brains (reviewed in Ref. 4). 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 and midtemporal gyrus in AD, and caudate in HD). In AD, the level of C1q mRNA was increased from 11–80-fold when compared with normal brain. The levels of C3, C4 and C9 mRNAs were also found to be upregulated in AD (Ref. 67) and the levels of C3 and C4 mRNAs were increased in HD caudate compared with the temporal lobe. Surprisingly, immunohistochemical and ISH analysis indicated that not only were reactive glial cells abundant sources of C (microglia≫astrocytes), but so were neurons.

It has been postulated that pro-inflammatory cytokines (e.g IL-1β and TNF-α) expressed in neurodegenerative disorders constitute a driving force in stimulating local C biosynthesis by resident cells (Ref. 4). RT-PCR, ISH and immunohistochemistry have indicated that the levels of C inhibitors are barely increased in neurodegenerative disorders, suggesting that brain cells would be highly susceptible to damage by increased local C biosynthesis (reviewed in Ref. 4). Moreover, there is now considerable evidence that C is synthesised and activated in the brain of several animal models of neurodegenerative diseases [e.g. amyloid precursor protein (APP)-transgenic mice and stroke models] (Refs 26, 86). Together, these data indicate that a full C system can be generated in situ to promote an innate immune response primarily involved in the safe clearance of toxic cell debris. If uncontrolled, C proteins might also contribute to cytotoxic and cytolytic activities against neurons.

Role of C1q-binding molecules and C activation in neurodegeneration

AD is the commonest cause of dementia and is a multifactorial syndrome rather than a single disease. Senile (neuritic) plaques and neurofibrillary tangles (NFTs) comprise the major neuropathological lesions, particularly in limbic and association cortices (reviewed in Refs 86, 87). Neuritic plaques contain extracellular deposits of βA4 as 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 βA4 in a β-sheet conformation. Plaques contain variable numbers of activated microglia as well as reactive astrocytes surrounding the core. Immunohistochemistry using antibodies against βA4 reveals an even larger number of thioflavine-negative plaques in AD brains; these plaques seem to lack microglia, astrocytes and dystrophic neurites. They are referred to as diffuse plaques and are exclusively composed of the highly amyloidogenic 42 amino acid form of the βA4 peptide.

Several groups have clearly demonstrated the presence of C proteins in senile amyloid plaques and NFTs in AD brains using immunohistochemical techniques (for comprehensive review see Ref. 4). By contrast, immunohistochemical staining for two alternative pathway proteins, fB and properdin, has not been observed in the AD brain. Interestingly, C1q immunostaining was co-localised to nearly all neuritic plaques, whereas no staining was detected in diffuse plaques (Ref. 88). It has since been shown in vitro that C1q can bind directly to fibrillar but not soluble βA4, resulting in activation of the classical pathway as seen in AD brains (Table 1). Thus, it appears that conversion from the nonfibrillar diffuse plaques correlates with the initiation of C activation. There is now some debate as to whether C1q binds to βA4 through its collagen stalk (Refs 89, 90) or by one of its globular heads (Fig. 4), as recently demonstrated using purified C1 components (Ref. 91).

Other molecules associated with AD lesions such as SAP and CRP are known to interact with the collagen part of C1q and could also contribute to activation of the C cascade (Fig. 4). NFTs were

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also immunopositive for C1q, C3 and C4, but not for fB and properdin. The mechanism involved in the activation of the classical pathway on NFTs remains unknown but it is possible that NFTs express a 'C1q receptor', allowing C1q binding and initiation of the classical pathway (Ref. 4).

C activation has also been detected in other human neurodegenerative disorders: HD and Pick's disease (PiD) (Refs 92, 93). HD is an autosomal dominant inherited neurodegenerative disease and the gene associated with the disease encodes a mutant protein named huntingtin, which has expanded polyglutamine repeats compared with its wild-type counterpart (Ref. 94). The neuropathological hallmark of HD is atrophy of the caudate nucleus with a profound loss of neurons in the putamen accompanied by reactive gliosis (loss of astrocytes and microglia). It has been shown that neurons, myelin and astrocytes in HD brains stain strongly with antibodies to C1q, C4, C3, iC3b-neoepitope and C9 neoepitope (Ref. 4). C activation takes place via the classical pathway on neurons and astrocytes, both of which express huntingtin. By analogy with the role of βA4 fibrils in initiating the classical pathway in AD, we have proposed that mutant huntingtin with a long glutamine stretch could be involved in C activation in HD caudate (Ref. 4). Moreover, since mutant huntingtin is involved in apoptosis of neurons, and since C1q can bind directly to the surface of apoptotic cells (Ref. 19), 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 could be tested using an

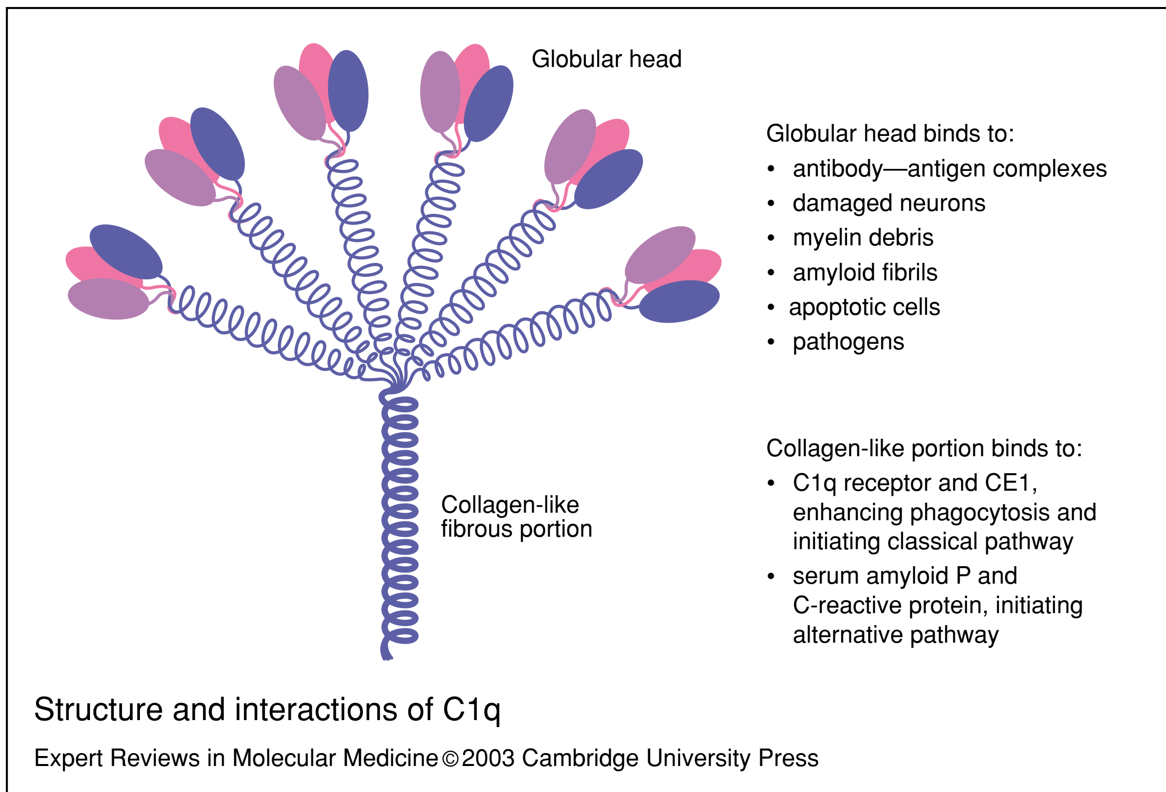

Figure 4. Structure and interactions of C1q. C1q is a key component of the classical complement pathway and acts as a recognition molecule that interacts with antibody–antigen complexes or nonimmune molecules to activate the complement cascade. C1q consists of six subunits, each with a collagen-like fibrous portion at its N-terminal and a globular head that is involved in binding to diverse targets. It has recently been determined that C1q may also opsonise damaged and dying cells, amyloid fibrils and myelin proteins, leading to their recognition and clearance **(fig004pgc)**.

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in vitro model of neurons hyperexpressing mutant huntingtin.

The histological hallmark of PiD is the neuronal Pick body, which strongly expresses aggregated forms of the microtubule-associated protein tau and ubiquitin. Neuronal loss and gliosis occur in the areas of disease that appear to be restricted to frontal and temporal lobes. Although it has been reported that Pick bodies stain strongly with antibodies to the MAC (Ref. 93), other data demonstrate strong staining for components of the classical pathway but little or no evidence for MAC (Ref. 92). Thus, the mechanism underlying the activation of the classical pathway in PiD is currently uncharacterised. 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. For instance, it is well known that C can be activated by a variety of intracellular components released by necrosed cells (e.g. nucleic acids, intermediate filaments, mitochondrial membranes) (reviewed in Ref. 18). It should also be stressed that neurons, at least in culture, seem to have a natural propensity to activate the C system spontaneously (see above). The hypothesis that the putative neuronal 'C1q receptor' mediates C activation on neurons, Pick bodies and NFTs is attractive and worthy of consideration.

Role of C1q receptors and other opsonins in neurodegeneration

The obvious and well-defined role of C1q is to bind to immune complexes and 'nonself' membranes (i.e. pathogens) to initiate activation of the classical pathway leading to lysis of the cell by the MAC. However, C1q is also an important opsonin that specifically labels a target cell so that it can be recognised by macrophages bearing C1q receptors, either CR1 or the phagocytic C1q receptor C1qRp (Table 1). CR1 is mainly expressed by phagocytes in the CNS (Ref. 48), although foetal astrocytes and some astrocyte cell lines were found to express CR1 in vitro (Ref. 95). By contrast, microglia, but not astrocytes, express C1qRp (Ref. 43). Interestingly, the immunoreactivity of microglia for C1qRp was particularly prominent in HD and PiD compared with age-matched normal brains, indicating that this receptor may be involved in brain inflammation. However, the function of CR1 and C1qRp on microglia in neurodegeneration remains to be identified. It is possible that microglia and astrocytes in concert are involved in the clearance by phagocytosis of C1q-opsonised cells (i.e. neurons, NFTs, Pick bodies) as well as C1q-opsonised 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 work along this promising line is warranted.

The role of other C receptors (CR3 and CR4) in mediating phagocytosis of C-opsonised target cells should not be underestimated. Macrophages and microglia express both receptors and will phagocytose C3-opsonised targets. At least in vitro, it is now well established that C and CRs expressed by glial cells are involved in the efficient clearance of amyloid fibrils (Refs 96, 97) (Table 1).

Taken together, 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. Pioneering studies have recently shown that active or passive vaccination of a transgenic mouse hyperexpressing mutant human APP using the βA4 peptide caused accelerated clearance of neuritic amyloid plaques from the mouse brain and reduced the extent and progression of the AD-like pathology (Refs 98, 99). It was proposed that microglia expressing a high level of immunoglobulin Fc receptors were able to phagocytose the Ig–βA4 complex. The role of C was not investigated in this model although, from data discussed above, it is likely that increased C biosynthesis and classical pathway activation were taking place. Therefore, the possibility of glial cells expressing C receptors to phagocytose the opsonised antibody–amyloid complex coated with C is an interesting and attractive hypothesis that remains to be tested.

Role of anaphylatoxins in neurodegeneration

As discussed earlier, C3a and C5a are important mediators of pro-inflammatory reactions involving the stimulation and chemotaxis of myeloid cells. Although it was thought that only myeloid cells (including microglia) expressed C5aR, it has now been shown that astrocytes and neurons also express the receptor (Refs

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100, 101, 102, 103, 104, 105, 106) (Table 1). C5a is an important chemoattractant molecule and stimulates cells to express increased level of cytokines, chemokines, adhesion molecules and C components (reviewed in Ref. 57). 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 exacerbated pathology. Interestingly, it has recently been shown that human astrocyte cell lines stimulated with C5a produced increased levels of IL-6, while the level of IL-1, TNF-α and transforming growth factor β (TGF-β) remained unaffected (Ref. 105).

Unexpectedly, C5a has been reported to induce apoptosis of neurons in vitro. Farkas 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 (Ref. 107). This experiment has not yet been confirmed using primary neurons but nevertheless indicates that C5a could contribute directly to neuronal damage. Conversely, C5a was recently found to be mitogenic for human neuroblastoma cells, involving several signalling pathways (Ref. 106).

The human C3aR was cloned in 1996 by several groups (reviewed in Ref. 4), allowing the production of specific reagents for ISH and immunohistochemistry. The distribution of the C3aR in the CNS is similar to that of the C5aR. However, the role of C3a in tissue inflammation is less certain (Ref. 108). 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 a chemoattractant but only for mast cells and not for either macrophages or microglia. Heese and collaborators have shown that a human microglia cell line stimulated with C3a expressed de novo nerve growth factor (NGF), a molecule involved in neuronal growth (Ref. 109). Moreover, it has been shown recently that C3a induces the release of a neuroprotective astroglial factor that has yet to be identified (Ref. 110). These types of study need to be extended to other glial and neuronal cell cultures and should assess the expression of anti-inflammatory cytokines and growth factors following C3a stimulation.

Roles of C5b-9

The MAC is by definition involved in cytotoxic and cytolytic activities. Aside from these functions, at sublytic level, the MAC is also involved in cell stimulation and programmed cell death (reviewed in Refs 111, 112) (Table 1). It was initially established that cultured glial cells release phospholipid and generate arachidonic acid (AA) and AA-derived pro-inflammatory mediators such as leukotriene B_4 in response to sublytic levels of C5b-9 (Ref. 113). Since these effects were observed on cell lines, it remains unproven as to whether the same effects can be reproduced on primary cultures of either astroglial cells or neurons. Sublytic MAC has also been shown to stimulate endothelial cells to express increased levels of C regulators and to protect against secondary C attack (Ref. 114). Taken together, these data suggest that sublytic MAC could act as a stress signal to stimulate cells to express increased levels of C inhibitors. It will be interesting to test whether the same effects are observed on glial and neuronal cultures. In addition, it remains to be ascertained whether brain cells stimulated with sublytic MAC alter their expression of pro-inflammatory or immunosuppressive molecules.

Conclusion: control of C in diseases and therapeutic intervention

The studies described here implicate C activation in the initiation and/or exacerbation of inflammation and tissue injury in diseases of the CNS such as AD, HD and PiD. It will soon be possible to ascertain the exact role of the C system using knockout animals. Until then, given the reported increase in local C biosynthesis, together with the strong propensity of C to be activated locally, it would be surprising if C was not a contributing factor in the pathology of these neurodegenerative disorders. Hence, 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 occurring C inhibitors. Soluble CR1 has been used successfully to control C activation in animal models of CNS disorders (Refs 115, 116), but the use of this molecule in chronic disorders such as AD would not be possible, since it is expensive, must be administered systemically (thereby also affecting the C system in the rest of the body) 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 either to be delivered specifically to the brain (by the use of specific targeting moieties) or to be expressed

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in the brain (by gene therapy). The former strategy is part of a unique technology platform that has been developed by Adprotech (Little Chesterford, Cambridge, UK) and consists of fusing a C inhibitor to a small targeting moiety (peptide) (Ref. 117). In addition, it will be interesting to search for new drugs that might control the pro-inflammatory activities, if any, of C-derived fragments such as C3a and C5a in the brain. The use of specific receptor antagonists (either peptides or chemical drugs) could prove to be useful for this purpose. A combination of these therapeutic approaches with anti-inflammatory drugs such as indomethacin might be of clinical benefit in the treatment of neurodegeneration.

By contrast, it is important to stress that there is a growing body of evidence that C plays a beneficial role in the early phase of neuroinflammation to induce the clearance of toxic cell debris and apoptotic cells. The efficient scavenging of these debris by C is of paramount importance to drive successful tissue repair together with the release of growth factors by neighbouring glial cells stimulated by C anaphylatoxins. Hence, a very discrete balance exits between the beneficial and detrimental roles of C in CNS inflammation, and novel therapeutic avenues will need to take this paradigm of a 'double-edged sword' into consideration.

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Features associated with this article

Figures

Figure 1. The blood–brain barrier (BBB) (fig001pgc).
Figure 2. Cells involved in the innate immune response in the brain (fig002pgc).
Figure 3. Activation and regulation of the complement system (fig003pgc).
Figure 4. Structure and interactions of C1q (fig004pgc).

Table

Table 1. Role of opsonins, anaphylatoxins and C5b-9 in the brain (tab001pgc).

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