The Complement System: An Unexpected Role in Synaptic Pruning During Development and Disease

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Abstract

An unexpected role for the classical complement cascade in the elimination of central nervous system (CNS) synapses has recently been discovered. Complement proteins are localized to developing CNS synapses during periods of active synapse elimination and are required for normal brain wiring. The function of complement proteins in the brain appears analogous to their function in the immune system: clearance of cellular material that has been tagged for elimination. Similarly, synapses tagged with complement proteins may be eliminated by microglial cells expressing complement receptors. In addition, developing astrocytes release signals that induce the expression of complement components in the CNS. In the mature brain, early synapse loss is a hallmark of several neurodegenerative diseases. Complement proteins are profoundly upregulated in many CNS diseases prior to signs of neuron loss, suggesting a reactivation of similar developmental mechanisms of complement-mediated synapse elimination potentially driving disease progression.

Contents

INTRODUCTION

The traditional view that the brain is an immune privileged organ has shifted dramatically with the growing realization that the nervous and immune systems interact on many levels in health and disease. Each system has an array of molecules and signaling pathways that have both novel and analogous functions in the other system. Among these proteins are components of the classical complement cascade, innate immune proteins traditionally associated with the rapid recognition and elimination of pathogens and harmful cellular debris.

New research reveals an unexpected role for the classical complement cascade in the developmental elimination, or pruning, of extranumerary synapses, a process critical for establishing precise synaptic circuits. Complement proteins are widely expressed in neurons and glia in the postnatal brain and are localized to subsets of developing synapses during periods of active synaptic remodeling (Stevens et al. 2007). Mice deficient in C1q, the initiating protein in the classical complement cascade, or the downstream complement protein C3 exhibit sustained defects in CNS synapse elimination and synaptic connectivity (Stevens et al. 2007). In the immune system, complement opsonizes, or tags, pathogenic microbes and unwanted cellular debris for rapid elimination by phagocytic macrophages or complement-mediated cell lysis. The surprising discovery that complement proteins are localized to developing synapses suggests that these classic immune molecules may be similarly opsonizing or tagging immature synapses for elimination during normal brain wiring.

Although synapse elimination is largely considered a developmental process, early synapse loss and dysfunction are becoming increasingly recognized as a hallmark of many CNS neurodegenerative diseases (Selkoe 2002, Mallucci 2009). Synapse degeneration associated with cognitive decline is also part of the normal aging process; however, the factors that trigger synapse loss in the aged and diseased brain remain elusive. One hypothesis is that synapse loss in CNS neurodegenerative diseases is caused by a reactivation, in the mature brain, of similar developmental mechanisms of synapse elimination. Indeed, components of the complement cascade are profoundly upregulated in Alzheimer's disease (AD), glaucoma, and other brain diseases (reviewed in Alexander et al. 2008, Rosen & Stevens 2010, Veerhuis et al. 2011) and are localized to synapses prior to signs of neuronal loss in animal models of neurodegenerative disease (Stevens et al. 2007). This notion suggests that the complement-mediated synapse degeneration process may be an early and critical event in driving the

neurodegenerative process in glaucoma and other brain diseases. Because synapse loss appears long before pathology or cognitive decline/behavioral deficits in most neurodegenerative diseases, understanding how synapses are normally pruned during development could provide mechanistic insight into how to prevent abberant synapse elimination during disease.

These provocative findings have spurred the search for molecular mechanisms underlying complement-mediated synaptic pruning. Emerging evidence implicates glial cells—microglia and astrocytes—as key players in this process. Glia are a major source of complement in the developing and diseased CNS, but they also express complement receptors that facilitate phagocytosis and secrete an array of cytokines and other factors that can initiate the complement cascade. Given that the appearance of reactive glia is a common early step in the progression of most CNS neurodegenerative diseases, further study of glia in complement-mediated synapse elimination in the diseased and normal CNS is likely to provide important insight into mechanisms underlying complement cascade regulation and function.

In this review we focus on recent progress in understanding the role of complement in regulating CNS synapse elimination and regression and discuss mechanisms of complement cascade regulation and potential targets for therapeutic intervention in CNS neurodegenerative diseases and disorders.

FUNCTION OF THE COMPLEMENT CASCADE: CLUES FROM THE INNATE IMMUNE SYSTEM

In the periphery, complement is our first line of defense against infection through the rapid elimination of invading pathogens and regulation of the slower adaptive immune response. In addition, the complement system clears modified self cells, such as apoptotic cells and cellular debris, to protect against autoimmunity (reviewed in Medzhitov & Janeway 2002, Carroll 2004, Zipfel et al. 2007, Ricklin et al. 2010). The complement system is composed of a large family (~60) of circulating and membrane-associated proteins that act synergistically in a sequential cascade-like manner to execute and regulate its functions. Circulating complement proteins, most of which are produced in the liver, are inactive proteins or zymogens until they encounter a cell membrane or biological surface. Binding results in structural modifications, proteolytic cleavage, and assembly into active enzyme complexes (convertases) that can then activate downstream substrates in a cascade-like fashion (**Figure 1**). Thus, complement zymogens can be widely distributed until locally activated.

Complement is activated by three major routes: the classical, the alternative, and the lectin pathways, all of which converge on complement component C3, a central molecule in the complement system that ultimately drives complement effector functions, including the elimination of pathogens, debris, and cellular structures (**Figure 1**). The classical pathway is canonically triggered when C1q, the initiating protein of the cascade, interacts with one of its multiple ligands, which include antigen-antibody complexes, C reactive protein (CRP), serum pentraxins, polyanions (DNA, RNA, and lipopolysaccharides), apoptotic cells, and viral membranes (Kishore & Reid 2000, Gal et al. 2009, Kang et al. 2009). The antibody-independent lectin pathway is triggered by the binding of mannose binding lectin (MBL) and a group of related proteins that recognize terminal sugar moieties expressed on polysaccharides or glycoproteins on cell membranes (Fujita 2002, Degn et al. 2007). In contrast with the classical and lectin pathways, the alternative pathway is spontaneously and continuously activated in plasma and serves to amplify the cascade initiated by classical and lectin pathways. Once C3 is cleaved, opsonization with activated C3 fragments (C3b and iC3b) leads to elimination of target structures by triggering of phagocytosis through C3 receptors on phagocytic cells (i.e., C3R/Cd11b) (**Figure 1**). Moreover, robust activation of C3 can trigger

the terminal activation of the complement cascade leading to cell lysis by inserting C5–C9 into the membrane to form the lytic membrane attack complex (MAC) (**Figure 1**).

In addition to opsonization, complement activation fragments, especially C3a, can mediate a multitude of functions including the recruitment and activation of circulating macrophages and effector cells (Nordahl et al. 2004, Zhou 2011). Much like C3a, C5a is also a potent neuroinflammatory anaphylatoxin that recruits cells expressing C5a receptors, such as macrophages in the periphery and microglia in the brain (Klos et al 2009, Zhou 2011). Although these active fragments are delivered nonspecifically to a cell surface, the progression and degree of activation of the complement cascade in the periphery is tightly controlled at

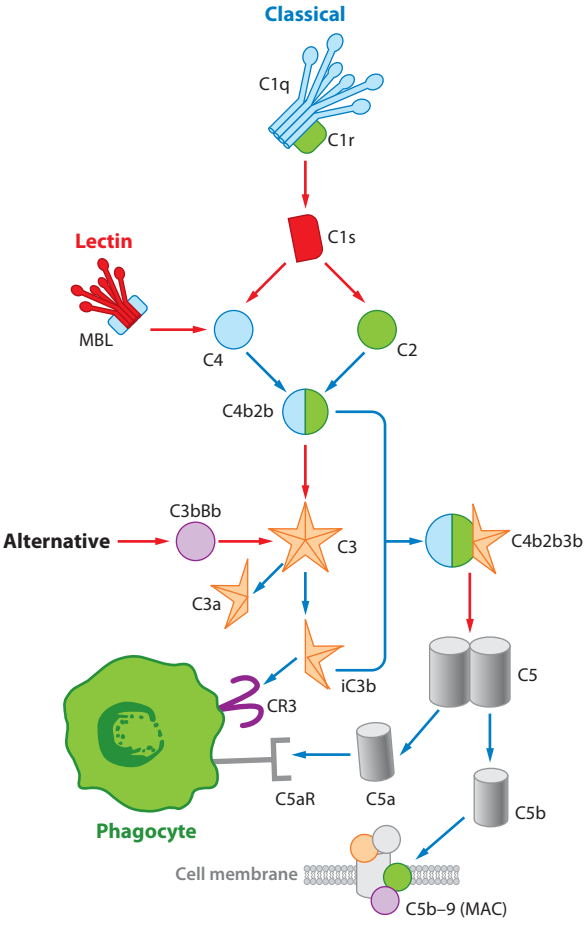

Figure 1

The complement cascade activation and function. The complement system consists of a large number of inactive components (zymogens) that are activated in a cascade-like manner to exert its biological effects in the innate immune system. Binding of complement zymogens to a membrane surface results in structural modifications, proteolytic cleavage, and the assembly into active enzyme complexes (convertases), which can then activate downstream substrates in a cascade-like fashion as shown. The complement cascade can be initiated by three major pathways: The classical pathway is induced when C1q interacts with antibodies or one of its many binding partners, such as serum pentraxins, polyanions (DNA, RNA, and lipopolysaccharides), or apoptotic cells. The C1q tail region of C1q binds proteases C1r and C1s to form the C1 complex. Binding of the C1q complex to an antibody/receptor on the cell surface induces a conformational change in the C1q molecule, which leads to activation of an autocatalytic enzymatic activity in C1r; C1r then cleaves C1s to generate the active serine protease. Once activated, C1s cleaves C4 and C2 to generate the C3 convertase, C3b2b, which in turn cleaves C3 and activates downstream cascade components. The lectin pathway is triggered by the binding of mannose binding lectin (MBL) to mannose residues on the cell surface. This activates the MBL-associated proteases mannose binding lectin serine protease 1 (MASP1) and MASP2, which then cleave C4 to generate the C4 convertase, C4b2b. The alternative pathway is spontaneously and continuously activated (via spontaneous C3 hydrolysis), which serves to amplify the cascade triggered by classical and lectin pathways. All three cascades converge on the major complement component, C3. Cleavage of C3 generates the anaphylactic peptide C3a and the opsonin C3b. Opsonization with C3b/iC3b leads to elimination of target structures by phagocytes that express C3 receptors (i.e., C3R/Cd11b). C3b later joins with C4b2a (the C3 convertase) to form the C5 convertase (C4b2a3b complex) that generates the anaphylatoxin C5a, which binds to C5a receptors (C5aR) on phagocytic/effector cells. Robust activation of complement can trigger activation of the terminal complement cascade, resulting in cell lysis through the insertion of the pore-forming C5b-C9 complex into the membrane, termed membrane attack complex (MAC).

every level by a battery of complement regulatory proteins that protect cells from aberrant elimination (Song 2006, Zipfel & Skerka 2009). Thus, a delicate balance between complement activation and inhibition is critical for proper complement function and tissue homeostasis.

COMPLEMENT EXPRESSION AND LOCALIZATION IN THE BRAIN

Complement has long been appreciated as a rapid and local immune surveillance system in the brain; however, new research has ascribed many new functions of complement in the brain that extend far beyond host defense and inflammatory processes (reviewed in Ricklin et al. 2010, Rutkowski et al. 2010b, Veerhuis et al. 2011). Although the blood brain barrier normally protects the brain from plasma-derived complement and infiltrating immune cells, many complement components can be locally produced in the brain, most often in response to injury or inflammatory signals (Zamanian et al. 2012, Veerhuis et al. 2011). Thus, local synthesis of complement is critical for local defense, neuroprotection, and homeostasis in the brain. Complement activation and its effector functions are tightly regulated by a large group of diverse molecules widely expressed throughout the organism (Zipfel & Skerka 2009), including the so-called neuroimmune regulators (NIReg), which are expressed on most nonneuronal cell types (Hoarau et al. 2011). These inhibitory molecules protect cells from uncontrolled complement-mediated damage by a range of complement inhibitor molecules, such as CD59, Complement Factor H (CFH), and Crry, which mainly interfere with C3 effector functions but also block earlier steps of complement activation. Paradoxically, inappropriate or uncontrolled complement activation can also promote inflammation, neurodegeneration, and ultimately, pathology (Sjöberg et al. 2009). This notion may be explained by the recent finding that CNS neurons, unlike most peripheral cell types, do not detectably express most of the known complement inhibitors (Cahoy et al. 2008). Thus, understanding where and when complement is normally expressed in the brain is likely to provide important insight into complement function and possible targets of intervention during development and disease.

In the CNS, complement proteins are locally synthesized by resident neurons and glial cells; however, microglia and astrocytes are the major producers of complement in the healthy and diseased CNS (Lampert-Etchells et al. 1993, Barnum 1995, Woodruff et al. 2010, Veerhuis et al. 2011). Microglia throughout the CNS express extremely high levels of C1q, as well as CR3 (CD11b) and CR5, complement receptors crucial for inducing phagocytosis of complement-coated structures and regulating cytokine signaling as well as chemotaxis, respectively (Veerhuis et al. 1999). Cultured and reactive astrocytes express high levels of C3 and other complement cascade proteins (Levi-Strauss & Mallat 1987, Cahoy et al. 2008). Complement expression by neurons has so far been described mainly in the disease context and upon injury to the CNS (Woodruff et al. 2010, Veerhuis et al. 2011). Neuronal stem cells express multiple complement receptors and differentiate and migrate in response to secreted complement. C3a–C3aR interactions were found to be a positive regulator of adult neurogenesis following ischemic injury (Rahpeymai et al. 2006, Bogestal et al. 2007, Shinjyo et al. 2009). In addition, CR2 (CD21), a receptor for activated C3 fragments, is also expressed in neural progenitor cells and regulates adult hippocampal neurogenesis (Moriyama et al. 2011).

We know that complement proteins are upregulated in neural cells following brain injury (reviewed in Veerhuis et al. 2011), but comparatively little is known about the normal function of complement proteins in the healthy brain. In situ hybridization and gene-profiling studies have unexpectedly revealed that many components of the complement system are expressed, albeit at lower levels, in the healthy brain (Stevens et al. 2007, Cahoy et al. 2008). Several components, including C1q and C3, are developmentally regulated and localized in

patterns that suggest novel functions (Stevens et al. 2007, Stephan et al. 2011). Indeed, complement has been recently implicated in several nonimmune functions during the embryonic and postnatal period, including neurogenesis, migration, neuronal survival (Benard et al. 2008, Shinjyo et al. 2009, Rutkowski et al. 2010a, Benoit & Tenner 2011), and synaptic development and elimination (Stevens et al. 2007)—the focus of this review.

THE CLASSICAL COMPLEMENT CASCADE REGULATES BRAIN WIRING DURING DEVELOPMENT

The developmental expression and synaptic localization of classical complement proteins in the postnatal brain were early clues that this family of immune proteins may function in synapse development. The first two weeks of postnatal development are a period of remarkable plasticity as immature synaptic circuits are actively remodeled (reviewed in Katz & Shatz 1996, Hua & Smith 2004, Huberman et al. 2008). There is a clear spatiotemporal correlation between the appearance and association of immature astrocytes with neurons at CNS synapses during this dynamic period. Immature astrocytes secrete an array of cytokines and other molecules that promote synapse formation and synaptic plasticity (Allen & Barres 2005, Bolton & Eroglu 2009, Eroglu & Barres 2010).

A screen to determine how astrocytes influence neuronal gene expression first identified C1q as one of the few genes that were highly upregulated in developing retinal ganglion cells (RGCs) in response to an astrocyte-derived secreted factor (Stevens et al. 2007). In contrast to microglia, which continue to express C1q in the mature brain, C1q expression in retinal neurons is developmentally restricted to the early postnatal period, when RGC axons and dendrites undergo active synaptic pruning and refinement. Indeed, immunohistochemical analyses and high-resolution imaging revealed C1q and downstream complement protein C3 are localized to subsets of synapses throughout the postnatal brain and retina (Stevens et al. 2007). Given complement's well-ascribed role as opsonins in the elimination of unwanted cells, these findings suggested that the complement cascade may be tagging weak or inappropriate synapses for elimination in the developing brain.

This idea was tested in the mouse retinogeniculate system—a classical model for studying activity-dependent developmental synapse elimination (reviewed in Shatz & Sretavan 1986, Huberman 2007, Guido 2008, Hong & Chen 2011). Early in development, RGCs form transient functional synaptic connections with relay neurons in the dorsal lateral geniculate nucleus (dLGN) of the thalamus. During the first two weeks of postnatal development, there is a robust period of synaptic remodeling in which many of these transient retinogeniculate synapses are eliminated while the remaining synaptic arbors are elaborated and strengthened (Sretavan & Shatz 1984, Campbell & Shatz 1992, Hooks & Chen 2006). Whereas the role of spontaneous and experience-driven synaptic activity in developmental synaptic pruning is well established (Katz & Shatz 1996, Sanes & Lichtman 1999, Hua & Smith 2004), surprisingly little is known about the cellular and molecular mechanisms that drive the elimination of inappropriate retinogeniculate synapses.

Consistent with a role for the classical complement cascade in synaptic pruning, neuroanatomical tracings of retinogeniculate projections and electrophysiological recordings in dLGN relay neurons showed that C1q and C3 knockout (KO) mice exhibited sustained defects in synaptic refinement and elimination, as shown by their failure to segregate into eye-specific territories and by their retention of multi-innervated LGN relay neurons (Stevens et al. 2007) (**Figure 2**). Moreover, C1q KOs show an increase in the number of presynaptic boutons and exuberant excitatory connectivity in the cortex (Chu et al. 2010), suggesting complement mediates synaptic pruning and/or remodeling in other brain regions. Although C1q and C3 KOs have sustained defects in synaptic

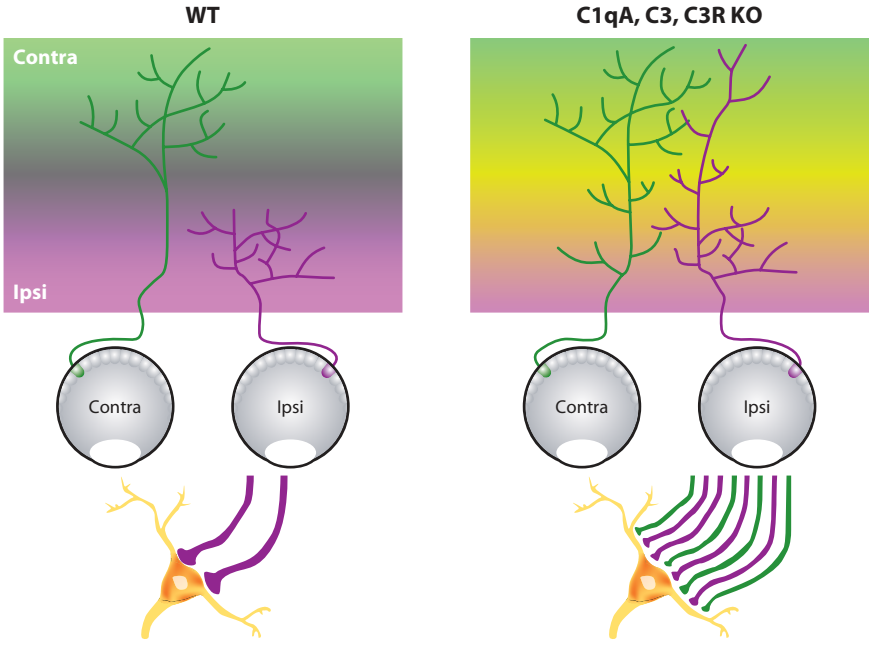

Figure 2

Classical complement cascade proteins mediate synaptic refinement in the developing retinogeniculate system. During the first postnatal week, overlapping inputs from both eyes (red versus green inputs) segregate into eye-specific territories in the dorsal lateral geniculate nucleus (dLGN) of the thalamus, resulting in the termination of ipsilateral (Ipsi) and contralateral (Contra) retinal ganglion cell (RGC) inputs in distinct nonoverlapping domains in the mature dLGN (*Left panel*). Eye-specific segregation involves the selective local pruning of overlapping parts of axonal arbors and the elaboration of the appropriate eye inputs to form the adult pattern of connections. A given relay neuron in the dLGN will ultimately receive 1–2 mature inputs from either the left or right eye (neuron, *bottom left*). Right panel: Mice deficient in classical complement cascade components, C1q and C3, and the microglia-specific complement receptor 3 (CR3) have sustained defects in eye-specific segregation compared with wild-type (WT) animals (*top*, *right*), depicted as increased overlap of ipsi- and contralateral RGC inputs in the dLGN (*yellow region*) and presence of binocularly innervated dLGN relay neurons (*bottom*, *right*).

pruning, these mice still undergo a substantial degree of synapse elimination (Stevens et al. 2007), suggesting that complement proteins cooperate with other pathways to regulate normal synaptic circuit development. Several immune-related molecules have recently been identified as mediators of synaptic refinement and plasticity in the visual system (reviewed in Boulanger 2009, Shatz 2009). These include neuronal pentraxins (e.g., NP1/2, NARP) and components of the adaptive immune system (e.g., MHC Class I (MHC-I) family of proteins and receptors) (Corriveau et al. 1998, Huh et al. 2000, Bjartmar et al. 2006, Datwani et al. 2009). Recent work suggests that C1q and MHC-I proteins colocalize at RGC synapses in the postnatal LGN (Datwani et al. 2009). Perhaps components of the complement cascade may be acting in concert with one of several of these immune-related pathways to mediate CNS synapse elimination. Together these findings raise many questions regarding the underlying cellular and molecular mechanisms.

Mechanisms of Complement-Dependent Synaptic Pruning: A Novel Role for Microglia

How are complement-tagged synapses eliminated? Emerging evidence implicates microglia

as key players in developmental synaptic pruning (Ransohoff & Stevens 2011, Tremblay & Stevens 2011). In the immune system, the activated C3 fragment C3b (iC3b) opsonizes the surface of cells/debris and tags them for elimination by phagocytic macrophages that express C3 receptors (C3R/cd11b) (Carroll 2004, Gasque 2004, van Lookeren Campagne et al. 2007). Microglia, the resident phagocytes of the CNS, are the only resident brain cells to express CR3 (Tenner & Frank 1987, Guillemin & Brew 2004, Ransohoff & Perry 2009, Graeber 2010). Indeed, process-bearing activated microglia and synaptically localized C3 have been observed in the dLGN and several other postnatal brain regions, including hippocampus, cerebellum, and olfactory bulb, undergoing active synaptic remodeling (Perry et al. 1985, Dalmau et al. 1998, Fiske & Brunjes 2000, Schafer et al. 2011); until recently, however, the function of microglia in a normal brain has remained a relative mystery.

Are microglia required for the elimination of extranumerary synapses? This question was recently investigated in the mouse retinogeniculate system. Using a combination of immune electron microscopy (EM) and high-resolution imaging, microglia were found to engulf RGC presynaptic inputs during a peak pruning period in the developing dLGN (Schafer et al. 2012). Genetic or pharmacological (minocycline) disruptions in microglia-mediated engulfment during early development result in sustained functional deficits in eye-specific segregation and synaptic pruning. Futhermore, microglia-mediated engulfment of synaptic inputs was dependent on signaling between phagocytic receptor CR3, expressed by microglia and CR3 ligand, the innate immune system molecule, and complement component C3. High-resolution quantitative analyses of structural synapses revealed that adult C3 KO and CR3 KOs have significantly more structural synapses in the visual system and other brain regions, indicating that altered complement signaling early in development results in sustained defects in synaptic connectivity (Schafer et al. 2012). Recent studies have demonstrated that microglia also engulf postsynaptic elements during synaptic remodeling in the hippocampus and visual cortex, raising the question of whether complement-dependent pruning is a more global mechanism of synaptic remodeling in the CNS (Tremblay et al. 2010, Paolicelli et al. 2011). Together these new findings suggest that microglia CR3, expressed on the surface of the microglia, and C3, enriched in synaptic compartments, interact to mediate engulfment of synaptic elements undergoing active pruning and raise several fundamental questions related to the underlying mechanisms.

Which Synapses Are Eliminated?

A longstanding question in neurobiology is what determines which synapses will be eliminated during development. Synapse elimination is thought to result from competition between neighboring axons for postsynaptic territory based on differences in patterns or levels of neuronal activity (reviewed in Shatz 1990, Sanes & Lichtman 1999, Huberman et al. 2008). Based on classic studies of the neuromuscular junction, the punishment model proposes that strong synapses, which are effective in driving postsynaptic responses, actively punish and eliminate nearby weaker, less-effective synapses by inducing two postsynaptic signals: a local protective signal and a longer-range elimination, or punishment, signal (Balice-Gordon & Lichtman 1994, Jennings 1994). To date, it is not clear whether this model is correct or relevant to CNS synapses. If it is, the identity of the putative activity-dependent punishment signal remains unknown.

Could complement tag and punish synapses destined for elimination? If so, how might selectivity occur for those synapses destined to be pruned? C1q and C3 could be preferentially tagging weaker or less-active synapses for elimination by phagocytic microglia (**Figure 3**). Alternatively, C1q/C3 could bind all synapses and only those synapses that are stronger or more active would be selectively protected by local membrane-bound complement regulatory

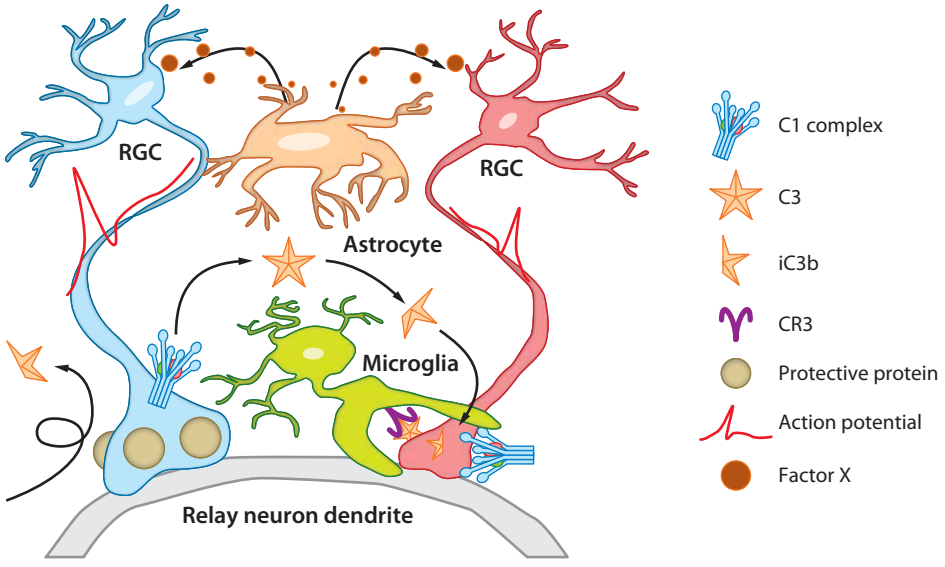

Figure 3

The complement punishment model of synapse elimination in the developing visual system. The punishment model proposes that strong synapses (*blue*), which are effective in driving postsynaptic responses, actively punish and eliminate nearby weaker, less-effective synapses (*red*) by inducing two postsynaptic signals: a local protective signal and a longer-range elimination or punishment signal (Balice-Gordon & Lichtman 1994, Jennings 1994). In the developing retina, complement is hypothesized to be upregulated in sensory neurons by unknown signals from immature astrocytes. An astrocyte-secreted factor (Factor X) upregulates C1q expression in postnatal retinal ganglion cell neurons (RGCs), which leads to deposition of C1q and the local activation of the classical complement cascade (cleavage of native C3). Synaptic deposition of activated C3 fragments (i.e., iC3b) could punish less-active retinogeniculate synapses (*red neuron*, *right*) of dLGN relay neurons in the thalamus (*target*). Activated microglia, which express high levels of CR3/cd11b, actively eliminate iC3b tagged synapses via CR3–C3-dependent phagocytosis. Strong synapses (*blue neuron*, *left*) may be protected from elimination by complement regulatory proteins or other activity-dependent protective signals).

molecules (Kim & Song 2006, Zipfel & Skerka 2009) or other activity-dependent factors. In addition, there may be a mechanism completely independent of synaptic tagging by C1q/C3 or complement regulatory molecules. For example, C3a, the anaphylatoxin and cleaved form of C3, may play a role in recruiting microglia to synaptically enriched regions (Klos et al. 2009).

In the retina, spontaneous, correlated neuronal activity from both eyes is thought to drive eye-specific segregation and retinogeniculate pruning (Penn et al. 1998, Stellwagen & Shatz 2002, Torborg & Feller 2005, Huberman 2007, Feller 2009). Although the specific properties of retinal activity that guide this process remain elusive, these findings are consistent with a model in which left- and right-eye retinal axons compete for territory on postsynaptic dLGN relay neurons. Complement and complement receptor–deficient mice have similar pruning deficits to mice in which this correlated firing has been disrupted (reviewed in Huberman 2007), suggesting that complement could act downstream of neural activity to prune inappropriate synaptic connections. Consistent with this idea, recent studies reveal that microglia-mediated pruning of RGC inputs in vivo is an activity-dependent process (Schafer et al. 2012). Indeed, microglia preferentially engulf inputs from the weaker eye, suggesting

that microglia are active participants in synaptic pruning.

In vivo imaging studies in the mouse cortex have revealed that microglial dynamics and interactions with neuronal compartments change in response to neural activity and experience (Davalos et al. 2005, Nimmerjahn et al. 2005, Wake et al. 2009, Tremblay et al. 2010), but the underlying mechanisms remain elusive. Future studies will aim to address how specific synapses are eliminated by complement and microglia-dependent mechanisms and whether neuronal activity plays a role in this process.

Candidate Complement Receptors and Interacting Proteins

Although it is clear that CR3 is an important mediator of developmental synaptic engulfment, microglia have been shown to, in some instances, express the phagocytic iC3b receptor CR4 (CD11c/CD18) (Chiu et al. 2009). In addition, the newly identified iC3b/C3 receptor immunoglobulin (Ig)-superfamily member CRig (Z39Ig, VSIG4), although not yet assessed in microglia, has been shown to mediate complement-mediated phagocytosis in subpopulations of resident tissue macrophages (Helmy et al. 2006, Gorgani et al. 2008). In some instances, C1q itself can facilitate engulfment via specific C1q receptors expressed on phagocytic cells, raising the possibility that microglia may phagocytose some synapses independently of C3 or CR3 (Bobak et al. 1986, Guan et al. 1991, Nepomuceno & Tenner 1998, Tenner 1998, Eggleton et al. 2000).

The molecular characteristics of C1q enable its interaction with a wide variety of molecules (Kishore & Reid 2000, Kojouharova et al. 2010, Nayak et al. 2011). In the immune system, C1q receptors mediate the ability of C1q to activate the complement pathway and to opsonize apoptotic cells. C1q binds via its globular head domains to pathogens and cell surfaces by directly binding to certain lipids or surface proteins or to other molecules already opsonizing (coating) the pathogen or cell surface. These opsonins include IgM or IgG antibodies and the short pentraxins serum amyloid protein (SAP) and CRP, both of which acute-phase reactant proteins quickly released by the liver into the blood upon inflammation. The long pentraxin, PTX3, functions similarly. Binding of C1q to any of these molecules can initiate the complement cascade, leading to either lysis or phagocytosis, because C1q has recently been identified as crucial for promoting phagocytosis of apoptotic cells in vivo (Taylor et al. 2000, Nauta et al. 2003). Its ability to recognize a wide range of molecular patterns suggests that C1q may interact with a variety of CNS molecules in health and disease. However, the synaptic receptors that recruit C1q and, thus, complement to synapses doomed for elimination are still unknown.

Synaptic changes occur at very early stages in most neurological diseases, which may initiate an extracellular synaptic profile that attracts C1q and which in turn mediates synapse elimination. This action potentially includes the reactivation of the molecular mechanism that marks synapses for elimination in the developing CNS or the synaptic expression of disease-specific C1q-interacting molecules that activate C1q at synapses, potentially associated with the downregulation of complement inhibitors. Alternatively, detrimental structural synaptic changes, similar to the ones observed in apoptotic cells, could trigger C1q activation and subsequent complement-dependent synapse elimination in development and disease. For example, *N*-methyl-D-aspartate (NMDA) receptor stimulation induces long-term depression transiently and locally activates caspase-3 in dendrites without causing cell death (Li et al. 2010, Jo et al. 2011). These important findings raise the interesting possibility that C1q and other complement proteins preferentially tag activated apoptotic synapses to mediate their elimination just as C1q is critical for the elimination of apoptotic cells (Taylor et al. 2000).

Lastly, there is emerging evidence for other C1q homologous, secreted proteins that play important roles in synaptic plasticity and synapse formation (Yuzaki 2010). These

include C1ql2, which is a synaptically localized protein (Iijima et al. 2010, Shimono et al. 2010), and other C1ql family members (C1ql1–4) that are expressed in the CNS and implicated in synapse formation or maintenance/elimination (Bolliger et al. 2011). In addition, the cerebellin family (Cbln1–4) is another family of C1q-like molecules that are secreted presynaptically and promote synapse formation and plasticity (Yuzaki 2010, Matsuda & Yuzaki 2011). Both presynaptic and postsynaptic receptors have been identified for Cbln1—neurexin-1β and GluRδ2 (Matsuda et al. 2010)—suggesting that cbln1 plays a critical synapse-organizing role during development (Martinelli & Sudhof 2011). Taken together, it seems likely that C1q-like molecules, including C1q, C1ql2, and Cbln1, are all likely to be part of the long mysterious mechanism through which activity-dependent competitive interactions between synapses lead some synapses to be maintained and others to be eliminated (Watanabe 2008).

Potential Cross Talk in Between Complement and Other Immune Pathways

Several other immune-related molecules have recently been identified as mediators of synaptic refinement and plasticity in the developing and mature brain (Boulanger 2009), including neuronal pentraxins (e.g., NP1/2, NARP) and components of the adaptive immune system (e.g., MHC-I family of proteins and receptors). It is intriguing to speculate that components of the complement pathway may be interacting with one of several of these immune-related molecules to mediate CNS synapse elimination.

Neuronal pentraxins are synaptic proteins with homology to pentraxins of the peripheral immune system, which are traditionally involved in opsonization and phagocytosis of dead cells in the immune system (Nauta 2003). Mice deficient in neuronal pentraxins, NP1 and NP2, and the receptor, NPR, have transient defects in eye-specific segregation in the dLGN (Bjartmar et al. 2006). In fact, an immune system pentraxin, the long pentraxin PTX3, which has homology to neuronal pentraxins, can enhance microglial phagocytic activity (Jeon et al. 2010). In addition, neuronal pentraxins are significantly homologous to short pentraxins such as CRP, which is a well-described binding partner of C1q. Thus neuronal pentraxins may serve as synaptic binding partners for C1q during synapse development and complement-mediated synaptic pruning.

Classical MHC-I molecules represent a large family of transmembrane immune proteins best known for their roles in the recognition and removal of foreign (non self) antigens. The MHC-I molecules and receptors were the first immune-related molecules implicated in developmental synapse elimination (Corriveau et al. 1998, Huh et al. 2000). MHC-I genes are highly expressed in brain regions undergoing activity-dependent synaptic remodeling (Corriveau et al. 1998, Huh et al. 2000, Datwani et al. 2009, McConnell et al. 2009, Shatz 2009). The MHC-I protein is enriched in synaptic compartments (i.e., dendrites), where it colocalizes with postsynaptic proteins, such as PSD95 (Corriveau et al. 1998, Huh et al. 2000, Goddard et al. 2007). Moreover, animals deficient in MHC-I molecules have defects in eye-specific segregation in the retinogeniculate pathway and ocular dominance plasticity, suggesting a role in developmental elimination of CNS synapses (Huh et al. 2000, Syken et al. 2006). MHC-I is highly expressed by activated microglia, including during normal development, which renders it possible that microglial MHC-I plays a role in synaptic pruning. In this context it is of particular interest that neuronal activity can regulate the surface expression of the other class of MHCs, MHC-II, specifically on microglia (Neumann et al. 1998, Biber et al. 2007).

Functional Consequences of Aberrant Complement Activation During Development

Insight into complement-mediated synaptic remodeling could have important implications for understanding the molecular basis of synapse

loss and dysfunction in cognitive and neurodevelopmental disorders in which the balance of excitation and inhibition is altered. Consistent with this idea, mice deficient in a functional classical complement cascade component (C1qA KO mice) exhibit enhanced excitatory synaptic connectivity in the mature cortex (Chu et al. 2010).

By comparison, inappropriate complement activation during synapse development could alter neural connectivity by excessively targeting synapses for elimination. Activation of microglia and the innate inflammatory process occurs after acute seizures. Indeed, complement (C1q and C3) is chronically upregulated and activated in the adult brain during early phases of epileptogenesis in both experimental and human temporal lobe epilepsy, suggesting that aberrant complement activation could play a role in destabilizing neural networks. Similarly, maternal infection during fetal development may activate immune system genes within the fetal brain (Patterson 2011), which may alter synaptic development and lead to autism or other neuropsychiatric diseases.

Recent genome-wide association studies and analyses of postmortem human brain tissue have suggested that abnormal microglial function and/or complement cascade activation may play a role in autism and psychiatric disorders such as schizophrenia (Pardo et al. 2005, Vargas et al. 2005, Hashimoto 2008, Monji et al. 2009, Chen et al. 2010, Morgan et al. 2010, Havik et al. 2011). Thus, important future questions are whether and how microglia and/or the complement cascade underlie disruptions in neuronal connectivity associated with these psychiatric disorders.

THE ROLE OF COMPLEMENT IN NEURODEGENERATIVE DISEASES AND CNS INJURY

Does a normal mechanism of developmental synapse elimination become reactivated in and drive adult neurodegenerative diseases? The hallmark of many neurodegenerative diseases is the vast loss of neurons, induced by a wide range of molecular and cellular defects, many of which are still unknown or only partially understood. However, it has recently emerged that neuron death is preceded by aberrant synaptic functioning and massive synapse loss (Selkoe 2002, Mallucci 2009). Therefore, synaptic dysfunction and synapse loss likely directly lead to neuron death and drive disease progression. This is critical when considering how best to treat these diseases, as it will obviously be pointless to develop therapies that keep neurons alive when their synapses are dysfunctional or degenerated.

Neuroinflammation, including microglial activation, reactive gliosis, and massive and early activation of the classical complement cascade, is a cardinal feature of AD and many or most other neurodegenerative diseases (Nguyen et al. 2002, Wyss-Coray & Mucke 2002). This striking degree of neuroinflammation has, however, long been considered to be a secondary event caused by neurodegeneration. The important role of the classical complement cascade and activated microglia in eliminating synapses throughout the normal developing brain, however, raises the intriguing hypothesis that complement activation actively drives the loss of synapses early in neurodegenerative disease, which in turn drives the loss of neurons and, thus, disease progression (**Figure 4**). Below we review emerging evidence that supports this possibility. The relatively low level, or possibly total lack, of complement inhibitor expression by CNS neurons likely makes them much more vulnerable to the action of the complement cascade compared with other body cell types.

Glaucoma is one of the most common neurodegenerative diseases, characterized by the elevation of intraocular pressure, the loss of RGC neurons, and optic nerve degeneration in humans, eventually resulting in blindness (see John & Howell 2012, this volume). Glaucomatous DBA/2J mice closely resemble the human disease, and this model system revealed that synapse loss precedes neuronal loss and may contribute to disease progression (Whitmore et al. 2005, Stevens et al. 2007). Classical complement component expression is

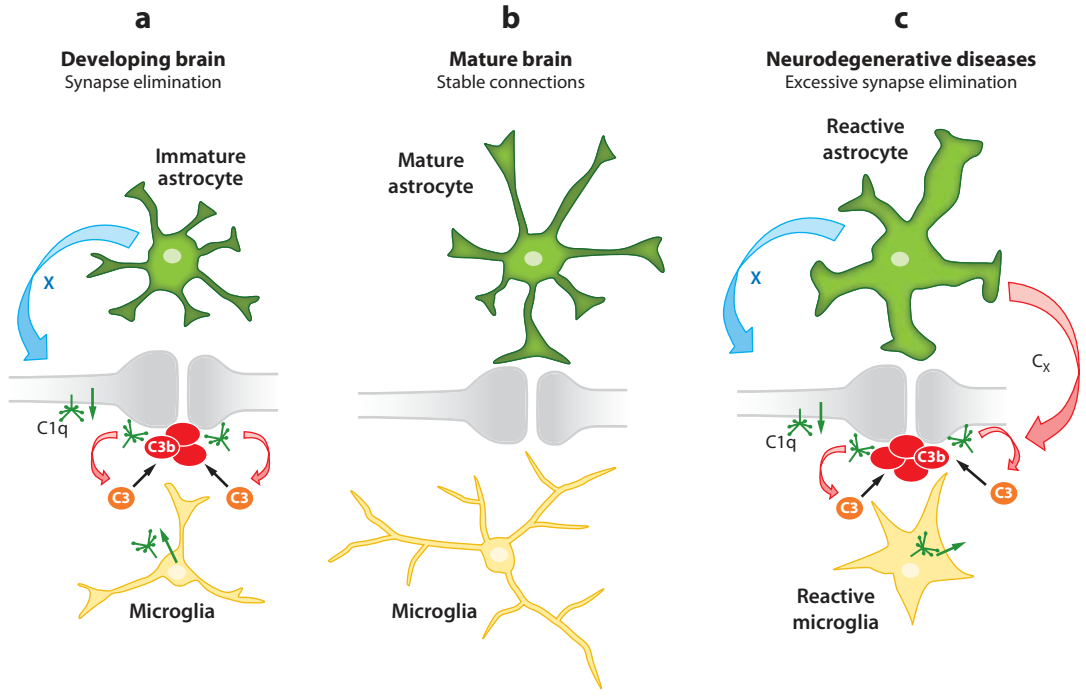

Figure 4

Complement-mediated synapse elimination during development and in neurodegenerative diseases. (*a*) In the developing brain, astrocytes induce the production of C1q in neurons through an unidentified molecular signal ("X"). Neuron and microglia-derived C1q tags weak or superfluous synapses for removal through the classical complement pathway, resulting in C3 cleavage and synaptic C3b deposition. Complement-tagged synapses are removed through phagocytosis by microglia. (*b*) In the absence of activated complement, synapses remain stable. (*c*) We propose that complement-mediated synapse elimination drives the development/progression of neurodegenerative diseases. As observed in the developing brain, reactive astrocytes release signal(s) ("X") that induce C1q production in neurons. Neuronal and microglia-derived C1q is recruited to synapses; recruitment then triggers the activation of downstream classical complement components, produced in excess by reactive astrocytes (C_X), reactive microglia, and neurons, resulting in microglia-mediated synapse elimination. Modified from Schafer & Stevens (2010).

upregulated in the murine retina during early glaucoma stages, before signs of neurodegeneration are detectable (Steele et al. 2006, Howell et al. 2011), and has also been found to be elevated in human glaucomatous retina tissue (Stasi et al. 2006). Furthermore, in the glaucomatous mouse eye, C1q immunoreactivity was shown to be upregulated in the inner plexiform layer of the retina, the synapse-rich compartment host to postsynaptic connections of RGCs. This increase in C1q immunoreactivity was temporally correlated with a decrease in synapse density, which preceded the first signs of dendrite atrophy and RGC loss (Stevens et al. 2007). Most importantly, C1q deficiency in the DBA/2J background conveys significant neuroprotection in the glaucomatous eye (Howell et al. 2011). This compelling evidence suggests that complement-mediated synapse elimination may be an early and critical event in driving the neurodegenerative process in glaucoma.

AD is the most common form of neurodegenerative dementia. A key molecular characteristic of AD is the increased generation of the amyloid-beta peptide (Aβ), a proteolytically generated derivative of the amyloid precursor protein, which accumulates in the extracellular milieu to form amyloid plaques (Glenner & Wong 1984). Many studies have

established that pronounced synaptic dysfunction and synapse loss are early features of this disease in both rodents and humans (Selkoe 2002, Spires-Jones et al. 2007, Koffie et al. 2011). Gliosis, microglia activation, and an increased expression and activation of virtually all complement components occur in the AD brain (Veerhuis et al. 2011). C1q is up to 80-fold upregulated in human AD brains (Yasojima et al. 1999). C1q deficieny in a mouse model of AD causes decreased synapse loss, AD pathology, and an improvement in cognitive function, providing direct evidence for a detrimental role of the classical complement pathway in this disease (Fonseca et al. 2004). Furthermore, several complement cascade interactors have recently emerged as susceptibility genes in AD, including ApoJ/Clusterin, a complement inhibitor, and CR1 (Jun et al. 2010, Chibnik et al. 2011, Degn et al. 2011), and enhanced levels of several complement components were detected in the cerebrospinal fluid of even presymptomatic individuals that carry familial AD disease mutations (Ringman et al. 2012). Finally, Aβ oligomers cause synapse degeneration (Wilcox et al. 2011), which is of particular interest because binding of Aβ to C1q activates the classical arm of the complement cascade (Tacnet-Delorme et al. 2001, Sim et al. 2007). Oligomeric Aβ-induced synapse loss can be detected in close proximity to amyloid plaques (Spires-Jones et al. 2007, Koffie et al. 2009), which constitutes, as the authors propose, a reservoir for Aβ-oligomers. These toxic oligomers colocalize with a subset of excitatory, degenerating synapses in vivo. Given that complement components can already be detected on amyloid plaques during early AD stages in humans (Zanjani et al. 2005), when synapse loss drives neurodegeneration, the interaction of complement with oligomeric Aβ at synapses may cause the microglia-mediated loss of these structures to drive disease progression. Taken together, these findings strongly support the idea that synaptic activation of the classical complement cascade in AD may drive disease progression by synapse elimination.

Synapse loss and neuroinflammation, including complement upregulation, are also major events in many other neurodegenerative diseases, including Huntington's disease, Parkinson's disease, and multiple sclerosis. In contrast with these neurodegenerative diseases, although C1q is highly elevated in both the mouse and human diseases, it is less clear if the loss of central synapses is a crucial early component of amyotrophic lateral sclerosis (ALS), a fatal adult-onset disorder confined to the voluntary motor system. However, neither C4 deficiency (Chiu et al. 2009) nor C3 deficiency (J.W. Lewcock, unpublished observation) is protective in the mouse SOD1 ALS model, arguing against a prominent role of complement at least in murine models of ALS. However the situation may be different for spinal muscular atrophy, an often fatal autosomal-recessive disorder of infancy caused by homozygous deletion or rare missense mutations in the survival MN 1 (*SMN1*) gene, accompanied by selective loss of motor neurons within the anterior horns of the spinal cord and early reactive gliosis (Papadimitriou et al. 2010). A recent study demonstrated that prominent CNS synapse loss precedes motor neuron degeneration in a mouse model of this disease (Mentis et al. 2011). This is one of the best examples of early synapse degeneration in a mouse model of neurodegenerative disease and may open up new avenues of future research that may reveal a role for complement in this disorder.

Although we have focused on the classical complement cascade in this review, emerging evidence implicates other limbs of the complement cascade in neurological disease susceptibility and pathophysiology. In particular, the alternative complement cascade contributes to damage after brain trauma (Leinhase et al. 2006), and genetic variations of several alternative complement cascade genes, including the complement regulator Factor H, Factor B, C2, and Factor I, confer a risk for age-related macular degeneration (AMD), a common form of blindness (Klein et al. 2005). Although it is not yet clear whether loss of retinal synapses is an

early component of AMD, this will be an important avenue of future investigation.

CONCLUSIONS AND PERSPECTIVES

The role of immune cells and immune pathways in the developing, adult, and diseased brain, long neglected, has emerged as an exciting area of research. Studying the role of the classical complement cascade pathway and proteins that interact with it is leading to a better understanding of some classical questions in neurobiology: How do synapses form, how are they eliminated, how does an activity-dependent competition sculpt the synaptic wiring of the developing brain, and why do synapses degenerate in neurodegenerative disease? The studies we have reviewed raise many exciting questions for future research. What are the glial signals that control activation of the complement cascade in the brain? Why are some synapses targeted by the complement cascade and not others? What are the critical synaptic receptors for C1q? Do neurons express novel molecules that control complement activation? Does the complement cascade drive synapse loss that accompanies normal aging? Might there be nonclassical roles for complement proteins in the CNS? Perhaps most importantly, will the development of therapeutic inhibitors of the classical complement cascade lead to a new therapy for neurodegenerative disorders and other neurological diseases and injuries? A better understanding of the roles of complement proteins in the CNS has the potential to broaden our understanding of neurodegenerative disease development and progression as well as open up new treatment strategies to interfere with the detrimental course common to all these diseases.

DISCLOSURE STATEMENT

B. Barres is a cofounder of Annexon Inc., a new company that will develop therapeutics for neurological diseases.

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