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ABSTRACT

The brain is considered to be an immune privileged site, because the blood-brain barrier limits entry of blood borne cells and proteins into the central nervous system (CNS). As a result, the detection and clearance of invading microorganisms and senescent cells as well as surplus neurotransmitters, aged and glycated proteins, in order to maintain a healthy environment for neuronal and glial cells, is largely confined to the innate immune system. In recent years it has become clear that many factors of innate immunity are expressed throughout the brain. Neuronal and glial cells express Toll like receptors as well as complement receptors, and virtually all complement components can be locally produced in the brain, often in response to injury or developmental cues. However, as inflammatory reactions could interfere with proper functioning of the brain, tight and fine tuned regulatory mechanisms are warranted. In age related diseases, such as Alzheimer's disease (AD), accumulating amyloid proteins elicit complement activation and a local, chronic inflammatory response that leads to attraction and activation of glial cells that, under such activation conditions, can produce neurotoxic substances, including pro-inflammatory cytokines and oxygen radicals. This process may be exacerbated by a disturbed balance between complement activators and complement regulatory proteins such as occurs in AD, as the local synthesis of these proteins is differentially regulated by pro-inflammatory cytokines. Much knowledge about the role of complement in neurodegenerative diseases has been derived from animal studies with transgenic overexpressing or knockout mice for specific complement factors or receptors. These studies have provided insight into the potential therapeutic use of complement regulators and complement receptor antagonists in chronic neurodegenerative diseases as well as in acute conditions, such as stroke. Interestingly, recent animal studies have also indicated that complement activation products are involved in brain development and synapse formation. Not only are these findings important for the understanding of how brain development and neural network formation is organized, it may also give insights into the role of complement in processes of neurodegeneration and neuroprotection in the injured

or aged and diseased adult central nervous system, and thus aid in identifying novel and specific targets for therapeutic intervention.

Keywords: Complement; brain; neurons; glia; neurodegeneration; neuroprotection

1. INTRODUCTION

1.1 Complement

Complement (C) is a major component of innate immunity, recognizing danger, as well as discriminating self from non-self (Ricklin *et al.*, 2010). The C system is best known for its role in the recognition and killing of pathogenic microbes. Activation of the C system, which consists of over 30 soluble and cell-associated factors, can occur through three pathways, each triggered by different types of agents. All three pathways lead to the assembly of C3 convertases that, in turn, can cleave C3 resulting in formation of C3b and C3a activation products. The larger C3b fragment, a major effector molecule of the C system, acts as an opsonin and, in addition, together with other factors can assemble the C5 convertase, which enables further activation of the C cascade ultimately leading to generation of the chemotactic C5a fragment and the formation of the terminal complement complex C5b-9, also called membrane attack complex (Figure 1). Thus, when the cascade is fully activated, C leads to assembly of the membrane attack complex (C5b-9; MAC) and lysis of invading microorganisms. However, if C5b-9 production is excessive or targeted to host cells, C5b-9 can induce host cell death. On the other hand, some C activation products also facilitate the generation of adaptive immune responses (Carroll, 2004;Erdei *et al.*, 2009), while other components contribute to the control of autoimmunity during the clearance of apoptotic cells (Sjoberg *et al.*, 2009;Fraser *et al.*, 2009;van Kooten *et al.*, 2008). Interestingly, sublytic amounts of C5b-9 on host cells may cause an influx of extracellular calcium that leads to activation and/or proliferation of the cells and resistance to induction of apoptosis (Cole and Morgan, 2003), further illustrating the diverse functions of this ancient pathway.

Binding of C1, a Ca^{2+}-dependent complex of the recognition unit C1q and a tetramer of the proenzymes C1r and C1s, to an activator is the initial event in classical pathway (CP) activation of C. Activators can be immune complexes, certain microbes, apoptotic cells, and other specific protein motifs, such as amyloid in a fibrillar beta sheet structure found in the plaques in brain from Alzheimer's disease (AD) patients.

Mannose-binding lectin (MBL) and ficolins (Ficolin-1,-2 and -3) bind to mannan and other carbohydrate moieties or acetylated moieties on microorganisms or dying cells initiating C activation, through the lectin pathway (LP). MBL and ficolins share homology with C1q and, like C1q, are associated with proenzymes, MBL-associated serine proteases (MASPs).

The alternative pathway (AP) is initiated by spontaneous hydrolysis of the internal thioester within C3, resulting in C3b-like C3 ("tick-over") or by recruitment of C3 by properdin bound to specific targets (Kemper and Hourcade, 2008). A range of microbial and also eukaryotic cell surfaces with a low sialic acid content allow AP C activation, whereas the inactivation of C3b by the C regulatory proteins (Creg) factor H (fH) and factor I (fI) is more efficient on surfaces rich in sialic acid (AUSTEN and Fearon, 1979). C3b generated by the CP or MP activation pathways can enlist the alternative pathway components, thereby amplifying the amount of downstream complement cascade events (Figure 1).

1.2 Complement in the brain

The brains of organisms with a well developed central nervous system are shielded by the blood-brain-barrier (BBB) with tight junction formations at three principal barrier sites i) the BBB formed by endothelial cells in the cerebral capillaries ii) the arachnoid barrier formed by the arachnoid multi-layered epithelium and iii) the blood-CSF barrier formed by the CSF-secreting choroid plexus epithelium. The integrity properties are further defined by BBB-associated cells including pericytes and astrocytes. Together these brain barriers efficiently prevent infiltration of circulating immune cells, such as B- and T lymphocytes, and minimize influx of plasma proteins as well as neuroexcitatory and neurotoxic substances from the blood (reviewed in (Abbott *et al.*, 2010)). The functions of immune surveillance and differentiation between "self" and "nonself" in non-CNS tissue, provided by neutrophils, dendritic cells, macrophages and natural killer cells in the periphery, are in the CNS attributed to resident glial cells including astrocytes, microglia, oligodendrocytes, and NG2 chondroitin sulphate (NG2) and platelet-derived growth factor-α

receptor (PDGFα) positive oligodendrocyte precursor cells ($NG2^+PDGF\alpha^+$ OPCs) ((Butt *et al.*, 2005) and reviewed in (Dong and Benveniste, 2001;Griffiths *et al.*, 2009)). Much of our understanding of the occurrence and role of complement in the CNS derives from studies into the pathogenic mechanisms involved in various diseases affecting the brain. In a variety of CNS diseases, including bacterial meningitis, transmissible spongiform encephalopathies (TSE; prion disease), stroke and in more chronic conditions such as multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), Huntington's (HD) and Parkinson's disease (PD), AD, as well as age-related macular degeneration (AMD), C contributes to the inflammatory process (Woodruff *et al.*, 2008;Bonifati and Kishore, 2007). In this review we discuss the findings that have led to insight on the role of C in acute and chronic brain disorders and, importantly, its role in the normal homeostasis and brain functioning, as more recent studies (Stevens *et al.*, 2007) indicate that C is also involved in brain development and synapse pruning.

2. Local Synthesis of Complement and Complement Regulators in the Brain

The liver is the main source of C proteins, but although the BBB is not absolute and some macromolecules can by-pass the barriers by use of extracellular routes (Broadwell and Sofroniew, 1993) most C proteins are unlikely to penetrate the brain parenchyma unless the BBB integrity is disrupted. Therefore, local synthesis of C components by resident cells in the brain is crucial to appropriate functions of the local defense system. Local synthesis of C proteins in the brain was suggested after identification of C in human brain tissue. In situ hybridization studies (Lampert-Etchells *et al.*, 1993;Rozovsky *et al.*, 1994;Veerhuis *et al.*, 1998) confirmed that C factors are all locally produced and that the presence of C is not merely due to leakage of plasma proteins because of BBB damage.

2.1 Glial cells

Astrocytes are the most numerous cell type in the human brain and are dedicated to various functions such as regulation of synaptogenesis, metabolic support and control of the homeostatic system including regulation of extracellular ion concentrations and neurotransmitters, especially glutamate, and regulation of brain water homeostasis (Verkhratsky and Parpura, 2010). Microglia, the macrophages of the brain, are scattered throughout the brain tissue at a density of about $6x10^6$ cells per mm^3 and, when in a "resting" state are highly dynamic and estimated to completely scan the brain parenchyma once every few hours (Nimmerjahn *et al.*, 2005). Microglia as well as astrocytes are considered CNS immune effector cells and are able to produce cytokines and chemokines as well as to phagocytose up targets upon stimulation (Dong and Benveniste, 2001;Nielsen *et al.*, 2010;Familian *et al.*, 2007;Nielsen *et al.*, 2009;Fraser *et al.*, 2010;Bohlson *et al.*, 2007;Yang *et al.*, 2010;Watabe *et al.*, 1989). Another group of macroglial cells are the oligodendrocytes, which are responsible for myelinating axons in the CNS.

More than two decades ago Levi-Strauss and Mallat reported that primary cultures of murine astrocytes were capable of producing C components of the AP (Levi-Strauss and Mallat, 1987). Extensive research during the 1990's, initially performed in several human astrocyte-derived tumor cell lines (118MG, T193, T98G), demonstrated the expression of components of the CP and also of terminal C components (Gasque *et al.*, 1995;Gasque *et al.*, 1993) by human glial cells. In further studies with cell lines and also primary cells isolated from mouse and adult human brain (Walker and McGeer, 1992;Veerhuis *et al.*, 1999;Walker *et al.*, 1995;Veerhuis *et al.*, 1998) convincing evidence was obtained for local synthesis of most C components of both the classical and alternative pathway, including Cregs, by human astrocytes and microglia (Table 1). What cell type that is responsible for local production of fluid-phase C inhibitor C4b binding protein (C4bp) has yet to be determined in the brain as detectable levels of secreted C4bp appeared to be absent in cultures of primary human astrocytes as well as several cell lines (Trouw *et al.*, 2008). Further, results from *in vitro* studies on primary human microglia and astrocytes suggest that synthesis of several C components, C1 subcomponents C1s and C1r, C3, C4 and C1-

inh can be modulated by various factors like pro-inflammatory cytokines but as well as by the AD-related amyloid-β peptide (Aβ) perhaps via TLR stimulation (Veerhuis *et al.*, 1999). The same study, in support of earlier investigations, also suggested that microglia, but not astrocytes, are a significant source of locally secreted C1q in the brain (Lampert-Etchells *et al.*, 1993;Veerhuis *et al.*, 1999).

Results from initial studies on rodent oligodendrocytes suggested that these cells are vulnerable to C lysis due to a deficiency of C inhibitor expression (Wren and Noble, 1989;Piddlesden *et al.*, 1994). Indeed, oligodendrocytes are susceptible to C attack which is particularly evident in multiple sclerosis (MS) (Schwab and McGeer, 2002). In one study, adult primary human oligodendrocyte cultures were found to produce only a limited Creg repertoire, which suggests that a relative deficiency in Creg expression may render oligodendrocytes sensitive to C damage in MS (Scolding *et al.*, 1998). Interestingly, oligodendrocytes seem to not only be susceptible to C attack but also to themselves be a source of a large number of C proteins including C1q, C1s, C4, C2, C3, C5, C6, C7, C8 and C9 (Hosokawa *et al.*, 2003). Little is known about C expression in various other cells in the CNS. However, initial studies suggest that primary human pericytes *in vitro* produce C1q (Verbeek *et al.*, 1999) and that cultured endothelial cells from human brain microvessels produce soluble regulators fH and C1-inh and components of both the classical (C4), and the alternative (fB) complement pathway (Vastag *et al.*, 1998). Ependymal cells, ciliated epithelial cells that line the lumen of the brain ventricular system, express Cregs CD59 and at a low level CD55, but no CD46 or CD35, however in inflammatory conditions (meningitis) CD46 and CD35 are highly expressed on epithelial cells of the ependymal lining, as well as in the choroid plexus (Canova *et al.*, 2006). To what extent these cell types contribute to the levels of C factors in the brain parenchyma and of the CSF is unknown. Thus, future studies extending the knowledge on C expression in various cells of the CNS are warranted.

2.2 Neurons

Neuronal cells have long been considered innocent victims of C activation in neurodegenerative conditions, as a result of activation of C factors that had passed the BBB or that had been synthesized by activated glial cells. Robust activated complement system with C5b-9 insertion can lead to lysis and death of a targeted cell, a process which can be prevented by appropriate expression of complement regulators. However, neuronal cells were also found to be capable of de novo synthesis of complement factors both *in vivo* and *in vitro*. Neuronal mRNA expression of C1q, C2, C3, C4, C5, C6, C7, C8 and C9 was minimally detected using in situ hybridization in the temporal cortex and hippocampus in post mortem control brain tissue, with increased expression in AD tissue. The strongest signals were recorded over pyramidal neurons (Shen *et al.*, 1997). Neuronal expression of C1-Inh was detectable in brain tissue from postmortem AD and control subjects as well as in the neuroblastoma cell line SK-N-SH (Veerhuis *et al.*, 1998). In vitro expression of C1-Inh could only be upregulated by treatment with interferon γ (Veerhuis *et al.*, 1998;Veerhuis *et al.*, 1999), whereas expression of the fluid-phase regulators, MCP and CD59 in human neuroblastoma cell lines could be modulated by treatment with pro-inflammatory cytokines (Gasque *et al.*, 1996). Extending those *in vitro* studies, Fontaine and colleagues showed that the above mentioned neuroblastoma cell lines and the human neuroblastoma cell lines SH-SY5Y and KELLY were able to express a complete set of C proteins and further suggested that the rate of synthesis was cell differentiation-dependent (Thomas *et al.*, 2000). Interestingly, primary fetal human neurons *in vitro* were shown to spontaneously and independent of antibody activate the CP, possibly by expressing a molecule with affinity for C1q, leading to assembly of the cytolytic C5b-9 on their membranes. Limited neuronal expression of Cregs MCP and CD59, and lack of DAF and CR1 expression was suggested to underlie this vulnerability to complement damage (Singhrao *et al.*, 2000). CD59 has been shown crucial to protection of for example NT2-N neurons (human NT2 cell line differentiated into post-mitotic neurons) against C attacks (Pedersen *et al.*, 2007) and current strategies aiming at increasing neuronal protection

against C include attempts of upregulation of Cregs like CD59. For example, the CD59 expression-regulating neural-restrictive silencer factor (REST) protected neurons from C-mediated lysis by a five-fold upregulation of CD59 expression in neuronal cultures (Kolev *et al.*, 2010). Whether modulation of neuronal production of Cregs is a successful neuroprotective strategy remains to be elucidated as recent *in vivo* studies suggest that C activation products, including the anaphylatoxins C3a and C5a and sublytic levels of the MAC, may in fact have several neuroprotective functions ((Osaka *et al.*, 1999;Tocco *et al.*, 1997;O'Barr *et al.*, 2001;Van Beek *et al.*, 2003) and reviewed in (Woodruff *et al.*, 2010).

3. The role of complement in normal CNS

Similar to other proteins that are part of the immune system, such as proinflammatory cytokines (e.g., TNFα, IL-6) and proteins of the adaptive immune system (e.g. major histocompatibility complex class I [MHCI] molecules and MHCI-binding immunoreceptors and their components (e.g., PIRB, Ly49, DAP12, CD3ζ) (for a review see (Boulanger, 2009)), C factors are now thought to also have nonimmune functions in the brain. Complement proteins were found to promote proliferation and regeneration in various tissues (reviewed in (Ricklin *et al.*, 2010)) and may exert similar functions in the CNS, as neuronal stem cells differentiate and migrate in response to C. C3a-C3aR interactions were found to be a positive regulator of adult neurogenesis (Bogestal *et al.*, 2007;Shinjyo *et al.*, 2009).

Recent studies have also shown that C activation products can modulate synapse formation during brain development (Stevens *et al.*, 2007;Chu *et al.*, 2010). Neurons isolated from the developing eye were found to express high levels of C1q mRNA. Using C1q and C3 knock-out mice, it was shown that whereas relay neurons in wild type (wt) mice are innervated by one or two axons, relay neurons in C deficient (C1q -/- , C3 -/-) mice have four or more functional inputs. This lead to the conclusion that C1q and C3 may tag synapses for elimination, leading to remodelling of synaptic connections in the developing visual system (Stevens *et al.*,

2007). In a subsequent study, a complete genetic deficiency of C1q resulted in enhanced circuitry that led to epileptogenesis in mouse models (Chu *et al.*, 2010). C1q, both alone and in conjunction with C3, can facilitate microglial clearance of misfolded proteins, apoptotic neurons and damaged cells such as neuronal blebs (Fraser *et al.*, 2010;Trouw *et al.*, 2008) and modulate cytokine profiles to subdue potentially neurotoxic inflammatory gene expression (Fraser *et al.*, 2010). Thus, depending on the timing and local environment, the C cascade can facilitate proper neuronal development or accelerate chronic inflammatory response contributing to neurodegeneration (see below).

Proteins related to C1q, cerebellins (Cbln) (Yuzaki, 2010) and C1ql (Bolliger *et al.*, 2011) have been found to be expressed in the cerebellum, as well as other brain regions of developing and mature brain. Cbln members may serve as regulators of synapse development and synaptic plasticity through regulation of the post synaptic endocytosis pathway of AMPA receptors (Yuzaki, 2008), and C1ql proteins have recently been shown to interact with a neuronal surface receptor BAI3 also involved in regulation of synapse formation and/or maintenance (Bolliger *et al.*, 2011). As C1q is expressed by neurons in the hippocampus and temporal cortex ((Afagh *et al.*, 1996;Rozovsky *et al.*, 1994) and Veerhuis, unpublished, and reviewed in (Alexander *et al.*, 2008)) of injured brain, it is tempting to speculate that C1q in the neocortex may serve a function similar to that of the Cbln proteins in the cerebellum. In addition, *in vitro* C1q enhances neuronal survival and is neuroprotective in response to certain toxic agents, such as fibrillar amyloid and serum amyloid P (Pisalyaput and Tenner, 2008). Whether these BAI3-C1ql interactions are influenced by C1q itself (which has been shown to influence neuron survival and neurite outgrowth in vitro (Benoit and Tenner, 2011;Pisalyaput and Tenner, 2008)) remains to be seen. Interestingly, half of more than 50 genes encoding putative Cregs predicted in the mouse genome, are expressed in the CNS, consistent with at least some of the uncharacterized C control protein domain (CCP)-bearing proteins in mammals may be involved in synapse organization (Gendrel *et al.*, 2009).

4. Complement during acute brain injury

Acute brain injuries including infections, brain trauma, ischemic and hemorrhagic stroke and subsequent reperfusion injuries are to date associated with a limited repertoire of effective treatments and high morbidity. Neurodegeneration and death in these acute conditions can be via necrosis or via apoptosis. For example, apoptosis was recently shown to be dominant in the peri-infarct area after ischemic stroke in humans (Sairanen *et al.*, 2006). Most likely neuronal death following acute conditions occurs via a combination of both necrosis and apoptosis.

4.1 Brain infections

Various pathogens including bacteria, virus and fungi can invade the CNS and cause life-threatening diseases. In the immune privileged brain C functionality can be crucial to fight off and kill invading microbes. However, C activation and regulation needs to be delicately balanced as excessive C activation might be detrimental to bystander cells. Intriguingly, several CNS invading microorganisms have developed mechanisms to avoid the destructive actions of C and in fact even to use C to their advantage. One of these mechanisms is mimicry of human Cregs which enables control and down-regulation of C activation against invading microbes (Cooper and Nemerow, 1989). Further strategies to circumvent C include the use of membrane-bound C receptors and Cregs to enter the host cell and acquisition of Cregs during budding from the membranes of the host cell or by binding to soluble Cregs (reviewed in (Speth *et al.*, 2002)). For example the meningitis causing bacteria *Neisseria meningitidis* invades the CNS through the nasopharyngeal mucosa and uses the membrane bound Creg CD46 which interacts with bacterial pili, to cross the blood-brain-barrier (Johansson *et al.*, 2003). Also, gram-negative *Escherichia coli K1* avoids C killing by binding to C4bp and promoting degradation of C3b and C4b (Wooster *et al.*, 2006). Similar to bacteria, several virus strains have developed protective strategies to avoid C (recently reviewed in (Stoermer and Morrison, 2011)) . The herpes virus Epstein-Barr

(EBV), which can cause encephalitis and aseptic meningitis, uses CR2 for viral entry by binding to the receptor at the same location as the C3 fragment C3dg (Carel *et al.*, 1989). HIV-1, detectable in the brains of >85% patients who died with AIDS (Johnson *et al.*, 1996), acquires Cregs CD46, CD55 and CD59 upon budding from the host cell (Frank *et al.*, 1996) and binding to soluble Creg fH (Stoiber *et al.*, 1995) thereby avoiding C mediated lysis of the virion particles. In addition, recent studies indicate that CNS invading fungi also have developed C evasion mechanisms (reviewed in Speth and colleagues (Speth *et al.*, 2008)). Although resident brain cells including astrocytes, neurons, oligodendrocytes, but to a lesser extent microglia, produce highly increased levels of C1q, C4 and C3 in response to fungus infection, as in the case of cerebral aspergillosis, fungal hyphae can limit surface deposition of C3 and thereby interfere with C-mediated phagocytosis of this pathogen (Rambach *et al.*, 2008;Speth *et al.*, 2008). Taken together, these examples illustrate that C plays various roles in brain infections and that the C evasion strategies by microbial pathogens invading the CNS may be a target for therapeutic intervention.

4.2 Trauma, stroke and reperfusion injuries

The diverse roles played by the C system in acute brain disorders are not fully elucidated, however a growing body of evidence suggests an important role in secondary brain damage (Stahel *et al.*, 1998). During some conditions of acute brain damage the BBB integrity is disrupted allowing influx of plasma proteins, including C proteins, and immune cells from the periphery, whereas in others CNS injuries C synthesis is induced by CNS insults (including oxidative stress). Activation of the CP in human brain following traumatic brain injury has been shown by increased immunoreactivity for C1q, C3b, C3d and C5b-9in the immediate vicinity of neurons in the penumbra area of the cerebral contusion (Bellander *et al.*, 2001). Further, C3 mRNA and upregulation of clusterin was found in the penumbra, indicating local de novo synthesis of C and Cregs following injury. The authors suggested that an unknown component,

possibly in the debris from injured neurons or myelin breakdown products, might be able to trigger C activation and formation of the following brain contusions. Investigation of brain tissue of patients with acute brain ischaemia or ischaemic stroke further revealed deposition of C1q, C3c and C4d in all ischaemic lesions, further supporting activation of the CP. In necrotic zones of the brains from the same patients, C9, C-reactive protein and IgM were found. The possibility of uncontrolled C activation following ischaemic insults, which might be harmful was underlined by the findings of virtually absent CD59 and CD55 in ischaemic lesions (Pedersen *et al.*, 2009). Consistent with a detrimental role of a fully activated C cascade, in an animal model of traumatic brain injury the C5a receptor antagonist (CD88-specific) was shown to reduce disease activity (Sewell *et al.*, 2004) suggesting that the generation of the chemotactic C5a activation fragment contributes to the detrimental consequences of C activation in this model.

4.3 Role of C5a in Neuroinflammation

The role of the activation fragment C5a in the brain has recently been reviewed (Woodruff *et al.*, 2010) and thus will not be extensively discussed here. However, it should be noted that seemingly contradictory results of the influence of the C component C5 on inflammation have been reported. In contrast to the detrimental effect of C5a mentioned above in the traumatic brain injury models (and below in chronic neurodegenerative disorders), C5a, when given with kainic acid intraventricularly or 24 hours prior to glutamate treatment in neuronal mouse cultures, was shown to be neuroprotective against glutamate mediated caspase-3 activation (Osaka *et al.*, 1999). It was subsequently hypothesized that the C5a mediated protection may be dependent on the modulation of Ca^{2+} and MAP-kinase activity (Mukherjee and Pasinetti, 2000;Mukherjee and Pasinetti, 2001). In other systems, C5a, as well as C3a, provided direct neuroprotection (Van Beek *et al.*, 2001;Mukherjee and Pasinetti, 2001;O'Barr *et al.*, 2001); however, these were cell lines and/or neurons perhaps at different stages of maturation which may align with the studies of Fontaine and colleagues in newborn rat brain. These researchers

demonstrate that in the developing cerebellar cortex brain, C5aR stimulation triggered increased BrdU incorporation by granule neurons, and a C3aR agonist promoted migration of cells to their proper location (Jauneau *et al.*, 2006;Benard *et al.*, 2004;Benard *et al.*, 2008). However, since defects in cerebellum have not been reported in C3, C3aR, C5 or C5aR deficient animals, further study will be necessary to determine if these systems are redundant, residual or involved in facilitating survival and development during infection. The underlying basis for the differences in outcome due to C5a/C3a engagement of their receptors are likely different differentiation states of the cells and/or the cell signaling resulting from mixed cell interaction. Another example of the complexity of these responses is the report that C5a (but not C3a) upregulates expression of microglial (but not astrocyte) glutamate receptor (GLT-1) which should provide increased glutamate uptake and thus protect neurons in the environment against glutamate toxicity (Humayun *et al.*, 2009). Since there are two C5aR (CD88 and C5L2) and there are suggestions that these receptors may "cooperate" with other receptors (reviewed in (Klos *et al.*, 2009), it is possible that a diverse, but precise, set of responses to a changing environment could be orchestrated depending on the repertoire of interacting receptors available in the sensing cell. Clearly a systematic approach using carefully characterized reagents with defined cells and differentiation states is needed to clarify these pathways and identify potential targets for therapeutic interventions.

5. Complement during chronic conditions of brain injury

Substantial advances in understanding the effects of C in the brain comes from research in neurodegenerative diseases (ND) and subsequent studies with animal models for ND including C knock outs or genetically manipulated animals over expressing certain C factors bred to AD mouse models. Here we will focus on only a few diseases that demonstrate some of the mechanisms of disease acceleration and begin to provide insight on potential therapeutic targets. Recent reviews of the pathogenesis of age related macular degeneration (Charbel *et al.*, 2010), as

well as contributions of C to systemic lupus erythematosus and spinal cord injury (Alexander *et al.*, 2008), and prion disease (Veerhuis *et al.*, 2005) provide additional examples of a substantial role of C in neurodegenerative disease.

5.1 Complement in AD

Characteristic neuropathological changes seen in AD brain include synaptic and neuronal loss, neurofibrillary tangles (NFTs), extracellular senile plaques composed of amyloid (Aß) protein deposits and evidence of inflammatory events (Querfurth and LaFerla, 2010). The relative contributions of these pathological markers to the cognitive dysfunction in AD remains controversial, but results from studies in both AD patients and transgenic mouse models of AD make it likely that multiple, overlapping processes contribute to the ultimate cognitive loss in this disorder. Evidence of neuroinflammation as a substantial component in the development of AD has been accumulating since the 1990's and immune activation in the brain has been identified as a potential target for therapeutic intervention ((Craft *et al.*, 2006;Hu *et al.*, 2007) and reviewed in (Shaftel *et al.*, 2008;Eikelenboom *et al.*, 2011)), although the presence of beneficial as well as detrimental effects requires care in selection of targets (Lucin and Wyss-Coray, 2009;Gasparini *et al.*, 2005).

The association of C factors with amyloid deposits in Alzheimer's disease (AD) was first described in immunohistochemical studies in the early '80s (Eikelenboom and Stam, 1982;Ishii and Haga, 1984). The development of monoclonal antibodies improved the specific detection of C activation products and the use of component specific knockout mice to validate antibodies used in mouse models of neurodegeneration further strengthened the validity of the reports of C component association with fibrillar amyloid containing plaques, thus providing stronger evidence that amyloid plaques do activate complement in vivo, and suggesting a role for C in AD pathophysiology (Eikelenboom *et al.*, 1989;Fonseca *et al.*, 2004b;Rogers *et al.*, 1992;McGeer *et al.*, 1989).

Interestingly, when different neuropathological Braak stages, representing different stages of disease progression (from control to severe AD), are compared, C factors C1q, C4d and C3d were found in early AD stages in plaques, but later C factors such as C5b-9 were absent or much less prominent. In later AD stages along with more prominent immunostaining for C1q, C4d and C3d, and some C5b-9 is seen in neuritic plaques and on neurofibrillary tangles (NFT) (Fonseca *et al.*, 2004a;Veerhuis *et al.*, 2003;Webster *et al.*, 1997;Zanjani *et al.*, 2005;Veerhuis *et al.*, 1995), suggesting a major role for CP activation and C3. In a post mortem study comparing young, middle aged and old Down syndrome (DS) cases, as a temporal model for studying the development of AD, similar results were obtained as in AD brain (Head *et al.*, 2001;Stoltzner *et al.*, 2000). The observed prominent presence of earlier activation products and relative absence of C5b-9 (Stoltzner *et al.*, 2000;Zhan *et al.*, 1995;Eikelenboom and Veerhuis, 1996;Zanjani *et al.*, 2005) is in line with the results from a mouse study comparing APP23 Tg mice and wild type mice (Reichwald *et al.*, 2009), and with *in vitro* data, showing lower than expected levels of C5b-9 upon activation of the C cascade by Aß (Cadman and Puttfarcken, 1997). Alternatively, the C5b-9 may be cleared since it associates either with membranes, clusterin or vitronectin ("S Protein") (Itagaki *et al.*, 1994;McGeer *et al.*, 1992;Verbeek *et al.*, 1998) rather than becoming covalently linked to the more long lived plaque as occurs with C4b/d and C3b/d.

Strong immunostaining for C1q and C activation products C4b/c/d and C3b/c/d is observed in the majority of highly fibrillar, dense-cored and primitive neuritic plaques in the temporal cortex of AD cases (Loeffler *et al.*, 2008;Veerhuis *et al.*, 1996) and in mouse models of AD and/or neurodegeneration (Fan *et al.*, 2007;Zhou *et al.*, 2008;Loeffler *et al.*, 2008) (Figure 2). In contrast, the C1 subcomponents C1r and C1s are only occasionally observed in neuritic plaques in AD (Veerhuis *et al.*, 1996), undoubtedly due to their dissociation from the activator-bound C1q by C1-Inh (Ziccardi and Cooper, 1979). Some positive C immunostaining has been seen in thioflavine-negative, cognitively normal brains, (Lue *et al.*, 2001;Zanjani *et al.*, 2005) but to a far lesser extent (Zhan *et al.*, 1995). Further indications that C1q binding to Aß depends on the

degree of Aß fibril formation, came from an immunohistochemical study in a preclinical familial AD case with only diffuse Aß plaques, where in contrast to advanced AD cases, immunostaining for C1q was only seen in neurons (Fonseca *et al.*, 2004a). In vitro studies in which interactions of purified C1q and synthetic Aß peptides were investigated (Tacnet-Delorme *et al.*, 2001;Snyder *et al.*, 1994;Velazquez *et al.*, 1997), validated the activation of C by beta sheet amyloid fibrils and identified candidate amino acids on both the amyloid and the C1q molecule that are involved in the interaction (Velazquez *et al.*, 1997;Jiang *et al.*, 1994;Tacnet-Delorme *et al.*, 2001).

Predominantly CP C activation products were found to co-localize with most cerebral Aß deposits in AD brain, as well as extracellular neuronal tangles, although AP components have been found associated with amyloid plaques in both human AD ((Strohmeyer *et al.*, 2000) and reviewed in (Veerhuis, 2011)) and in murine models of AD (Fonseca *et al.*, 2011). Additional *in vitro* studies have shown that Aß can activate C via the AP pathway ((Bradt *et al.*, 1998), reviewed in (Alexander *et al.*, 2008;Veerhuis, 2011)). In addition, in C1q-/- AD mouse models, while there was essentially no CP deposition, cleaved C3 products and properdin were prominently present on the fibrillar amyloid plaques (Zhou *et al.*, 2008;Fonseca *et al.*, 2011).

The observed presence of CP products up to iC3b, and limited further C activation (C5b-9 and AP amplification loop) seen in AD, could be due to the presence of Cregs fH (Strohmeyer *et al.*, 2002) and C4bp (Trouw *et al.*, 2008;Zhan *et al.*, 1995) that accumulate in Aß deposits associated with C activation and covalently bound C4b and C3b. Factor H and C4bp enhance the conversion of C3b into iC3b, thereby preventing further C activation and enhancing Aß uptake by microglia via CR3 and CR4 (Sjoberg *et al.*, 2009;Strohmeyer *et al.*, 2002). Clusters of activated microglia that express the ß2-integrin C receptors CR3 and CR4, can be found surrounding fibrillar amyloid plaques (Rozemuller *et al.*, 1989;Akiyama and McGeer, 1990;Kobayashi *et al.*, 1998) suggesting that these phagocytes may be trying to ingest complement-tagged plaque material.

However, clearly the role of the C system in AD pathogenesis and progression is complex, as in animal models both C-dependent detrimental and protective effects have been observed. When AD mouse models were made C3 deficient or overexpressing Crry, pathology was enhanced relative to the C3 sufficient mice or to mice with normal levels of Crry suggesting a protective contribution of C3 (Wyss-Coray *et al.*, 2002;Maier *et al.*, 2008). Enhanced pathology in these mice was likely due to the loss of the opsonic effect of C3b for amyloid and/or cellular debris. This protective role of early components of C is also consistent with the recent report demonstrating a correlation between the induction of C1q and C3 and the suppression of Aß deposition in the TgCRND8 AD mouse model (Chakrabarty *et al.*, 2010). However, deletion of C1q in the Tg2576 and APPPS1 models of AD suggested a detrimental role for C activation since the Tg2576C1q-/- and APPPS1C1q-/- mice showed less reactive glia surrounding plaques and increased synaptophysin than the C1q-sufficient Tg2576 or APPPS1 (Fonseca *et al.*, 2004b). The protection given by the lack of C1q was substantial (~50%) but not complete, suggesting that the AP and/or other non C mediated events contribute to the inflammatory reaction around the plaques. Consistent with a role for C in contributing to the rate of progression of the disease, the development of pathology was accelerated in the 3xTg AD mouse model on BUB background (a strain with higher serum hemolytic activity *in vitro*) (Fonseca *et al.*, 2011) .

Perhaps the most compelling evidence that cleavage of C5 plays a substantial detrimental role in AD progression was the decrease in pathology and the suppression of behavioral deficits in AD mice treated with a C5a receptor antagonist, PMX205 (Fonseca *et al.*, 2009). It has been demonstrated that receptors for C5a are expressed in the brain (recently reviewed in (Klos *et al.*, 2009)) and that CNS cells do respond to C5a (Sayah *et al.*, 2003). The genetic deficiency of C5 has been shown to be one of a limited number of genetic differences that are associated with decreased amyloid deposition in DBA/2J mice vs. C57Bl6 mice transgenic for the human APP gene (Ryman *et al.*, 2008) and more recently, the AD mouse model 3xTg was shown to lack pathology when crossed onto the C5-deficient FVB strain for 6 generations (Morrissette, 2009).

Recent studies have demonstrated that C5a-C5aR signaling synergizes with other receptor signaling, including TLR (Zhang *et al.*, 2007) and P2Y6 (Flaherty *et al.*, 2008), in multiple tissues including the brain. Some of these receptors, specifically TLR2 and TLR4 (Jana *et al.*, 2008;Jin *et al.*, 2008;Udan *et al.*, 2008) have been shown to mediate detrimental effects of ß-amyloid. Thus, in addition to recruiting glia to the site of plaque deposition, inflammation generated in response to Aß interactions (or other proinflammatory signals) with receptors on C5a-recruited glial cells (Tahara *et al.*, 2006) may be significantly enhanced by the binding of C5a to C5aR, thus accelerating pathology and/or neuronal dysfunction. Thus, developing inhibitors of C5a activation of myeloid cells, such as receptor antagonists of C5a, should be further investigated as a therapeutic strategy in human AD as this would specifically inhibit the detrimental consequences of complete activation of the C cascade but leave the beneficial effects of C1q and C3 intact.

Finally, recent reports of the potential polymorphisms in CR1 and clusterin associated with human AD also suggests a point of control of C activation (Lambert *et al.*, 2009). While CR1 is a critical regulator of C3 convertase activity in humans, it is expressed predominantly in the periphery. Clusterin (Apo J) is a soluble inhibitor highly expressed in brain. How these regulators influence disease progression remains to be investigated.

5.2 Complement in other dementias and neurodegenerative diseases

Although in Parkinson's disease (PD) C activation was described to be associated with Lewy bodies (the intraneuronal inclusion bodies consisting of aggregates of α-synuclein) as well as axonal spheroids in the substantia nigra (Yamada *et al.*, 1992), no indications for C activation were found in another study, investigating cortical Lewy bodies in the cingulated gyrus (Rozemuller *et al.*, 2000). Whether this is due to region specific forms of α-synuclein, or to regional differences in expression of C proteins and Cregs, remains to be determined. In Pick's disease neuronal inclusions can be found in the frontal and temporal cortex. Pick's bodies

consisting of filaments of tau protein and can evoke inflammatory reactions and C activation. Complement activation products including C1q, C4, C2, C3, C5, C6, C8, but not C9 or C5b-9 were seen co-localized with astrocytes, cytoplasmic ballooned neurons and Pick bodies. Fluid phase Cregs vitronectin and clusterin, as well as the membrane bound regulatory protein CD59, but not other Cregs as CR1, DAF and MCP, were also found at these sites, suggesting sufficient protection to TCC mediated cell lysis. The intracellular localization of many C factors in ballooned neurons and the Pick bodies, was suggested to be caused by the internalization C targeted cell membranes (Singhrao *et al.*, 1996). While animal models of these diseases are far from perfect, the reports of disease reduction (pathology and in some cases behavior) by treatment with C5a receptor antagonist of models of amyotrophic lateral sclerosis (ALS) (Woodruff *et al.*, 2008) and Huntington-like neurodegeneration (Woodruff *et al.*, 2006) warrant further investigation.

In other neurodegenerative diseases such as in familial British and Danish chromosome 13 dementia cases, termed ABri and ADan respectively, amyloid deposits were immunopositive for CP activation products C1q, C4d and also C5b-9 (Rostagno *et al.*, 2002). When aggregated synthetic ABri and ADan, the peptides that form cerebral amyloid deposits in chromosome 13 dementia, were incubated with human serum, sC5b-9 was generated of which 25% could be attributed to AP activation (Rostagno *et al.*, 2002)**.** Taken together these findings suggest that C activation can be a general reaction to a number of proteins of different etiology that form highly fibrillar aggregates with specific motifs that interact with and activate the C cascade (Velazquez *et al.*, 1997). Whether the neurtoxicity is the direct (primary) result of the aggregates or the result from the secretion of neurotoxic factors by glial cells activated by the fibrillar protein deposits or both, remains to be determined.

5.3 Multiple sclerosis

Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system (CNS), resulting in progressive loss of motor and sensory function. Focal areas (lesions or plaques) of myelin and partial axonal loss within the CNS parenchyma are hallmarks of MS (Bo *et al.*, 2003). Deposition of C and IgG in white matter MS lesions was reported by a number of groups. While the diffuse presence of C activation products probably results from leakage through a damaged BBB (Gay and Esiri, 1991), C activation products (C1q, C3d, and C5b-9) and IgG are also detectable in capillary walls in active MS lesions, and, although less consistently, on myelin sheaths (Lumsden, 1971) and on degraded myelin as well as in microglia /macrophages containing myelin (Compston *et al.*, 1989;Brink *et al.*, 2005;Barnett *et al.*, 2009;Storch *et al.*, 1998) , all of which is consistent with the possible involvement of C in myelin degradation in MS. Based on observed differences in occurrence of C deposition between cases, a classification with 4 pathological subtypes of MS was proposed (Lucchinetti *et al.*, 2000). However in subsequent studies, the heterogeneous presence of IgG and of C activation products was found to be due to different stages in evolution of lesions within cases, rather than heterogeneity between cases (Barnett *et al.*, 2009;Breij *et al.*, 2008;Barnett and Prineas, 2004).

C activation in MS probably is lesion and location dependent. In viturally all white matter lesions C3d and C4d were prominently present on myelin sheath and C3d, C1q and C5b-9 are on disrupted myelin and in macrophage / microglia, astrocytes and vessel walls (Compston *et al.*, 1989;Brink *et al.*, 2005;Prineas *et al.*, 2001;Breij *et al.*, 2008). C3d and C4d probably are covalently bound to the myelin, in contrast to other factors that are rapidly turned over, which may explain the inability to detect C1q and C5b-9 on myelin sheaths in many studies (Prineas *et al.*, 2001;Brink *et al.*, 2005;Compston *et al.*, 1989;Breij *et al.*, 2008). In mixed white and grey matter lesions much less frequent C activation was seen, with C3d and C4d on myelin sheaths on the border of the lesions, and C3d in blood vessel walls only. Moreover, in pure grey matter lesions the extent of C deposition was found to be extremely low (Brink *et al.*, 2005). While not much is known concerning the source of C proteins in MS lesions, enhanced expression of

mRNAs for C1q and to a lesser extent C3 in MS lesions demonstrates again that in response to injury at least part of the C proteins in areas of active demyelination are produced locally. Astrocytes in all lesion areas were immunopositive for C proteins, but C immunoreactive myeloid cells were restricted to inflammatory demyelinating areas, suggesting that macrophages are responsible for enhanced local production of C1q and C3 (Lock *et al.*, 2002;Breij *et al.*, 2008;Brink *et al.*, 2005). Additional roles for complement uncovered in the murine model for MS, experimental autoimmune encephalomyelitis (EAE) have been recently reviewed by Alexander and colleagues (Alexander *et al.*, 2008).

5.4 Disturbed protease / protease inhibitor balance in AD and other ND

Neurons and astrocytes express a number of serine protease inhibitors, including C1-Inh (Veerhuis *et al.*, 1998), thrombin inhibitors, such as protease nexin 1 (PN-1) (Choi *et al.*, 1995) and inhibitors of plasminogen activation (including PAI-1 (Soeda *et al.*, 2008)), neuroserpin (Osterwalder *et al.*, 1998) and alpha2-macroglobulin (α2M) (Bauer *et al.*, 1991) However, in neurodegenerative diseases like AD, expression levels of several regulatory proteins including C1-Inh (Veerhuis *et al.*, 1998;Yasojima *et al.*, 1999a) and AP Cregs fH and fI (Strohmeyer *et al.*, 2000) remain low or are decreased (PN-1) (Choi *et al.*, 1995) which may lead to uncontrolled actions of the proteases. Functions of some regulatory proteins can be taken over by others, as many proteases and protease inhibitors act in the C, the coagulation, the kallikrein-kinin, as well as in fibrinolytic systems. Examples are α2M, which regulates thrombin, plasmin and kallikrein activation, PN-1 which inhibits thrombin and also forms complexes with activated C1s (Van Nostrand *et al.*, 1988), and especially C1-Inh, that except for being the only known physiological regulator of C1 activation, it is a major inhibitor of MASP2 of the lectin pathway and of the contact system of coagulation (kallikrein-kinin system) (Beinrohr *et al.*, 2008). In AD Aß can initiate the C cascades and the kallikrein-kinin system (Bergamaschini *et al.*, 1998). Therefore, Aß-induced activation of one system may lead to a disturbed protease - protease inhibitor balance

in another system, especially when simultaneously the synthesis of the proteases (thrombin, C1s,C1r, and other C factors) increase, as is seen in AD (Yasojima *et al.*, 1999b;Veerhuis *et al.*, 1999). Such disturbed protease – protease inhibitor balances may then initiate subsequent steps in neurodegenerative processes in AD, including APP metabolism, maintenance of BBB integrity and neuritic outgrowth. In an attempt toward a therapeutic strategy, administration of C1-Inh was found to restrict infarct size in experimental models (Storini *et al.*, 2005). A recombinant form was shown to have a much wider time window of efficacy compared to plasma purified C1-Inh when applied in transient and permanent cerebral ischemia studies in mice. This difference probably is due to the selective binding of the recombinant protein to MBL (Gesuete *et al.*, 2009). However, getting C1Inh into the brain in cases of an intact BBB is currently problematic. Another approach is to enhance C1-Inh functioning with low molecular weight heparin, which was found to be effective in reducing Aß plaque load, as well as to reduce the number of activated astrocytes and activation of C and contact systems in an AD model (Bergamaschini *et al.*, 2004)

Summary and Future Directions

In summary, various cell types in the CNS were shown to synthesize C factors, and the synthesis rates of many factors increase during development and within the injured brain. Data from human immunohistochemistry, animal models of diseases, and *in vitro* studies suggest that the role of C in AD is complex, with evidence for both detrimental and beneficial functions, presumably dependent on location, timing, and environmental signals. The potential disease associated polymorphisms of C factors also suggests that control of C activation may have substantial effect on the rate of progression of neurodegenerative diseases. As a result, with precise understanding of the interrelationships between these processes in the CNS in health and disease, C proteins and Cregs can be targeted for therapeutic intervention. The use of inhibitors of selective events downstream of potentially beneficial C cascade events would avoid interfering

with these beneficial consequences of C activation (Fonseca *et al.*, 2009). Some therapeutic approaches utilizing large recombinant molecules may work only when the BBB is compromised, but small molecule drugs, such as known receptor antagonists and low molecular weight heparin, are candidates for chronic disorders that may maintain an intact BBB. Indeed, while challenges of specificity and balance of multiple coincident cascades cannot be over emphasized, the cocktail approach of both promoting beneficial effects and preventing detrimental activities is an attractive and realistic goal for developing treatments for human neurological disorders.

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Figure Legends

Figure 1: **Complement activation and regulation.**

Binding of the C1 macromolecule to the immune complexes, DNA, SAP and Aß can initiate the CP, binding of mannose-binding lectin (MBL) or ficolins, complexed with a homodimer of MASP2, to carbohydrates (on bacterial cell walls) or attachment of spontaneously hydrolyzed C3 via active thio-ester to permissive surfaces or to properdin bound to an activating surface, generates a C3 convertase (C4b2a or C3bBb), and subsequently C5 convertases (C4b2a3b or C3bBb3b). Soluble and membrane-bound complement inhibitors regulate C activation. The soluble inhibitors C1-Inhibitor regulates activated C1, while factor I (fI) and C4b-binding protein (C4bp) control activation at the C4 and C3 level of the CP and LP, and fI together with factor H (fH) at the C3 and C5 convertase level of the AP. In addition, the membrane bound inhibitors CD35 and CD46 act as co-factors for fI, and CD55, decay accelerating factor (DAF) that accelerates the decay of C3 convertases. The fluid phase regulators vitronectin and clusterin and the membrane bound regulator CD59 can prevent formation of the C5b-9 complex on host cell membranes. [Adapted from (Veerhuis, 2011).]

Figure 2: Complement proteins C1q and C3 are associated with plaque structures in human AD and in transgenic mouse models of AD.

C1q immunostaining (brown) in hippocampus of an AD case (90 years old) (top left) and in cortex of 20 mo Tg2576 (bottom left) using anti human (Dako) and anti mouse (1151) C1q antibodies respectively. Activated C3 immunostaining in frontal cortex of an 68 year old AD case (Dako, red, top right) and in cortex of an 18m Tg2576 (brown, bottom right) using an anti human C3d and an anti mouse C3b/iC3b/C3c (2/11, Hycult) antibody respectively. Scale bar: 50 um. Photomicrographs courtesy of Dr. M.I. Fonseca, UC, Irvine.

Reference List

1. Abbott,N.J., Patabendige,A.A., Dolman,D.E., Yusof,S.R., Begley,D.J., 2010. Structure and function of the blood-brain barrier. Neurobiol. Dis. 37**,** 13-25.

2. Afagh,A., Cummings,B.J., Cribbs,D.H., Cotman,C.W., Tenner,A.J., 1996. Localization and cell association of C1q in Alzheimer's disease brain. Exp. Neurol. 138**,** 22-32.

3. Akiyama,H., McGeer,P.L., 1990. Brain microglia constitutively express B-2 integrins. J. Neuroimmunol. 30**,** 81-93.

4. Alexander,J.J., Anderson,A.J., Barnum,S.R., Stevens,B., Tenner,A.J., 2008. The complement cascade: Yin-Yang in neuroinflammation--neuro-protection and -degeneration. J Neurochem. 107**,** 1169-1187.

5. AUSTEN,K.F., Fearon,D.T., 1979. A molecular basis of activation of the alternative pathway of human complement. Adv. Exp Med Biol 120B**,** 3-17.

6. Barnett,M.H., Parratt,J.D., Cho,E.S., Prineas,J.W., 2009. Immunoglobulins and complement in postmortem multiple sclerosis tissue. Ann Neurol 65**,** 32-46.

7. Barnett,M.H., Prineas,J.W., 2004. Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. Ann. Neurol. 55**,** 458-468.

8. Bauer,J., Strauss,S., Schreiter-Gasser,U., Ganter,U., Schlegel,P., Witt,I., Yolk,B., Berger,M., 1991. Interleukin-6 and alpha-2-macroglobulin indicate an acute-phase state in Alzheimer's disease cortices. FEBS Lett. 285**,** 111-114.

9. Beinrohr,L., Dobo,J., Zavodszky,P., Gal,P., 2008. C1, MBL-MASPs and C1-inhibitor: novel approaches for targeting complement-mediated inflammation. Trends Mol. Med. 14**,** 511-521.

10. Bellander,B.M., Singhrao,S.K., Ohlsson,M., Mattsson,P., Svensson,M., 2001. Complement activation in the human brain after traumatic head injury. J Neurotrauma 18**,** 1295-1311.

11. Benard,M., Gonzalez,B.J., Schouft,M.T., Falluel-Morel,A., Vaudry,D., Chan,P., Vaudry,H., Fontaine,M., 2004. Characterization of C3a and C5a receptors in rat cerebellar granule neurons during maturation. Neuroprotective effect of C5a against apoptotic cell death. J Biol Chem 279**,** 43487-43496.

12. Benard,M., Raoult,E., Vaudry,D., Leprince,J., Falluel-Morel,A., Gonzalez,B.J., Galas,L., Vaudry,H., Fontaine,M., 2008. Role of complement anaphylatoxin

receptors (C3aR, C5aR) in the development of the rat cerebellum. Mol. Immunol. 45**,** 3767-3774.

13. Benoit,M.E., Tenner,A.J., 2011. Complement Protein C1q-Mediated Neuroprotection Is Correlated with Regulation of Neuronal Gene and MicroRNA Expression. J Neurosci. 31**,** 3459-3469.

14. Bergamaschini,L., Parnetti,L., Pareyson,D., Canziani,S., Cugno,M., Agostoni,A., 1998. Activation of the contact system in cerebrospinal fluid of patients with Alzheimer disease. Alzheimer Dis. Assoc. Disord. 12**,** 102-108.

15. Bergamaschini,L., Rossi,E., Storini,C., Pizzimenti,S., Distaso,M., Perego,C., De Luigi,A., Vergani,C., De Simoni,M.G., 2004. Peripheral treatment with enoxaparin, a low molecular weight heparin, reduces plaques and beta-amyloid accumulation in a mouse model of Alzheimer's disease. J Neurosci. 24**,** 4181-4186.

16. Bo,L., Vedeler,C.A., Nyland,H.I., Trapp,B.D., Mork,S.J., 2003. Subpial demyelination in the cerebral cortex of multiple sclerosis patients. J Neuropathol. Exp Neurol. 62**,** 723-732.

17. Bogestal,Y.R., Barnum,S.R., Smith,P.L., Mattisson,V., Pekny,M., Pekna,M., 2007. Signaling through C5aR is not involved in basal neurogenesis. J Neurosci Res. 85**,** 2892-2897.

18. Bohlson,S.S., Fraser,D.A., Tenner,A.J., 2007. Complement proteins C1q and MBL are pattern recognition molecules that signal immediate and long-term protective immune functions. Mol Immunol 44**,** 33-43.

19. Bolliger,M.F., Martinelli,D.C., Sudhof,T.C., 2011. The cell-adhesion G protein-coupled receptor BAI3 is a high-affinity receptor for C1q-like proteins. Proc Natl Acad Sci U S A 108**,** 2534-2539.

20. Bonifati,D.M., Kishore,U., 2007. Role of complement in neurodegeneration and neuroinflammation. Mol Immunol 44**,** 999-1010.

21. Boulanger,L.M., 2009. Immune proteins in brain development and synaptic plasticity. Neuron 64**,** 93-109.

22. Bradt,B.M., Kolb,W.P., Cooper,N.R., 1998. Complement-dependent proinflammatory properties of the Alzheimer's disease beta-peptide. J Exp Med 188**,** 431-438.

23. Breij,E.C., Brink,B.P., Veerhuis,R., van den Berg,C., Vloet,R., Yan,R., Dijkstra,C.D., Van der Valk,P., Bo,L., 2008. Homogeneity of active demyelinating lesions in established multiple sclerosis. Ann. Neurol. 63**,** 16-25.

24. Brink,B.P., Veerhuis,R., Breij,E.C., Van der Valk,P., Dijkstra,C.D., Bo,L., 2005. The pathology of multiple sclerosis is location-dependent: no significant complement activation is detected in purely cortical lesions. J. Neuropathol. Exp. Neurol. 64**,** 147-155.

25. Broadwell,R.D., Sofroniew,M.V., 1993. Serum proteins bypass the blood-brain fluid barriers for extracellular entry to the central nervous system. Exp Neurol. 120**,** 245-263.

26. Butt,A.M., Hamilton,N., Hubbard,P., Pugh,M., Ibrahim,M., 2005. Synantocytes: the fifth element. J Anat. 207**,** 695-706.

27. Cadman,E.D., Puttfarcken,P.S., 1997. Beta-amyloid peptides initiate the complement cascade without producing a comparable effect on the terminal pathway in vitro. Exp Neurol. 146**,** 388-394.

28. Canova,C., Neal,J.W., Gasque,P., 2006. Expression of innate immune complement regulators on brain epithelial cells during human bacterial meningitis. J. Neuroinflammation. 3**,** 22.

29. Carel,J.C., Frazier,B., Ley,T.J., Holers,V.M., 1989. Analysis of epitope expression and the functional repertoire of recombinant complement receptor 2 (CR2/CD21) in mouse and human cells. j immunol 143**,** 923-930.

30. Carroll,M.C., 2004. The complement system in regulation of adaptive immunity. Nat. Immunol 5**,** 981-986.

31. Chakrabarty,P., Ceballos-Diaz,C., Beccard,A., Janus,C., Dickson,D., Golde,T.E., Das,P., 2010. IFN-gamma promotes complement expression and attenuates amyloid plaque deposition in amyloid beta precursor protein transgenic mice. J. Immunol. 184**,** 5333-5343.

32. Charbel,I.P., Victor,C.N., Scholl,H.P., 2010. The significance of the complement system for the pathogenesis of age-related macular degeneration - current evidence and translation into clinical application. Graefes Arch. Clin Exp Ophthalmol.

33. Choi,B.H., Kim,R.C., Vaughan,P.J., Lau,A., Van Nostrand,W.E., Cotman,C.W., Cunningham,D.D., 1995. Decreases in protease nexins in Alzheimer's disease brain. Neurobiol. Aging 16**,** 557-562.

34. Chu,Y., Jin,X., Parada,I., Pesic,A., Stevens,B., Barres,B., Prince,D.A., 2010. Enhanced synaptic connectivity and epilepsy in C1q knockout mice. Proc. Natl. Acad. Sci. U. S. A 107**,** 7975-7980.

35. Cole,D.S., Morgan,B.P., 2003. Beyond lysis: how complement influences cell fate. Clin Sci (Lond) 104**,** 455-466.

36. Compston,D.A.S., Morgan,B.P., Campbell,A.K., Wilkins,P., Cole,G., Thomas,N.D., Jasani,B., 1989. Immunocytochemical localization of the terminal complement complex in multiple sclerosis. Neuropathol. Appl. Neurobiol. 15**,** 307-316.

37. Cooper,N.R., Nemerow,G.R., 1989. Complement and infectious agents: A tale of disguise and deception. Comple. and Inflam. 6**,** 249-258.

38. Craft,J.M., Watterson,D.M., Van Eldik,L.J., 2006. Human amyloid beta-induced neuroinflammation is an early event in neurodegeneration. Glia 53**,** 484-490.

39. Dong,Y., Benveniste,E.N., 2001. Immune function of astrocytes. Glia 36**,** 180-190.

40. Eikelenboom,P., Hack,C.E., Rozemuller,J.M., Stam,F.C., 1989. Complement activation in amyloid plaques in Alzheimer's dementia. Virchows Archiv B Cell Pathol. 56**,** 259-262.

41. Eikelenboom,P., Stam,F.C., 1982. Immunoglobulins and complement factors in senile plaques. An immunoperoxidase study. Acta Neuropathol. 57**,** 239-242.

42. Eikelenboom,P., Veerhuis,R., 1996. The role of complement and activated microglia in the pathogenesis of Alzheimer's disease. Neurobiol. Aging 17**,** 673-680.

43. Eikelenboom,P., Veerhuis,R., Van Exel,E., Hoozemans,J.J., Rozemuller,A.J., van Gool,W.A., 2011. The Early Involvement of the Innate Immunity in the Pathogenesis of Alzheimer's Disease: Neuropathological, Epidemiological and Genetic Evidence. Curr Alzheimer Res.

44. Erdei,A., Isaak,A., Torok,K., Sandor,N., Kremlitzka,M., Prechl,J., Bajtay,Z., 2009. Expression and role of CR1 and CR2 on B and T lymphocytes under physiological and autoimmune conditions. Mol Immunol 46**,** 2767-2773.

45. Familian,A., Eikelenboom,P., Veerhuis,R., 2007. Minocycline does not affect amyloid beta phagocytosis by human microglial cells. Neurosci. Lett. 416**,** 87-91.

46. Fan,R., DeFilippis,K., Van Nostrand,W.E., 2007. Induction of complement proteins in a mouse model for cerebral microvascular A beta deposition. J. Neuroinflammation. 4**,** 22.

47. Flaherty,P., Radhakrishnan,M.L., Dinh,T., Rebres,R.A., Roach,T.I., Jordan,M.I., Arkin,A.P., 2008. A dual receptor crosstalk model of G-protein-coupled signal transduction. PLoS. Comput. Biol 4**,** e1000185.

48. Fonseca,M.I., Ager,R.R., Chu,S.H., Yazan,O., Sanderson,S.D., LaFerla,F.M., Taylor,S.M., Woodruff,T.M., Tenner,A.J., 2009. Treatment with a C5aR

antagonist decreases pathology and enhances behavioral performance in murine models of Alzheimer's disease. J. Immunol 183**,** 1375-1383.

49. Fonseca,M.I., Chu,S.H., Berci,A.M., Benoit,M.E., Peters,D.G., Kimura,Y., Tenner,A.J., 2011. Contribution of complement activation pathways to neuropathology differs among mouse models of Alzheimer's disease. J Neuroinflammation. 8**,** 4.

50. Fonseca,M.I., Kawas,C.H., Troncoso,J.C., Tenner,A.J., 2004a. Neuronal localization of C1q in preclinical Alzheimer's disease. Neurobiol. Dis. 15**,** 40-46.

51. Fonseca,M.I., Zhou,J., Botto,M., Tenner,A.J., 2004b. Absence of C1q leads to less neuropathology in transgenic mouse models of Alzheimer's disease. J Neurosci. 24**,** 6457-6465.

52. Frank,I., Stoiber,H., Godar,S., Stockinger,H., Steindl,F., Katinger,H.W., Dierich,M.P., 1996. Acquisition of host cell-surface-derived molecules by HIV-1. AIDS 10**,** 1611-1620.

53. Fraser,D.A., Laust,A.K., Nelson,E.L., Tenner,A.J., 2009. C1q differentially modulates phagocytosis and cytokine responses during ingestion of apoptotic cells by human monocytes, macrophages, and dendritic cells. J. Immunol. 183**,** 6175-6185.

54. Fraser,D.A., Pisalyaput,K., Tenner,A.J., 2010. C1q enhances microglial clearance of apoptotic neurons and neuronal blebs, and modulates subsequent inflammatory cytokine production. J Neurochem. 112**,** 733-743.

55. Gasparini,L., Ongini,E., Wilcock,D., Morgan,D., 2005. Activity of flurbiprofen and chemically related anti-inflammatory drugs in models of Alzheimer's disease. Brain Res. Brain Res. Rev. 48**,** 400-408.

56. Gasque,P., Fontaine,M., Morgan,B.P., 1995. Complement expression in human brain. Biosynthesis of terminal pathway components and regulators in human glial cells and cell lines. J. Immunol. 154**,** 4726-4733.

57. Gasque,P., Ischenko,A., Legoedec,J., Mauger,C., Schouft,M.T., Fontaine,M., 1993. Expression of the complement classical pathway by human glioma in culture. A model for complement expression by nerve cells. J Biol. Chem. 268**,** 25068-25074.

58. Gasque,P., Thomas,A., Fontaine,M., Morgan,B.P., 1996. Complement activation on human neuroblastoma cell lines in vitro: route of activation and expression of functional complement regulatory proteins. J Neuroimmunol. 66**,** 29-40.

59. Gay,D., Esiri,M., 1991. Blood-brain barrier damage in acute multiple sclerosis plaques. An immunocytological study. Brain 114 (Pt 1B)**,** 557-572.

60. Gendrel,M., Rapti,G., Richmond,J.E., Bessereau,J.L., 2009. A secreted complement-control-related protein ensures acetylcholine receptor clustering. Nature 461**,** 992-996.

61. Gesuete,R., Storini,C., Fantin,A., Stravalaci,M., Zanier,E.R., Orsini,F., Vietsch,H., Mannesse,M.L., Ziere,B., Gobbi,M., De Simoni,M.G., 2009. Recombinant C1 inhibitor in brain ischemic injury. Ann. Neurol. 66**,** 332-342.

62. Griffiths,M.R., Gasque,P., Neal,J.W., 2009. The multiple roles of the innate immune system in the regulation of apoptosis and inflammation in the brain. J Neuropathol. Exp Neurol 68**,** 217-226.

63. Head,E., Azizeh,B.Y., Lott,I.T., Tenner,A.J., Cotman,C.W., Cribbs,D.H., 2001. Complement association with neurons and beta-amyloid deposition in the brains of aged individuals with Down Syndrome. Neurobiol. Dis 8**,** 252-265.

64. Hosokawa,M., Klegeris,A., Maguire,J., McGeer,P.L., 2003. Expression of complement messenger RNAs and proteins by human oligodendroglial cells. Glia 42**,** 417-423.

65. Hu,W., Ranaivo,H.R., Roy,S.M., Behanna,H.A., Wing,L.K., Munoz,L., Guo,L., Van Eldik,L.J., Watterson,D.M., 2007. Development of a novel therapeutic suppressor of brain proinflammatory cytokine up-regulation that attenuates synaptic dysfunction and behavioral deficits. Bioorg. Med Chem Lett. 17**,** 414-418.

66. Humayun,S., Gohar,M., Volkening,K., Moisse,K., Leystra-Lantz,C., Mepham,J., McLean,J., Strong,M.J., 2009. The complement factor C5a receptor is upregulated in NFL-/- mouse motor neurons. J Neuroimmunol.

67. Ishii,T., Haga,S., 1984. Immuno-electron-microscopic localization of complement in amyloid fibrils of senile plaques. Acta Neuropathol. 63**,** 296-300.

68. Itagaki,S., Akiyama,H., Saito,H., McGeer,P.L., 1994. Ultrastructural localization of complement membrane attack complex (MAC)-like immunoreactivity in brains of patients with Alzheimer's disease. Brain Res. 645**,** 78-84.

69. Jana,M., Palencia,C.A., Pahan,K., 2008. Fibrillar amyloid-beta peptides activate microglia via TLR2: implications for Alzheimer's disease. J Immunol. 181**,** 7254-7262.

70. Jauneau,A.C., Ischenko,A., Chatagner,A., Benard,M., Chan,P., Schouft,M.T., Patte,C., Vaudry,H., Fontaine,M., 2006. Interleukin 1b and anaphylatoxins exert a synergistic effect on NGF expression by astrocytes. J Neuroinflammation. 3**,** 8.

71. Jiang,H., Burdick,D., Glabe,C.G., Cotman,C.W., Tenner,A.J., 1994. β-amyloid activates complement by binding to a specific region of the collagen-like domain of the C1q A chain. J. Immunol. 152**,** 5050-5059.

72. Jin,J.J., Kim,H.D., Maxwell,J.A., Li,L., Fukuchi,K., 2008. Toll-like receptor 4-dependent upregulation of cytokines in a transgenic mouse model of Alzheimer's disease. J Neuroinflammation. 5**,** 23.

73. Johansson,L., Rytkonen,A., Bergman,P., Albiger,B., Kallstrom,H., Hokfelt,T., Agerberth,B., Cattaneo,R., Jonsson,A.B., 2003. CD46 in meningococcal disease. Science 301**,** 373-375.

74. Johnson,R.T., Glass,J.D., McArthur,J.C., Chesebro,B.W., 1996. Quantitation of human immunodeficiency virus in brains of demented and nondemented patients with acquired immunodeficiency syndrome. Ann. Neurol. 39**,** 392-395.

75. Kemper,C., Hourcade,D.E., 2008. Properdin: New roles in pattern recognition and target clearance. Mol. Immunol 45**,** 4048-4056.

76. Klos,A., Tenner,A.J., Johswich,K.O., Ager,R.R., Reis,E.S., Kohl,J., 2009. The role of the anaphylatoxins in health and disease. Mol. Immunol 46**,** 2753-2766.

77. Kobayashi,K., Muramori,F., Aoki,T., Hayashi,M., Miyazu,K., Fukutani,Y., mukai,m., Koshino,F., 1998. KP-1 is a marker for extraneuronal neurofibrillary tangles and senile plaques in Alzheimer diseased brains. Dement. Geriatr. Cogn Disord. 9**,** 13-19.

78. Kolev,M.V., Ruseva,M.M., Morgan,B.P., Donev,R.M., 2010. Targeting neural-restrictive silencer factor sensitizes tumor cells to antibody-based cancer immunotherapy in vitro via multiple mechanisms. j immunol 184**,** 6035-6042.

79. Lambert,J.C., Heath,S., Even,G., Campion,D., Sleegers,K., Hiltunen,M., Combarros,O., Zelenika,D., Bullido,M.J., Tavernier,B., Letenneur,L., Bettens,K., Berr,C., Pasquier,F., Fievet,N., Barberger-Gateau,P., Engelborghs,S., De,D.P., Mateo,I., Franck,A., Helisalmi,S., Porcellini,E., Hanon,O., de Pancorbo,M.M., Lendon,C., Dufouil,C., Jaillard,C., Leveillard,T., Alvarez,V., Bosco,P., Mancuso,M., Panza,F., Nacmias,B., Bossu,P., Piccardi,P., Annoni,G., Seripa,D., Galimberti,D., Hannequin,D., Licastro,F., Soininen,H., Ritchie,K., Blanche,H., Dartigues,J.F., Tzourio,C., Gut,I., Van,B.C., Alperovitch,A., Lathrop,M., Amouyel,P., 2009. Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. Nat Genet. 41**,** 1094-1099.

80. Lampert-Etchells,M., Pasinetti,G.M., Finch,C.E., Johnson,S.A., 1993. Regional localization of cells containing complement C1q and C4 mRNAs in the frontal cortex during Alzheimer's disease. Neurodegeneration 2**,** 111-121.

81. Levi-Strauss,M., Mallat,M., 1987. Primary cultures of murine astrocytes produce C3 and Factor B, two components of the alternative pathway of complement activation. J. Immunol. 139**,** 2361-2366.

82. Lock,C., Hermans,G., Pedotti,R., Brendolan,A., Schadt,E., Garren,H., Langer-Gould,A., Strober,S., Cannella,B., Allard,J., Klonowski,P., Austin,A., Lad,N.,

Kaminski,N., Galli,S.J., Oksenberg,J.R., Raine,C.S., Heller,R., Steinman,L., 2002. Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. Nat Med 8**,** 500-508.

83. Loeffler,D.A., Camp,D.M., Bennett,D.A., 2008. Plaque complement activation and cognitive loss in Alzheimer's disease. J Neuroinflammation. 5**,** 9.

84. Lucchinetti,C., Bruck,W., Parisi,J., Scheithauer,B., Rodriguez,M., Lassmann,H., 2000. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. Ann. Neurol. 47**,** 707-717.

85. Lucin,K.M., Wyss-Coray,T., 2009. Immune activation in brain aging and neurodegeneration: too much or too little? Neuron 64**,** 110-122.

86. Lue,L.F., Rydel,R., Brigham,E.F., Yang,L.B., Hampel,H., Murphy,G.M., Jr., Brachova,L., Yan,S.D., Walker,D.G., Shen,Y., Rogers,J., 2001. Inflammatory repertoire of Alzheimer's disease and nondemented elderly microglia in vitro. Glia 35**,** 72-79.

87. Lumsden,C.E., 1971. The immunogenesis of the multiple sclerosis plaque. Brain Res. 28**,** 365-390.

88. Maier,M., Peng,Y., Jiang,L., Seabrook,T.J., Carroll,M.C., Lemere,C.A., 2008. Complement C3 deficiency leads to accelerated amyloid beta plaque deposition and neurodegeneration and modulation of the microglia/macrophage phenotype in amyloid precursor protein transgenic mice. J. Neurosci. 28**,** 6333-6341.

89. McGeer,P.L., Akiyama,H., Itagaki,S., McGeer,E.G., 1989. Activation of the classical complement pathway in brain tissue of Alzheimer patients. Neurosci. Lett. 107**,** 341-346.

90. McGeer,P.L., Kawamata,T., Walker,D.G., 1992. Distribution of clusterin in Alzheimer brain tissue. Brain Res. 579**,** 337-341.

91. Morrissette,D.A. Effects of mouse genetic background strain on Alzheimer-like pathology and behavior in the triple transgenic mouse model of Alzheimer Disease. 2009. University of California, Irvine.
Ref Type: Thesis/Dissertation

92. Mukherjee,P., Pasinetti,G.M., 2000. The role of complement anaphylatoxin C5a in neurodegeneration: implications in Alzheimer's disease. J. Neuroimmunol. 105**,** 124-130.

93. Mukherjee,P., Pasinetti,G.M., 2001. Complement anaphylatoxin C5a neuroprotects through mitogen-activated protein kinase-dependent inhibition of caspase 3. J. Neurochem. 77**,** 43-49.

94. Nielsen,H.M., Mulder,S.D., Belien,J.A., Musters,R.J., Eikelenboom,P., Veerhuis,R., 2010. Astrocytic A beta 1-42 uptake is determined by A beta-aggregation state and the presence of amyloid-associated proteins. Glia 58**,** 1235-1246.

95. Nielsen,H.M., Veerhuis,R., Holmqvist,B., Janciauskiene,S., 2009. Binding and uptake of A beta1-42 by primary human astrocytes in vitro. Glia 57**,** 978-988.

96. Nimmerjahn,A., Kirchhoff,F., Helmchen,F., 2005. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 308**,** 1314-1318.

97. O'Barr,S.A., Caguioa,J., Gruol,D., Perkins,G., Ember,J.A., Hugli,T., Cooper,N.R., 2001. Neuronal expression of a functional receptor for the C5a complement activation fragment. J. Immunol. 166**,** 4154-4162.

98. Osaka,H., Mukherjee,P., Aisen,P.S., Pasinetti,G.M., 1999. Complement-derived anaphylatoxin C5a protects against glutamate-mediated neurotoxicity. J Cell Biochem 73**,** 303-311.

99. Osterwalder,T., Cinelli,P., Baici,A., Pennella,A., Krueger,S.R., Schrimpf,S.P., Meins,M., Sonderegger,P., 1998. The axonally secreted serine proteinase inhibitor, neuroserpin, inhibits plasminogen activators and plasmin but not thrombin. J Biol Chem 273**,** 2312-2321.

100. Pedersen,E.D., Aass,H.C., Rootwelt,T., Fung,M., Lambris,J.D., Mollnes,T.E., 2007. CD59 efficiently protects human NT2-N neurons against complement-mediated damage. Scand. J. Immunol. 66**,** 345-351.

101. Pedersen,E.D., Loberg,E.M., Vege,E., Daha,M.R., Maehlen,J., Mollnes,T.E., 2009. In situ deposition of complement in human acute brain ischaemia. Scand. J Immunol 69**,** 555-562.

102. Piddlesden,S.J., Storch,M.K., Hibbs,M., Freeman,A.M., Lassmann,H., Morgan,B.P., 1994. Soluble recombinant complement receptor 1 inhibits inflammation and demyelination in antibody-mediated demyelinating experimental allergic encephalomyelitis. j immunol 152**,** 5477-5484.

103. Pisalyaput,K., Tenner,A.J., 2008. Complement component C1q inhibits beta-amyloid- and serum amyloid P-induced neurotoxicity via caspase- and calpain-independent mechanisms. J. Neurochem. 104**,** 696-707.

104. Prineas,J.W., Kwon,E.E., Cho,E.S., Sharer,L.R., Barnett,M.H., Oleszak,E.L., Hoffman,B., Morgan,B.P., 2001. Immunopathology of secondary-progressive multiple sclerosis. Ann. Neurol. 50**,** 646-657.

105. Querfurth,H.W., LaFerla,F.M., 2010. Alzheimer's disease. N. Engl. J Med 362**,** 329-344.

106. Rambach,G., Maier,H., Vago,G., Mohsenipour,I., Lass-Florl,C., Defant,A., Wurzner,R., Dierich,M.P., Speth,C., 2008. Complement induction and complement evasion in patients with cerebral aspergillosis. Microbes. Infect. 10**,** 1567-1576.

107. Reichwald,J., Danner,S., Wiederhold,K.H., Staufenbiel,M., 2009. Expression of complement system components during aging and amyloid deposition in APP transgenic mice. J Neuroinflammation. 6**,** 35.

108. Ricklin,D., Hajishengallis,G., Yang,K., Lambris,J.D., 2010. Complement: a key system for immune surveillance and homeostasis. Nat Immunol 11**,** 785-797.

109. Rogers,J., Cooper,N.R., Webster,S., Schultz,J., McGeer,P.L., Styren,S.D., Civin,W.H., Brachova,L., Bradt,B., Ward,P., Lieberburg,I., 1992. Complement activation by beta-amyloid in Alzheimer disease. Proc. Natl. Acad. Sci. 89**,** 10016-10020.

110. Rostagno,A., Revesz,T., Lashley,T., Tomidokoro,Y., Magnotti,L., Braendgaard,H., Plant,G., Bojsen-Moller,M., Holton,J., Frangione,B., Ghiso,J., 2002. Complement activation in chromosome 13 dementias. Similarities with Alzheimer's disease. J. Biol. Chem. 277**,** 49782-49790.

111. Rozemuller,A.J., Eikelenboom,P., Theeuwes,J.W., Jansen Steur,E.N., de Vos,R.A., 2000. Activated microglial cells and complement factors are unrelated to cortical Lewy bodies. Acta Neuropathol. 100**,** 701-708.

112. Rozemuller,J.M., Eikelenboom,P., Stam,F.C., Beyreuther,K., Masters,C.L., 1989. A4 protein in Alzheimer's disease: primary and secondary cellular events in extracellular amyloid deposition. J. of Neuropathology and Experimental Neurology 48**,** 674-691.

113. Rozovsky,I., Morgan,T.E., Willoughby,D.A., Dugichi-Djordjevich,M.M., Pasinetti,G.M., Johnson,S.A., Finch,C.E., 1994. Selective expression of clusterin (SGP-2) and complement C1qB and C4 during responses to neurotoxins in vivo and in vitro. Neuroscience 62**,** 741-758.

114. Ryman,D., Gao,Y., Lamb,B.T., 2008. Genetic loci modulating amyloid-beta levels in a mouse model of Alzheimer's disease. Neurobiol. Aging 29**,** 1190-1198.

115. Sairanen,T., Karjalainen-Lindsberg,M.L., Paetau,A., Ijas,P., Lindsberg,P.J., 2006. Apoptosis dominant in the periinfarct area of human ischaemic stroke--a possible target of antiapoptotic treatments. Brain 129**,** 189-199.

116. Sayah,S., Jauneau,A.C., Patte,C., Tonon,M.C., Vaudry,H., Fontaine,M., 2003. Two different transduction pathways are activated by C3a and C5a anaphylatoxins on astrocytes. Brain Res. Mol. Brain Res. 112**,** 53-60.

117. Schwab,C., McGeer,P.L., 2002. Complement activated C4d immunoreactive oligodendrocytes delineate small cortical plaques in multiple sclerosis. Exp. Neurol. 174**,** 81-88.

118. Scolding,N.J., Morgan,B.P., Compston,D.A., 1998. The expression of complement regulatory proteins by adult human oligodendrocytes. J Neuroimmunol. 84**,** 69-75.

119. Sewell,D.L., Nacewicz,B., Liu,F., Macvilay,S., Erdei,A., Lambris,J.D., Sandor,M., Fabry,Z., 2004. Complement C3 and C5 play critical roles in traumatic brain cryoinjury: blocking effects on neutrophil extravasation by C5a receptor antagonist. J Neuroimmunol. 155**,** 55-63.

120. Shaftel,S.S., Griffin,W.S., O'Banion,M.K., 2008. The role of interleukin-1 in neuroinflammation and Alzheimer disease: an evolving perspective. J Neuroinflammation. 5**,** 7.

121. Shen,Y., Li,R., McGeer,E.G., McGeer,P.L., 1997. Neuronal expression of mRNAs for complement proteins of the classical pathway in Alzheimer brain. Brain Res. 769**,** 391-395.

122. Shinjyo,N., Stahlberg,A., Dragunow,M., Pekny,M., Pekna,M., 2009. Complement-derived anaphylatoxin C3a regulates in vitro differentiation and migration of neural progenitor cells. Stem Cells 27**,** 2824-2832.

123. Singhrao,S.K., Neal,J.W., Gasque,P., Morgan,B.P., Newman,G.R., 1996. Role of complement in the aetiology of Pick's disease? J Neuropathol. Exp Neurol. 55**,** 578-593.

124. Singhrao,S.K., Neal,J.W., Rushmere,N.K., Morgan,B.P., Gasque,P., 2000. Spontaneous classical pathway activation and deficiency of membrane regulators render human neurons susceptible to complement lysis. Am. J Pathol. 157**,** 905-918.

125. Sjoberg,A.P., Trouw,L.A., Blom,A.M., 2009. Complement activation and inhibition: a delicate balance. Trends Immunol 30**,** 83-90.

126. Snyder,S.W., Wang,G.T., Barrett,L., Ladror,U.S., Casuto,D., Lee,C.M., Krafft,G.A., Holzman,R.B., Holzman,T.F., 1994. Complement C1q does not bind monomeric β-amyloid. Exp. Neurol. 128**,** 136-142.

127. Soeda,S., Koyanagi,S., Kuramoto,Y., Kimura,M., Oda,M., Kozako,T., Hayashida,S., Shimeno,H., 2008. Anti-apoptotic roles of plasminogen activator inhibitor-1 as a neurotrophic factor in the central nervous system. Thromb. Haemost. 100**,** 1014-1020.

128. Speth,C., Dierich,M.P., Gasque,P., 2002. Neuroinvasion by pathogens: a key role of the complement system. Mol. Immunol 38**,** 669-679.

129. Speth,C., Rambach,G., Wurzner,R., Lass-Florl,C., 2008. Complement and fungal pathogens: an update. Mycoses 51**,** 477-496.

130. Stahel,P.F., Morganti-Kossmann,M.C., Kossmann,T., 1998. The role of the complement system in traumatic brain injury. Brain Res. Brain Res. Rev 27**,** 243-256.

131. Stevens,B., Allen,N.J., Vazquez,L.E., Howell,G.R., Christopherson,K.S., Nouri,N., Micheva,K.D., Mehalow,A.K., Huberman,A.D., Stafford,B., Sher,A., Litke,A.M., Lambris,J.D., Smith,S.J., John,S.W., Barres,B.A., 2007. The classical complement cascade mediates CNS synapse elimination. Cell 131**,** 1164-1178.

132. Stoermer,K.A., Morrison,T.E., 2011. Complement and viral pathogenesis. Virology 411**,** 362-373.

133. Stoiber,H., Ebenbichler,C., Schneider,R., Janatova,J., Dierich,M.P., 1995. Interaction of several complement proteins with gp120 and gp41, the two envelope glycoproteins of HIV-1. AIDS 9**,** 19-26.

134. Stoltzner,S.E., Grenfell,T.J., Mori,C., Wisniewski,K.E., Wisniewski,T.M., Selkoe,D.J., Lemere,C.A., 2000. Temporal accrual of complement proteins in amyloid plaques in Down's syndrome with Alzheimer's disease. Am. J. Pathol. 156**,** 489-499.

135. Storch,M.K., Piddlesden,S., Haltia,M., Iivanainen,M., Morgan,P., Lassmann,H., 1998. Multiple sclerosis: in situ evidence for antibody- and complement-mediated demyelination. Ann. Neurol. 43**,** 465-471.

136. Storini,C., Rossi,E., Marrella,V., Distaso,M., Veerhuis,R., Vergani,C., Bergamaschini,L., De Simoni,M.G., 2005. C1-inhibitor protects against brain ischemia-reperfusion injury via inhibition of cell recruitment and inflammation. Neurobiol. Dis. 19**,** 10-17.

137. Strohmeyer,R., Ramirez,M., Cole,G.J., Mueller,K., Rogers,J., 2002. Association of factor H of the alternative pathway of complement with agrin and complement receptor 3 in the Alzheimer's disease brain. J Neuroimmunol. 131**,** 135-146.

138. Strohmeyer,R., Shen,Y., Rogers,J., 2000. Detection of complement alternative pathway mRNA and proteins in the Alzheimer's disease brain. Brain Res. Mol. Brain Res. 81**,** 7-18.

139. Tacnet-Delorme,P., Chevallier,S., Arlaud,G.J., 2001. Beta-amyloid fibrils activate the C1 complex of complement under physiological conditions: evidence for a binding site for A beta on the C1q globular regions. J. Immunol. 167**,** 6374-6381.

140. Tahara,K., Kim,H.D., Jin,J.J., Maxwell,J.A., Li,L., Fukuchi,K., 2006. Role of toll-like receptor signalling in Abeta uptake and clearance. Brain 129**,** 3006-3019.

141. Thomas,A., Gasque,P., Vaudry,D., Gonzalez,B., Fontaine,M., 2000. Expression of a complete and functional complement system by human neuronal cells in vitro. Int. Immunol 12**,** 1015-1023.

142. Tocco,G., Musleh,W., Sakhi,S., Schreiber,S.S., Baudry,M., Pasinetti,G.M., 1997. Complement and glutamate neurotoxicity. Genotypic influences of C5 in a mouse model of hippocampal neurodegeneration. Mol. Chem. Neuropathol. 31**,** 289-300.

143. Trouw,L.A., Nielsen,H.M., Minthon,L., Londos,E., Landberg,G., Veerhuis,R., Janciauskiene,S., Blom,A.M., 2008. C4b-binding protein in Alzheimer's disease: binding to Abeta1-42 and to dead cells. Mol Immunol 45**,** 3649-3660.

144. Udan,M.L., Ajit,D., Crouse,N.R., Nichols,M.R., 2008. Toll-like receptors 2 and 4 mediate Abeta(1-42) activation of the innate immune response in a human monocytic cell line. J Neurochem. 104**,** 524-533.

145. Van Beek,J., Elward,K., Gasque,P., 2003. Activation of complement in the central nervous system: roles in neurodegeneration and neuroprotection. Ann. N. Y. Acad. Sci. 992**,** 56-71.

146. Van Beek,J., Nicole,O., Ali,C., Ischenko,A., MacKenzie,E.T., Buisson,A., Fontaine,M., 2001. Complement anaphylatoxin C3a is selectively protective against NMDA-induced neuronal cell death. NeuroReport 12**,** 289-293.

147. van Kooten,C., Fiore,N., Trouw,L.A., Csomor,E., Xu,W., Castellano,G., Daha,M.R., Gelderman,K.A., 2008. Complement production and regulation by dendritic cells: molecular switches between tolerance and immunity. Mol. Immunol. 45**,** 4064-4072.

148. Van Nostrand,W.E., McKay,L.D., Baker,J.B., Cunningham,D.D., 1988. Functional and structural similarities between protease nexin I and C1 inhibitor. J Biol Chem 263**,** 3979-3983.

149. Vastag,M., Skopal,J., Kramer,J., Kolev,K., Voko,Z., Csonka,E., Machovich,R., Nagy,Z., 1998. Endothelial cells cultured from human brain microvessels produce complement proteins factor H, factor B, C1 inhibitor, and C4. Immunobiology 199**,** 5-13.

150. Veerhuis,R., 2011. Histological and Direct Evidence for the Role of Complement in the Neuroinflammation of AD. Curr Alzheimer Res. 8**,** 34-58.

151. Veerhuis,R., Boshuizen,R.S., Familian,A., 2005. Amyloid associated proteins in Alzheimer's and prion disease. Curr. Drug Targets. CNS. Neurol. Disord. 4**,** 235-248.

152. Veerhuis,R., Janssen,I., De Groot,C.J., Van Muiswinkel,F.L., Hack,C.E., Eikelenboom,P., 1999. Cytokines associated with amyloid plaques in Alzheimer's

disease brain stimulate human glial and neuronal cell cultures to secrete early complement proteins, but not C1-inhibitor. Exp Neurol. 160**,** 289-299.

153. Veerhuis,R., Janssen,I., Hack,C.E., Eikelenboom,P., 1996. Early complement components in Alzheimer's disease brains. Acta Neuropathol. (Berl.) 91**,** 53-60.

154. Veerhuis,R., Janssen,I., Hoozemans,J.J.M., De Groot,C.J.A., Hack,C.E., Eikelenboom,P., 1998. Complement c1-inhibitor expression in Alzheimer's disease. Acta Neuropathol. (Berl.) 96**,** 287-296.

155. Veerhuis,R., Van Breemen,M.J., Hoozemans,J.M., Morbin,M., Ouladhadj,J., Tagliavini,F., Eikelenboom,P., 2003. Amyloid beta plaque-associated proteins C1q and SAP enhance the Abeta(1-42)peptide-induced cytokine secretion by adult human microglia in vitro. Acta Neuropathol. (Berl) 105**,** 135-144.

156. Veerhuis,R., Van der Valk,P., Janssen,I., Zhan,S.S., VanNostrand,W.E., Eikelenboom,P., 1995. Complement activation in amyloid plaques in Alzheimer's disease brains does not proceed further than C3. Virchows Arch. 426**,** 603-610.

157. Velazquez,P., Cribbs,D.H., Poulos,T.L., Tenner,A.J., 1997. Aspartate residue 7 in amyloid beta-protein is critical for classical complement pathway activation: implications for Alzheimer's disease pathogenesis. Nat Med 3**,** 77-79.

158. Verbeek,M.M., Otte-Holler,I., Ruiter,D.J., de Waal,R.M., 1999. Human brain pericytes as a model system to study the pathogenesis of cerebrovascular amyloidosis in Alzheimer's disease. Cell Mol Biol (Noisy. -le-grand) 45**,** 37-46.

159. Verbeek,M.M., Otte-Höller,I., Veerhuis,R., Ruiter,D.J., De Waal,R.M.W., 1998. Distribution of Aβ-associated proteins in cerebrovascular amyloid of Alzheimer's disease. Acta Neuropathol. (Berl.) 96**,** 628-636.

160. Verkhratsky,A., Parpura,V., 2010. Recent advances in (patho)physiology of astroglia. Acta Pharmacol. Sin. 31**,** 1044-1054.

161. Walker,D.G., Kim,S.U., McGeer,P.L., 1995. Complement and cytokine gene expression in cultured microglial derived from postmortem human brains. J. Neurosci. Res. 40**,** 478-493.

162. Walker,D.G., McGeer,P.L., 1992. Complement gene expression in human brain: comparison between normal and Alzheimer disease cases. Brain Res. Mol. Brain Res. 14**,** 109-116.

163. Watabe,K., Osborne,D., Kim,S.U., 1989. Phagocytic activity of human adult astrocytes and oligodendrocytes in culture. J Neuropathol. Exp Neurol. 48**,** 499-506.

164. Webster,S., Lue,L.F., Brachova,L., Tenner,A.J., McGeer,P.L., Terai,K., Walker,D.G., Bradt,B., Cooper,N.R., Rogers,J., 1997. Molecular and cellular

characterization of the membrane attack complex, C5b-9, in Alzheimer's disease. Neurobiol. Aging 18**,** 415-421.

165. Woodruff,T.M., Ager,R.R., Tenner,A.J., Noakes,P.G., Taylor,S.M., 2010. The role of the complement system and the activation fragment C5a in the central nervous system. Neuromolecular. Med 12**,** 179-192.

166. Woodruff,T.M., Costantini,K.J., Crane,J.W., Atkin,J.D., Monk,P.N., Taylor,S.M., Noakes,P.G., 2008. The complement factor C5a contributes to pathology in a rat model of amyotrophic lateral sclerosis. J Immunol. 181**,** 8727-8734.

167. Woodruff,T.M., Crane,J.W., Proctor,L.M., Buller,K.M., Shek,A.B., de,V.K., Pollitt,S., Williams,H.M., Shiels,I.A., Monk,P.N., Taylor,S.M., 2006. Therapeutic activity of C5a receptor antagonists in a rat model of neurodegeneration. FASEB J 20**,** 1407-1417.

168. Wooster,D.G., Maruvada,R., Blom,A.M., Prasadarao,N.V., 2006. Logarithmic phase Escherichia coli K1 efficiently avoids serum killing by promoting C4bp-mediated C3b and C4b degradation. Immunology 117**,** 482-493.

169. Wren,D.R., Noble,M., 1989. Oligodendrocytes and oligodendrocyte/type-2 astrocyte progenitor cells of adult rats specifically susceptible to the effects of complement in absence of antibody. Proc. Natl. Acad. Sci. USA 86**,** 9025-9029.

170. Wyss-Coray,T., Yan,F., Lin,A.H., Lambris,J.D., Alexander,J.J., Quigg,R.J., Masliah,E., 2002. Prominent neurodegeneration and increased plaque formation in complement-inhibited Alzheimer's mice. Proc. Natl. Acad. Sci. U. S. A 99**,** 10837-10842.

171. Yamada,T., McGeer,P.L., McGeer,E.G., 1992. Lewy bodies in Parkinson's disease are recognized by antibodies to complement proteins. Acta Neuropathol. 84**,** 100-104.

172. Yang,I., Han,S.J., Kaur,G., Crane,C., Parsa,A.T., 2010. The role of microglia in central nervous system immunity and glioma immunology. J Clin Neurosci. 17**,** 6-10.

173. Yasojima,K., McGeer,E.G., McGeer,P.L., 1999a. Complement regulators C1 inhibitor and CD59 do not significantly inhibit complement activation in Alzheimer disease. Brain Res. 833**,** 297-301.

174. Yasojima,K., Schwab,C., McGeer,E.G., McGeer,P.L., 1999b. Up-regulated production and activation of the complement system in Alzheimer's disease brain. Amer. J. Path. 154**,** 927-936.

175. Yuzaki,M., 2008. Cbln and C1q family proteins: new transneuronal cytokines. Cell Mol. Life Sci 65**,** 1698-1705.

176. Yuzaki,M., 2010. Synapse formation and maintenance by C1q family proteins: a new class of secreted synapse organizers. Eur. J Neurosci. 32**,** 191-197.

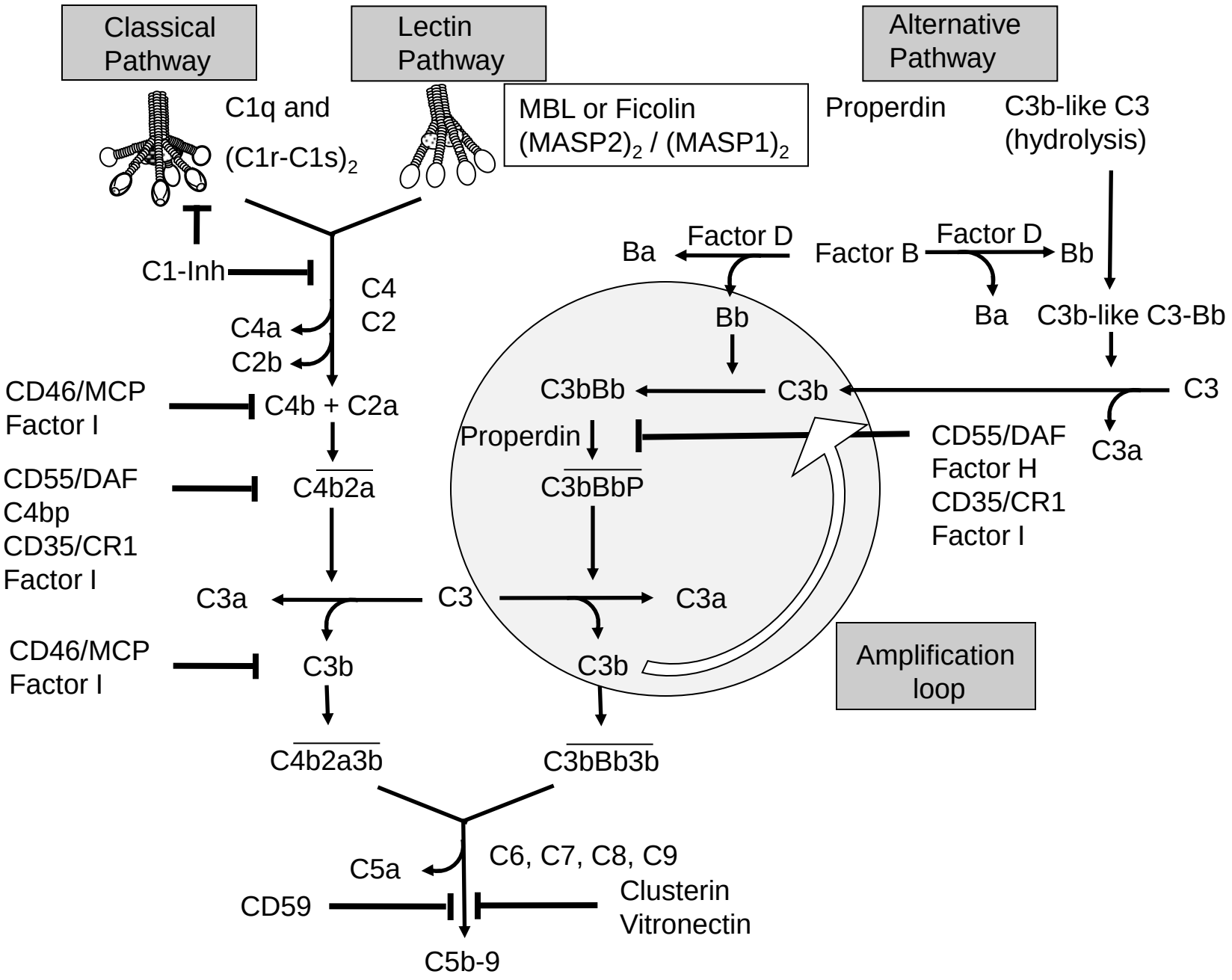
177. Zanjani,H., Finch,C.E., Kemper,C., Atkinson,J., McKeel,D., Morris,J.C., Price,J.L., 2005. Complement activation in very early Alzheimer disease. Alzheimer Dis. Assoc. Disord. 19**,** 55-66.

178. Zhan,S.S., Veerhuis,R., Kamphorst,W., Eikelenboom,P., 1995. Distribution of beta amyloid associated proteins in plaques in Alzheimer's disease and in the non-demented elderly. Neurodegeneration. 4**,** 291-297.

179. Zhang,X., Kimura,Y., Fang,C., Zhou,L., Sfyroera,G., Lambris,J.D., Wetsel,R.A., Miwa,T., Song,W.C., 2007. Regulation of Toll-like receptor-mediated inflammatory response by complement in vivo. Blood 110**,** 228-236.

180. Zhou,J., Fonseca,M.I., Pisalyaput,K., Tenner,A.J., 2008. Complement C3 and C4 expression in C1q sufficient and deficient mouse models of Alzheimer's disease. J. Neurochem. 106**,** 2080-2092.

181. Ziccardi,R.J., Cooper,N.R., 1979. Active Disassembly of the First Complement Component, C1, by C1 Inactivator. J. Immunol. 123**,** 788-792.


Classical Pathway
Lectin Pathway
Alternative Pathway
C1q and
(C1r-C1s)2
MBL or Ficolin
(MASP2)2 / (MASP1)2
Properdin
C3b-like C3
(hydrolysis)
C1-Inh
C4
C2
C4a
C2b
Ba
Factor D
Factor B
Factor D
Bb
Ba
C3b-like C3-Bb
Bb
CD46/MCP
Factor I
C4b + C2a
C3bBb
C3b
C3
Properdin
CD55/DAF
Factor H
CD35/CR1
Factor I
C3a
CD55/DAF
C4bp
CD35/CR1
Factor I
C4b2a
C3bBbP
C3a
C3
C3a
CD46/MCP
Factor I
C3b
C3b
Amplification loop
C4b2a3b
C3bBb3b
C5a
C6, C7, C8, C9
CD59
Clusterin
Vitronectin
C5b-9

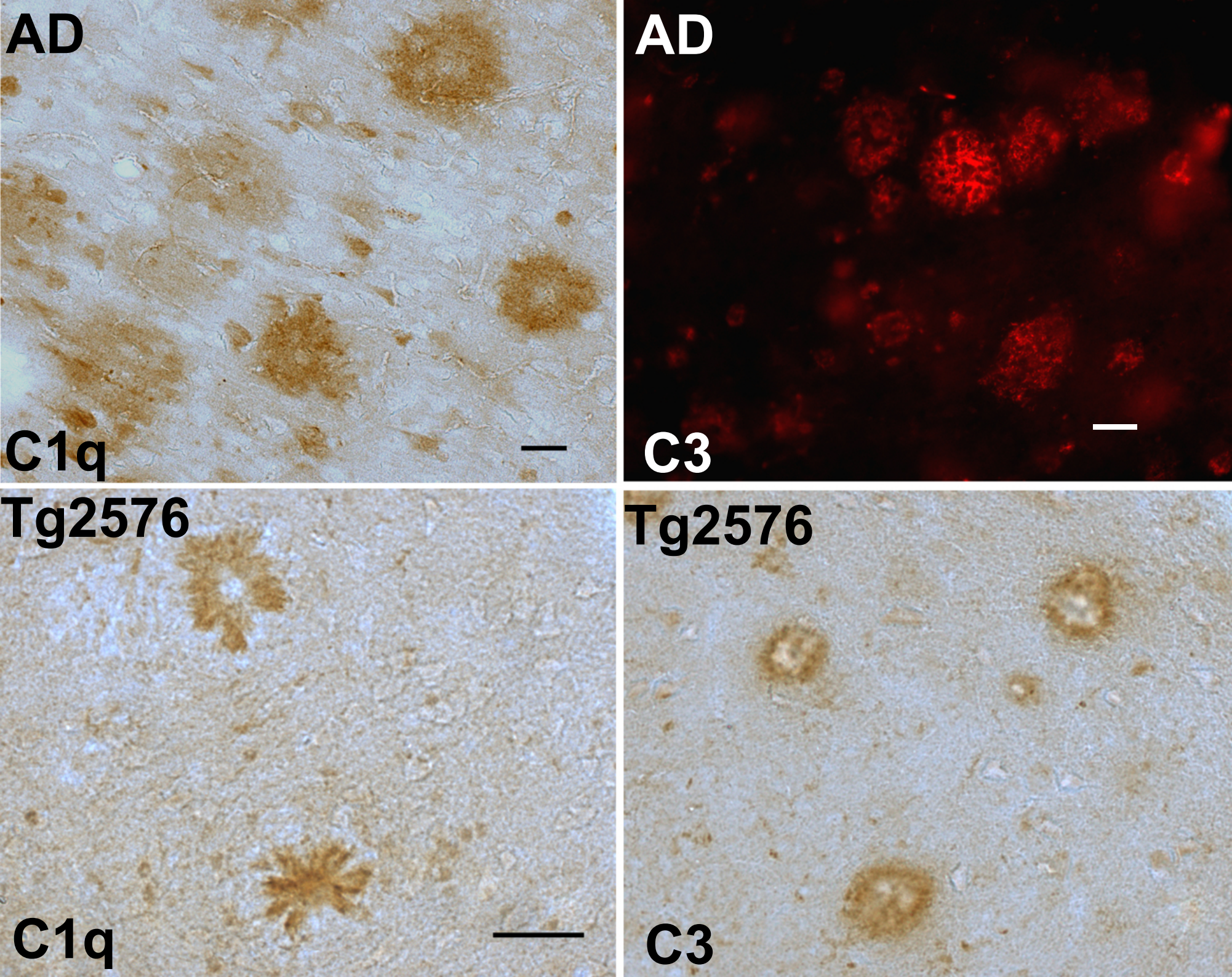

AD
C1q
AD
C3
Tg2576
C1q
Tg2576
C3

Table 1. Expression of C proteins by human astrocytes and microglia[1]

	Complement Components					
Cell Type	**Classical**	**Alternative**	**Terminal**	**C-receptors**	**C-regulators**	
					Soluble	*Membrane-bound*
Astrocytes	C1q,C1r, C1s C2, C3, C4	C3, fB, fD	C5, C6, C7, C8, C9	C1qR, CR2, C3aR, C5aR	C1-inh, fH, fI, clusterin	CD59, DAF, MCP, CR1
Microglia	C1q, C1r, C1s, C2, C3, C4	C3		C1qR, CR3, C3aR, CR4, C5aR,	C1-inh	CD59, CR1

[1] C components in the brain are inducible, most notably in response to injury. Recently reviewed in (Woodruff *et al.*, 2010).