Neurobiology of Disease 127 (2019) 223–232

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

Review

Revisiting the role of the innate immune complement system in ALS

Sandra E. Parker[a], Angela M. Hanton[a], Stephen N. Stefanou[a], Peter G. Noakes[a,b], Trent M. Woodruff[a,c], John D. Lee[a,d,*]

[a] School of Biomedical Sciences, The University of Queensland, St Lucia, Brisbane, QLD 4072, Australia
[b] Queensland Brain Institute, The University of Queensland, St Lucia, Brisbane, QLD 4072, Australia
[c] Wesley Medical Research, The Wesley Hospital, Brisbane, Auchenflower, Australia
[d] University of Queensland Centre for Clinical Research, The University of Queensland, Herston, QLD 4029, Australia

A B S T R A C T

Amyotrophic lateral sclerosis (ALS) is a fatal and rapidly progressing motor neuron disease without effective treatment. Although the precise mechanisms leading to ALS are yet to be determined, there is now increasing evidence implicating components of the innate immune complement system in the onset and progression of its motor phenotypes. This review will survey the clinical and experimental evidence for the role of the complement system in driving neuroinflammation and contributing to ALS disease progression. Specifically, it will explore findings regarding the different complement activation pathways involved in ALS, with a focus on the terminal pathway. It will also examine potential future research directions for complement in ALS, highlighting the targeting of specific molecular components of the system.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is the most common form of motor neuron disease (MND), and the third most prevalent adult-onset neurodegenerative disease (Renton et al., 2011). The pathogenesis of ALS involves the progressive and selective loss of upper cortical and lower α-motor neurons in the motor cortex, brainstem and spinal cord, as well as denervation of skeletal muscle, leading to symptoms of muscle weakness, fasciculation and ultimately paralysis (Bruijn and Cleveland, 1996; Lee et al., 2013; Zarei et al., 2015). ALS is rapidly fatal, with a median life expectancy of 2–4 years from diagnosis, most commonly as a result of loss of respiratory muscle function. The aetiology of ALS is poorly understood, and the number of ALS cases are predicted to increase in coming years largely due to an ageing population, particularly in developing nations, reaching an estimated 375,000 cases worldwide by 2040 (Arthur et al., 2016). There are only a few drugs, including Riluzole and Edaravone, that are approved to treat the disease, and these have limited efficacy in patients (Dharmadasa and Kiernan, 2018; Hardiman and van den Berg, 2017). Thus, there is an urgent need to develop new therapeutics that will significantly extend survival and decrease morbidity in ALS.

There are two forms of ALS - familial and sporadic, which make up approximately 10% and 90% of cases respectively. The two aetiologies are phenotypically indistinguishable from each other based on their clinical and pathological features, both resulting in progressive muscle weakness, atrophy and spasticity (Siddique and Siddique, 2008). For familial ALS, a few of the key genes implicated include mutations in C9orf72, SOD1, TARDBP, FUS and VCP (DeJesus-Hernandez et al., 2011; Koppers et al., 2012; Kwiatkowski Jr. et al., 2009; Renton et al., 2011; Rosen et al., 1993; Sreedharan et al., 2008). Asides from genetic predisposition, several theories have been put forward regarding the causes of motor neuron death and muscle denervation in ALS. These include glutamate excitotoxicity, impaired protein homeostasis, aberrant RNA metabolism, mitochondrial abnormalities, axonal defects, impaired DNA repair, dysregulated vesicle transport, radical-mediated oxidative stress and glial and immune dysfunction leading to neuroinflammation (Brown and Al-Chalabi, 2017; Hardiman et al., 2017; Zarei et al., 2015). This review will survey evidence for a role for the innate immune complement system in driving neuroinflammation and contributing to disease progression in ALS. Specifically, it will cover findings regarding the pathways involved, particularly the role of the terminal complement pathway. It will also examine potential future directions for research into the role of the complement system in ALS.

2. The immune system in neurodegenerative diseases

The immune system has evolved as the body's primary defence against foreign and potentially infectious agents (Hwang and McKenzie, 2013). In vertebrates, we observe two distinct, yet interlinked effector arms that comprise the host's overall immunity. The first of these is the innate immune response, an evolutionarily ancient process that acts through rapid and non-specific targeting of foreign antigens by

* Corresponding author at: School of Biomedical Sciences, The University of Queensland, St. Lucia, Brisbane, QLD 4072, Australia.
E-mail address: j.lee9@uq.edu.au (J.D. Lee).

https://doi.org/10.1016/j.nbd.2019.03.003
Received 17 December 2018; Received in revised form 21 February 2019; Accepted 4 March 2019
Available online 06 March 2019
0969-9961/

monocytes, macrophages, dendritic cells and granulocytes (Akira et al., 2006). The second is the adaptive immune response, which is primarily organised around two classes of lymphocytes known as T and B cells (Dunkelberger and Song, 2010). It is from the precise and dynamic interplay between these two systems that host cells are able to maintain homeostatic conditions to protect against invading pathogens.

As it is now recognised however, abnormalities in the same immunological cascades that accompany these responses have been linked to immuno-inflammatory disease. Additionally, in more recent years these same immune systems are believed to contribute to a large part to neurodegenerative disorders, including Alzheimer's disease and other dementias, Parkinson's disease, Huntington's disease and ALS. Compelling evidence has revealed how the pathologies of such diseases may not be restricted to cell-autonomous mechanisms of the neurons themselves, but rely crucially on non-neuronal involvement (Ilieva et al., 2009). In the case of ALS, this phenomenon is supported by investigations into mice overexpressing the ALS mutant SOD1 protein in motor neurons and non-neuronal cells (Gong et al., 2000; Lino et al., 2002; Pramatarova et al., 2001). Indeed, such studies identified the presence of the mutant protein in motor neurons and astrocytes alone, was not sufficient enough to cause neurodegeneration, and at most, the animals only experienced minor ALS-like phenotypes - a stark contrast to transgenic mice systemically overexpressing the same protein in all cells (Gong et al., 2000; Heurich et al., 2011; Lino et al., 2002; Pramatarova et al., 2001). Therefore, at least in this model, motor neuron toxicity is derived from external cell types and factors. One suggested candidate that may drive motor neuron toxicity in ALS is a pathogenic contribution from components and cells of the innate immune system (Boillee et al., 2006; Clement et al., 2003; Kang et al., 2013; Wang et al., 2009; Yamanaka et al., 2008a; Yamanaka et al., 2008b).

Until recently, the central nervous system (CNS) was considered an immunoprivileged region due to the presence of a blood brain and spinal cord barrier that prevents passage of the major immune cells and molecules (Louveau et al., 2015). Numerous studies have now demonstrated however, a local expression of immune molecules, as well as evidence of immune cell infiltration into the CNS from the periphery (Engelhardt et al., 1993; Greter et al., 2005; Kierdorf et al., 2013; Togo et al., 2002; Troost et al., 1990). During disease, the current hypothesis describes resident glial cells, such as microglia, astrocytes and oligodendrocytes, acting as CNS immune effectors in response to noxious self-derived molecules, so-called damage associated molecular patterns (DAMPS), or danger signals, from dying neurons. Early in the disease, these cells are thought to protect surrounding neuronal tissue, clearing any debris and secreting growth promoting factors into the cell's microenvironment, as would be expected under normal physiological conditions (Julier et al., 2017; Sochocka et al., 2017). However, sustained chronic neuroinflammation via continued stimulation from dying neurons as disease progresses can cause these glial cells, as well as those infiltrating immune cells via an increasingly compromised blood brain and spinal cord barrier, to shift to a neurotoxic status and release pro-inflammatory mediators, thereby accelerating degeneration (Hanisch and Kettenmann, 2007; Liddelow et al., 2017; Nagai et al., 2007). Whilst the triggers for this phenotypic shift remains unknown, there is evidence that the self-amplifying, and inflammatory properties of the complement system may be the key driver (Brennan et al., 2016; Morgan, 2018). The ability of complement effector molecules to not only modulate innate immune responses, but also to interface with adaptive immune biology, initiate synapse degeneration and regulate the blood brain and spinal cord barrier integrity, has become increasingly appreciated as a driving force behind neurodegenerative pathogenesis (Tenner et al., 2018).

3. Complement system activation in the CNS

The complement system is a key component of the innate immune system. It is comprised of a large collection of well over 50 identified blood-circulating and membrane-bound proteins, including receptors and regulators, which act in a cascade manner to augment the immune response and rid the body of exogenous threats in the form of foreign pathogens and noxious self-derived molecules generated by cellular stress responses and death (Kolev et al., 2014). The complement system undertakes its defensive role primarily through its ability to regulate inflammation through the activation of complement receptors on innate and adaptive immune cells, as well as its ability to eliminate pathogens directly through cytotoxic and cytolytic activity of the membrane attack complex (MAC (C5b-9); (Hess and Kemper, 2016). Although the CNS does not receive the same composition of circulating complement factors produced in the liver due to the blood brain and spinal cord barriers, many studies have demonstrated that multiple components of the complement cascade can be locally synthesised in the CNS, where it has been shown to be produced by neurons, astrocytes, oligodendrocytes and microglia (Barnum, 1995; Gasque et al., 1997; O'Barr et al., 2001). Similar to the periphery, one primary role of complement activation within the CNS is to protect neurons from toxic stimuli through the activation of inflammatory responses by surrounding glial cells. However, under-regulated and over-activation of the complement system may lead to unwarranted neuronal death observed in multiple neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, Huntington's disease and ALS (Kjaeldgaard et al., 2018; Loeffler et al., 2006; Morgan, 2018; Singhrao et al., 1999). In addition to their immune surveillance functions, it has also been noted that complement factors have a key role in nervous system development, cellular metabolism, regeneration and regulation of CNS homeostasis (Coulthard et al., 2018a; Coulthard et al., 2017; Coulthard et al., 2018b; Hawksworth et al., 2017; Stevens et al., 2007).

The complement system is known to be activated via four different pathways: the classical, lectin, alternative, as well as the increasingly recognised extrinsic pathway. Complement activation leads to the production of opsonins (i.e. C1q and C3b), anaphylatoxins (i.e. complement cleavage peptides C3a and C5a) and the formation of the cytolytic MAC (Fig. 1). The classical complement pathway is primarily activated by the recognition molecule C1q, which binds to antigen-antibody complexes and endogenous pattern recognition molecules such as immunoglobins (IgG and IgM) and pentraxins (C-reactive proteins) to initiate complement activation. It can also bind to non-pathogen surfaces such as beta-amyloid or liposomes to act as an opsonin (Jiang et al., 1994), and has recently been identified as forming complexes with ApoE in Alzheimer's disease (Yin et al., 2019). The opsonisation of foreign pathogens or DAMPs by C1q enhances their recognition and engulfment by immune cells through their complement receptor 1 (CR1) and the phagocytic receptor cC1qRp (Paidassi et al., 2008). C1q has also been shown to bind directly to apoptotic cells, necrotic cells and blebs of degenerating neurons in the CNS via its globular head domain and facilitate ingestion via CR1 and/or complement receptor 3 on microglia and macrophages (Fraser et al., 2010). This binding process is deemed critical in the CNS for rapid clearance of toxic cellular debris released by dying neurons (Galvan et al., 2012). The lectin pathway is activated in response to complexes formed when pattern recognition molecules such as mannose-binding lectin (MBL) and ficolins binds to carbohydrate groups called mannan, present on the surfaces of some pathogens (Shastri et al., 2013). MBL and ficolins are also known to opsonise exposed carbohydrate groups on apoptotic cells, which can promote phagocytosis by monocytes and macrophages, similar to C1q dependent phagocytosis (Tenner et al., 1995). The alternative pathway is initiated by bacteria and foreign surfaces, and leads to an amplification of the spontaneous hydrolysis of C3, forming C3 (H_2O). This then binds to factor B proteases and is cleaved by factor D to generate a C3 convertase. This constant 'tick-over' of C3 helps the system to stay alert and assists with normal surveillance of the surrounding environment (Ricklin et al., 2010). Similar to classical and lectin pathways, the alternative pathway is also activated in response to

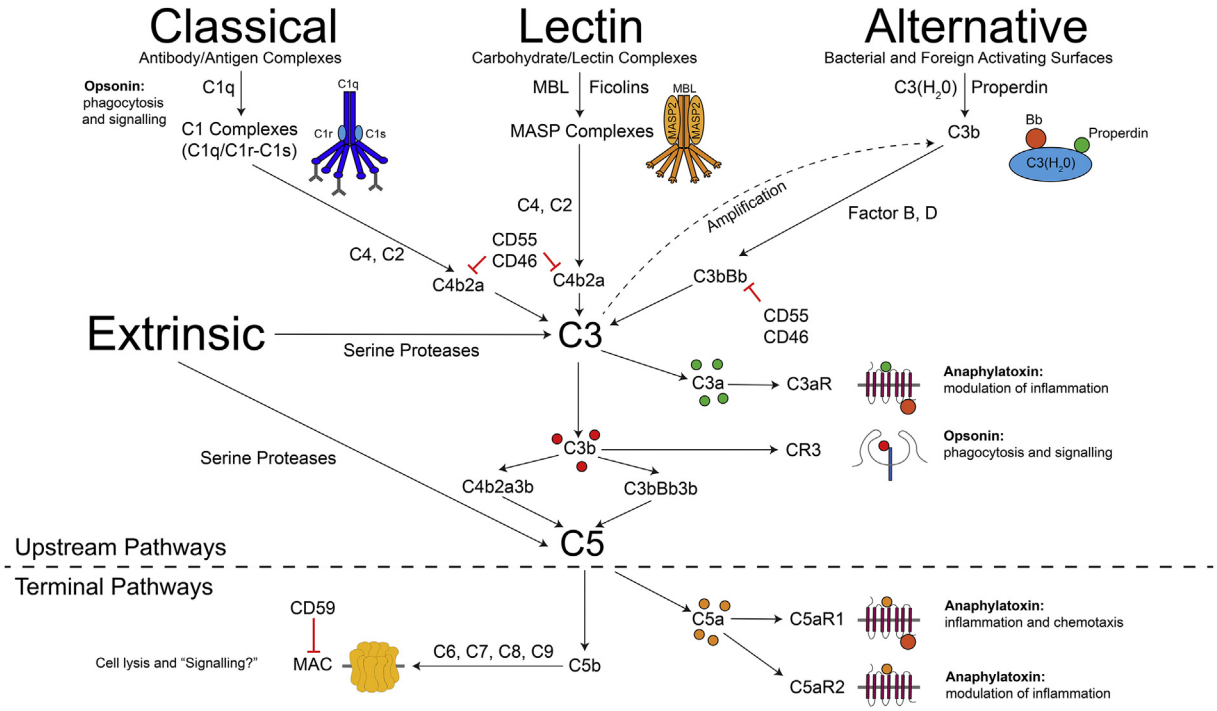

Fig. 1. The complement system. Schematic overview of the complement system, including properties of its major upstream and downstream effectors. Classical, lectin and alternative pathways converge on component C3, which is subsequently cleaved by convertase enzymes (C4b2a and C3bBb) to generate C3a and C3b. Independent cleavage of C3 and C5 by serine proteases may also occur via the extrinsic pathway. Subsequent C5 activation releases the system's two terminal molecules, C5a and C5b, both involved in immunity through formation of the membrane attack complex (MAC), and C5a receptors (C5aR) activation.

pattern recognition molecule such as Properdin, where it recognises several DAMPs on foreign and apoptotic cells. This leads to C3b binding to these pattern recognition molecules resulting in assembly and stabilisation of the C3 convertase complexes on these cells (Ricklin et al., 2010).

The three canonical complement pathways all converge on the central and most abundant complement molecule, C3, with its cleavage leading to generation of its active fragments C3a and C3b. C3a is a small peptide that triggers signalling through its corresponding seven-transmembrane G-protein coupled receptor C3aR. It is expressed on most myeloid cells like granulocytes, mast cells, monocytes, macrophages and microglia as well as on dendritic cells and astrocytes (Quell et al., 2017). The activation of the receptor leads to several immune modulatory actions, which include chemotaxis, pro-inflammatory cytokine production, degranulation and oxidative burst (Coulthard and Woodruff, 2015). However, there is also compelling evidence that also suggests that C3aR stimulation has anti-inflammatory effects (Coulthard and Woodruff, 2015; Kildsgaard et al., 2000; Wu et al., 2013). The other cleavage product, C3b, is an opsonin that, along with C1q, can directly target pathogens and dying neurons for degradation (Ricklin et al., 2016). Generation of C3b through cleavage of C3 exposes a specific short-lived thioester moiety which attaches to specific carbohydrate groups on activated surfaces, leading to preferential opsonisation of foreign pathogens and DAMPs released by dying neurons. It has also been shown to play a pathological role in immunological demyelination and neurodegeneration in the spinal cord (Ingram et al., 2014). Of equal importance, C3b is also an essential factor in generating C5 convertases to cleave C5, which initiates the terminal complement activation pathway (Daha et al., 1976; Vogt et al., 1978).

Another complement activation pathway called the extrinsic pathway bypasses the need for any upstream complement activity, and involves direct cleavage of C3 and C5 into its biological active peptides C3a/C3b and C5a/C5b via proteolytic enzymes such as thrombin, plasmin, kallikrein and factor XIIa (Huber-Lang et al., 2006), as well as intracellular enzymes such as cathepsin L (Liszewski et al., 2013). As a result, synthesis of C5 by local inflammatory cells can produce C5a via cleavage of C5 with cell derived proteases, even when devoid of the complement system precursor, C3 (Huber-Lang et al., 2006). This pathway may provide a source of terminal complement activation factors in the absence of upstream complement activation, and in a local tissue environment such as the CNS (Woodruff et al., 2014).

4. The terminal complement pathway

The cleavage of C5 leads to the formation of the two major effectors of complement via its two active fragments C5a and C5b. C5a, like C3a, is a small polypeptide, which is considered to be one of the most potent inflammatory molecules generated from the immune response, and exhibits a broad range of biological and pathological functions (Manthey et al., 2009). C5a exerts its effects through two high affinity receptors, the classical and generally predominant signalling receptor C5aR1, and the lesser investigated alternate receptor, C5aR2 (also referred to as C5L2).

C5aR1 is a member of the rhodopsin family of seven transmembrane domain receptors coupled to the hetero-metric G proteins of the G_i subtype: pertussis toxin-sensitive $G_{\alpha i}2$, $G_{\alpha i}3$ or pertussis toxin-insensitive $G_{\alpha 16}$. Cellular activation of C5aR1 leads to intracellular calcium mobilization and the initiation of a variety of downstream signalling pathways, all of which support pro-inflammatory responses. These include chemotaxis of immune cells, and their subsequent activation leading to superoxide production, release of proteases, kinases and cytokines/chemokines, thus setting off an immuno-inflammatory cascade (Gomez-Cambronero et al., 2007; Manthey et al., 2009; Melendez and Ibrahim, 2004; Torres and Forman, 1999). Its expression has been widely documented on immune cell types such as monocytes/macrophages, dendritic cells, neutrophils and eosinophils in the periphery (Karsten et al., 2015) as well as astrocytes, microglia and neurons in the CNS (Woodruff et al., 2010).

Since its discovery in 2000, the physiological role of the less understood second receptor C5aR2 has remained somewhat controversial

(Li et al., 2013). C5aR2 is a seven-transmembrane domain receptor that is grouped into the G-protein coupled receptor family like C5aR1. In contrast to the C5aR1, however, it is not coupled to intracellular G-protein activated signalling pathways due to an amino acid replacement of arginine by leucine in the DRY motif in the second intracellular loop structure (Croker et al., 2016). Previous studies have shown contradictory roles of C5aR2, where different groups have suggested either pro- or anti-inflammatory functions (Zhang et al., 2017). Early studies have presented a theory that C5aR2 acted as a decoy receptor for C5aR1, due to its inability to induce intracellular calcium mobilization, extracellular signal-related kinase phosphorylation or receptor internalization, which are all consequences of C5aR1 activation by C5a (Lee et al., 2012; Okinaga et al., 2003). This has led to the proposal that C5aR2 may regulate the availability of C5a binding to C5aR1, or by forming oligomers with C5aR1, to interrupt C5a – C5aR1 signalling and thus act in an anti-inflammatory manner (Croker et al., 2016). Furthermore, another study supported the anti-inflammatory function as it demonstrated C5aR2 may function as an intracellular receptor, which becomes activated once the ligand binds to C5aR1. It was suggested that C5aR2 could negatively modulate C5a – C5aR1 signalling and limit the signalling capacity of C5a via its interaction with C5aR1 and β-arrestin (Bamberg et al., 2010; Wang et al., 2013). In direct contrast to decoy receptor and β-arrestin models of C5aR2 function, several studies have shown that C5aR2 deficient mice had a diminished response to C5a, including a reduced influx of inflammatory cells and a attenuation of cytokines such as IL-6 and TNF-α compared to wild-type mice (Chen et al., 2007; Hsu et al., 2014). Furthermore, some studies have also found evidence for C5aR2 playing a direct pro-inflammatory role by acting as a trigger for the release of cytokines, including extracellular release of the pro-inflammatory transcription factor HMGB1 (Rittirsch et al., 2008).

The other fragment of C5, C5b, in conjunction with the remaining complement components C6 – C9 forms the terminal cytolytic MAC on cell membranes through non-enzymatic assembly. Once proteolytic cleavage of C5 into C5b has occurred, C6 binds to a labile binding site on C5b, quickly associating with C7, and forming a lipophilic complex (Hadders et al., 2012). Membrane insertion of the complex occurs when C8 binds, allowing it to penetrate through the cell membrane lipid bilayer. Once inserted, the complex acts as a receptor for C9, and when bound, catalyses its oligomerisation into multiple C9 molecules (Serna et al., 2016). These molecules of C9 allow the complex to form a pore that perforates the membrane, and permits the flux of ions, such as calcium and sodium, into the cell, ultimately leading to cell death via osmotic lysis or metabolic storm (Brennan et al., 2016; Hadders et al., 2012). Unlike C5aR2 with its seemingly contradicting functions, MAC has clearly identified pathogenic roles in various CNS disorders, including neurotrauma and multiple sclerosis, where MAC appears to play a role in facilitating neuronal death through direct cell lysis or potentially via inflammatory activation (Fluiter et al., 2014; Harhausen et al., 2010; Ingram et al., 2009; Michailidou et al., 2018).

5. Clinical and experimental evidence of upstream complement pathway involvement in ALS

Substantial research has been accumulated from ALS patients investigating levels of complement components during disease progression. Additionally, ALS animal models have been useful in corroborating findings from human patients, as well as allowing investigators to perform disease-modifying interventions in a controlled research environment. Components of various complement activation pathways have been found to be altered in serum samples, neurological tissues and skeletal muscles from ALS patients and also in mouse models of ALS (Kjaeldgaard et al., 2018).

5.1. Classical complement pathway

The classical complement pathway was initially implicated in ALS pathogenesis, as various studies demonstrated elevated levels of C1q and C4 in the serum, cerebrospinal fluid (CSF), CNS (motor cortex and spinal cord) and skeletal muscle tissue of ALS patients (Apostolski et al., 1991; Bahia El Idrissi et al., 2016a; Sta et al., 2011; Trbojevic-Cepe et al., 1998; Tsuboi and Yamada, 1994; Yamada et al., 1994). The first of these studies showed significantly increased levels of C4 in the serum from 37 ALS patients when compared to healthy volunteers (Apostolski et al., 1991). Similar results were found in subsequent studies, where upregulated levels of C4 and C4d were identified in the CSF and serum of ALS patients (Trbojevic-Cepe et al., 1998; Tsuboi and Yamada, 1994). More recently, increased levels of C1q and C4 were found on glial cells in the vicinity of motor neurons in the spinal cord and motor cortex of 16 ALS patients when compared to 10 control cases who had died from non-neurological diseases (Sta et al., 2011). Furthermore, another study also looked at the expression and localisation of C1q in the post-mortem intercostal muscle biopsies from ALS patients and control donors who did not suffer from neuromuscular or neurological disease (Bahia El Idrissi et al., 2016a). Increased levels of C1q on the nerves innervating the motor end plates and in the vicinity of nerve endings in the intercostal muscles of ALS patients was identified, while C1q was undetectable in control biopsies. Additionally, this study provided evidence that C1q was deposited on innervated motor end-plates early in the disease process, prior to denervation, suggesting that it may play a role in degradation of the neuromuscular junction through its opsonisation properties (Bahia El Idrissi et al., 2016a).

In addition to the clinical studies implicating the classical complement pathway in pathophysiology of ALS, many studies in ALS animal models have also confirmed the role of upstream classical complement pathways in disease progression. The first study to demonstrate the involvement of the classical complement pathway was performed by Olsen and colleagues in 2001. They demonstrated up-regulation of C1qA and C1qB mRNA transcripts in the spinal cord of $SOD1^{G93A}$ transgenic mice at later stages of disease (Olsen et al., 2001). Subsequent studies experimentally profiled the changes in complement components in isolated lumbar motor neurons from SOD1 transgenic mouse models including $SOD1^{G93A}$, $SOD1^{G37R}$ and $SOD1^{G85R}$ mice using microarray analysis and real time quantitative PCR (Ferraiuolo et al., 2007; Lobsiger et al., 2007; Perrin et al., 2005). An increase in the mRNA transcripts of classical pathway recognition component C1q (C1qA, C1qB and C1qC) was identified in motor neurons of SOD1 transgenic mice, when compared to motor neurons from wild-type mice. Furthermore, another study using $SOD1^{L126delTT}$ transgenic mice showed elevated mRNA levels of C1qB in lumbar spinal cord at post-symptomatic stages using microarray analysis (Fukada et al., 2007). Similarly, many studies have also demonstrated that mRNA levels of C1qB, as well as C4, were continually elevated in the lumbar spinal cords of $SOD1^{G93A}$ mice following onset of disease (Ferraiuolo et al., 2007; Heurich et al., 2011; Lee et al., 2013; Lobsiger et al., 2013). Another study demonstrated classical complement induction in a non-SOD1 animal model of ALS, with increases in C1qB and C4 mRNA levels in the lumbar spinal cord of $TDP43^{Q331K}$ transgenic mice identified (Lee et al., 2018). More recently, studies have also shown peripheral changes in classical complement proteins using $SOD1^{G93A}$ and $TDP43^{Q331K}$ ALS mice. These studies demonstrated upregulated mRNA expression of C1qB and C4 in the tibialis anterior (primarily fast-twitch) muscles of $SOD1^{G93A}$ and $TDP43^{Q331K}$ mice at the mid-symptomatic and end stages of disease (Lee et al., 2018; Wang et al., 2017). In support of this, another study also revealed an elevated presence of these C1q and proteins at the motor end-plates of $SOD1^{G93A}$ mice, suggesting that it could play a role in degradation of neuromuscular junction (Bahia El Idrissi et al., 2016a; Heurich et al., 2011).

5.2. Central component C3

Multiple studies have also shown upregulation of the central complement component, C3 in ALS patients. An early study showed through immunofluorescence techniques that C3 was deposited in the spinal cord and motor cortex of ALS patients, primarily on astrocyte-like cells (Donnenfeld et al., 1984). Similarly, C3 staining was increased in ALS patient glial cells within the spinal cord and motor cortex (Sta et al., 2011). In support of this, C3 was upregulated in the CSF of 71 ALS patients when compared to 40 healthy controls, and increased levels of C3 cleavage products C3c and C3dg in the serum of 186 ALS patients (Ganesalingam et al., 2011; Goldknopf et al., 2006). A recent seminal study demonstrated that C3 positive astrocytes, termed neurotoxic A1 astrocytes, were increased in the spinal cord of ALS patients. These A1 astrocytes are induced by factors released from activated microglia, and could drive neuronal death in culture (Liddelow et al., 2017). This suggests that C3 expressing A1 astrocytes may be integral for disease initiation and progression in ALS. Furthermore, the previously mentioned study by Bahia El Idrissi et al. (2016a, 2016b) also found deposition at the motor end plate of both C3 and C3b prior to onset of symptoms. The complement regulator CD55, (also known as decay accelerating factor), which prevents formation of C3 convertase, was also present at the motor end-plate of ALS patients but not control donors, suggesting an attempt at protecting the motor end-plates from further complement activity and degradation (Bahia El Idrissi et al., 2016a; Fig. 2A).

There have also been consistent reports implicating C3 involvement in experimental models of ALS. When compared to age-matched wild-type controls, an upregulation of C3 mRNA and protein expression is observed in SOD1^{G93A} rodents' spinal cord (Heurich et al., 2011; Lee et al., 2013; Woodruff et al., 2008). Notably, the activation fragment of C3, C3b was shown to co-localize with activated glial cell parenchyma, and was deposited in close proximity to dying motor neurons, suggesting that C3b could assist in the removal of dying motor neurons via opsonisation, during disease progression. The central component of all complement pathways, C3 was also increased in the lumbar spinal cord of TDP43^{Q331K} mice, suggesting that complement activation occurs in response to motor neuron death regardless of which ALS-related gene mutation is present (Lee et al., 2018). In terms of peripheral complement dysregulation, there have been documented increases in C3 deposition at the motor end plates and nerve terminals of SOD1^{G93A} mice, starting at the pre-symptomatic stage, and upregulated C3 mRNA in the SOD1^{G93A} and TDP43^{Q331K} tibialis anterior muscle (Heurich et al., 2011; Lee et al., 2018; Wang et al., 2017). Additionally, these studies showed decreased mRNA levels of the complement regulator CD55 in the spinal cord and increased levels in the skeletal muscles of SOD1^{G93A} and TDP43^{Q331K} mice, which suggests that homeostatic balance of complement system is perturbed in these models leading to over-activation of the complement system.

5.3. Alternative and lectin pathway

In comparison to the widely documented expression of classical and central components of the complement system during ALS disease progression, few studies to date have directly investigated complement components associated with alternative and lectin pathways (Kjaeldgaard et al., 2018). A moderate increase in mRNA levels of factor B from the alternative pathway was identified in the lumbar spinal cord and tibialis anterior muscles of SOD1^{G93A} and TDP43^{Q331K} mice (Lee et al., 2013; Lee et al., 2018). However, these studies failed to detect levels of mannose binding lectin (MBL), in the lumbar spinal cords of either SOD1^{G93A} or TDP43^{Q331K} mice (Lee et al., 2013; Lee et al., 2018)(Fig. 2B). It is possible that other lectin pathway initiators such as Ficolin could be involved (Xu et al., 2018). To date however, no study has investigated the functional role of the alternative or lectin pathways in ALS pathogenesis.

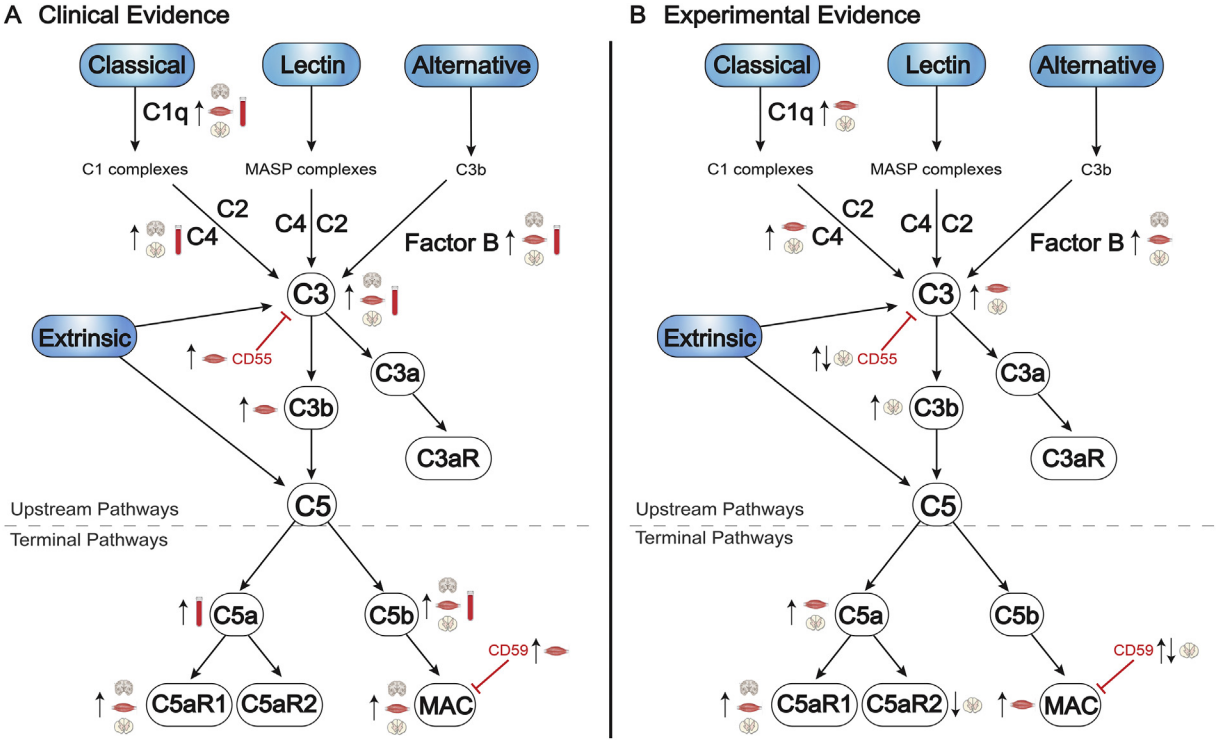

Fig. 2. Complement induction during amyotrophic lateral sclerosis (ALS). Components of the complement system accompanied by reported mRNA or protein expression changes in the central nervous system (brain and spinal cord) and the periphery (blood and muscle) of diseased subjects as compared to healthy controls. Shown are representative results from studies of human patients (Panel A) or animal models (Panel B) of ALS, recorded at varying stages of the disease.

6. Clinical and experimental evidence of terminal complement involvement in ALS

Somewhat surprisingly, in contrast to the above studies implicating an important role for classical and central complement component in the pathogenesis of ALS, prior studies using knockout mice have indicated no major role for these pathways in disease pathology. Genetic deletion of upstream complement components C1q, C3 and C4 in SOD1 transgenic ALS mouse models showed a reduction in macrophage levels, but ultimately did not show any significant beneficial effects on disease progression and survival (Chiu et al., 2009; Lobsiger et al., 2013). Although these were germline gene knockout studies, which may have some limitations, this indicates that the upstream complement pathway may not be an integral factor in the pathophysiology of ALS. To support the involvement of terminal complement pathway in the disease progression of ALS, previous studies by our group have shown that the genetic deletion of C5aR1, and the administration of a C5aR1 antagonist to $SOD1^{G93A}$ mice, significantly extended survival and improved motor function (Lee et al., 2017; Woodruff et al., 2014). One explanation for the differing outcomes from these studies could be that deleting the upstream complement components C1q, C3 and C4 does not eliminate the direct activation of C5 via the extrinsic pathway, and thus the biological function of the cleavage products, C5a and C5b are not inhibited (Woodruff et al., 2014). Indeed, a prior study using C3 knockout mice demonstrated a compensatory upregulation prothrombin to enable protease-mediated C5 cleavage (Huber-Lang et al., 2006). Regardless, the similar findings from C5aR1 knockout and C5aR1 antagonist-treated $SOD1^{G93A}$ mice strongly indicate that the terminal complement pathway may be more important in driving ALS pathophysiology.

Coinciding with the potential role for terminal complement activation products in ALS pathogenesis, a previous study demonstrated increased levels of C5a and terminal complement complex (C5b-9) in the serum of 54 ALS patients when compared to 49 age and gender-matched healthy controls (Mantovani et al., 2014). Additionally, elevated C5a levels within leukocytes from these patients was also identified, suggesting increased C5a – C5aR1 signalling on these immune cells. In addition to peripheral upregulation of terminal pathway components, an earlier study also found that C5aR1 immunostaining was significantly increased in motor neurons of ALS patients when compared to healthy controls, which was localised to the cell membrane and vesicular-like cytoplasmic structures (Humayun et al., 2009). Utilisation of in vitro studies suggested that heightened neuronal C5aR1 signalling could contribute to the motor neuron death in ALS. Previous studies have also demonstrated increased deposition of MAC in both neurological tissue (motor cortex and spinal cord) and intercostal muscle biopsies from ALS patients (Bahia El Idrissi et al., 2016a; Sta et al., 2011). Using immunohistochemistry, the first of these studies indicated the presence of C5b-9/MAC in neuronal and glial cells in the spinal cord and motor cortex of ALS patients, but not in control tissue (Sta et al., 2011). Furthermore, C5b-9/MAC deposition on the motor end-plates in intercostal muscle biopsies was identified in ALS patients, while no deposition was observed in control tissue. Like C1q and C3b, this deposition occurred early in the disease, prior to end-plate denervation, suggesting that the MAC may contribute to the loss of motor end-plates (Bahia El Idrissi et al., 2016a). CD59a, a regulator of C5b-9/MAC formation, was also detected at the motor end-plates of ALS patient muscle biopsies, but not controls, suggesting that the muscle could be attempting to counteract complement activation to prevent MAC deposition (Fig. 2A).

In addition to the role of terminal complement pathway in ALS patients, extensive investigation into the terminal pathway, particularly C5a – C5aR1 signalling, have demonstrated that this pathway has disease-modifying consequences in ALS rodent models. There have been several reports of C5aR1 up-regulation in the lumbar spinal cord of $SOD1^{G93A}$ rodents and $TDP43^{Q331K}$ mice with its expression localised to both motor neurons and glia (Lee et al., 2013; Lee et al., 2018; Woodruff et al., 2008). Additionally, up-regulation of C5aR1 mRNA and protein was also observed in mice deficient in the low molecular weight neurofilament (NFL) subunit protein, another mouse model of motor neuron degeneration in which NFL aggregates in a similar fashion to that of ALS patients (Humayun et al., 2009). In separate studies, $SOD1^{G93A}$ rats and mice were treated with the specific C5aR1 antagonist, PMX205. Chronic administration of PMX205 in $SOD1^{G93A}$ rats markedly delayed the onset of motor symptoms and increased survival, compared to untreated animals (Woodruff et al., 2008). Furthermore, PMX205 treatment in $SOD1^{G93A}$ mice also extended survival, improved motor functions (i.e. hind-limb grip strength) and slowed disease progression (Lee et al., 2017). These results using pharmacological inhibition of C5aR1 are consistent with previous findings where deletion of the *C5ar1* gene in $SOD1^{G93A}$ mice extended survival and improved hind-limb grip strength (Wang et al., 2017; Woodruff et al., 2014). More recently, it was also demonstrated that C5aR1 was increased in the tibialis anterior muscle of $SOD1^{G93A}$ and $TDP43^{Q331K}$ mice during disease progression where it was localised to infiltrating macrophages (Lee et al., 2013; Wang et al., 2017). Furthermore, a dramatic decrease in the number of macrophages infiltrating into the tibialis anterior muscle of $SOD1^{G93A}$ animals lacking C5aR1 was demonstrated, in line with the improvements shown in muscle strength (Wang et al., 2017). Hence, this suggests that C5a – C5aR1 signalling could potentially accelerate ALS disease progression through increasing the recruitment of pro-inflammatory macrophages to sites of motor neuron death and neuromuscular denervation (Fig. 2B).

While extensive research has been conducted on the role of C5aR1 in ALS progression in ALS rodent models, the other by-product of C5 cleavage, C5b, has received more limited attention to date. Despite this, there have been numerous studies that have investigated CD59a, a regulatory complement protein that inhibits MAC formation. Up-regulation of mRNA levels of CD59a in the lumbar spinal cord of $SOD1^{G93A}$ mice was observed early in disease, indicating an initial attempt at controlling excessive complement activation (Lee et al., 2013). However, mRNA levels of CD59a ultimately decreased in the lumbar spinal cord of $SOD1^{G93A}$ mice at later stages of disease, causing a disruption in complement homeostasis, leading to excessive complement activation due to defective regulatory mechanisms (Baalasubramanian et al., 2004; Lee et al., 2013). Similarly, another study also demonstrated increased CD59a mRNA levels in the tibialis anterior muscle of $SOD1^{G93A}$ mice, while interestingly MAC deposition was also increased in the neuromuscular junction of $SOD1^{G93A}$ mice (Bahia El Idrissi et al., 2016a; Wang et al., 2017). This strongly suggests that a dysregulation in MAC formation during ALS disease progression exists. To further validate a role for MAC in ALS progression, another study showed that targeting complement C6 with an antisense oligonucleotide and thus inhibiting MAC formation resulted in delayed disease progression and extended survival (Bahia El Idrissi et al., 2016b). Collectively, these findings suggest that MAC may also have a pathogenic role in ALS progression (Fig. 3).

7. Future directions towards therapeutic application of complement-targeted drugs in ALS

Current therapies for ALS are inadequate. The only clinically approved medications for ALS are Riluzole (Rilutek, Aventis Pharmaceuticals Inc.) and Edaravone (Radicava, Mitsubishi. Tanabe). Riluzole has been shown to extend survival of patients by a modest 2–3 months while a subset of patients receiving Edaravone showed a 33% reduction in the decline of their physical abilities (Dharmadasa and Kiernan, 2018; Writing and Edaravone, 2017). Despite these therapies, there is still a distinct lack of truly effective disease-modifying treatments for ALS, and thus, there is an urgent need to develop new therapeutics to meet these needs. As a result, an increased understanding into the pathophysiology of ALS is vital for the

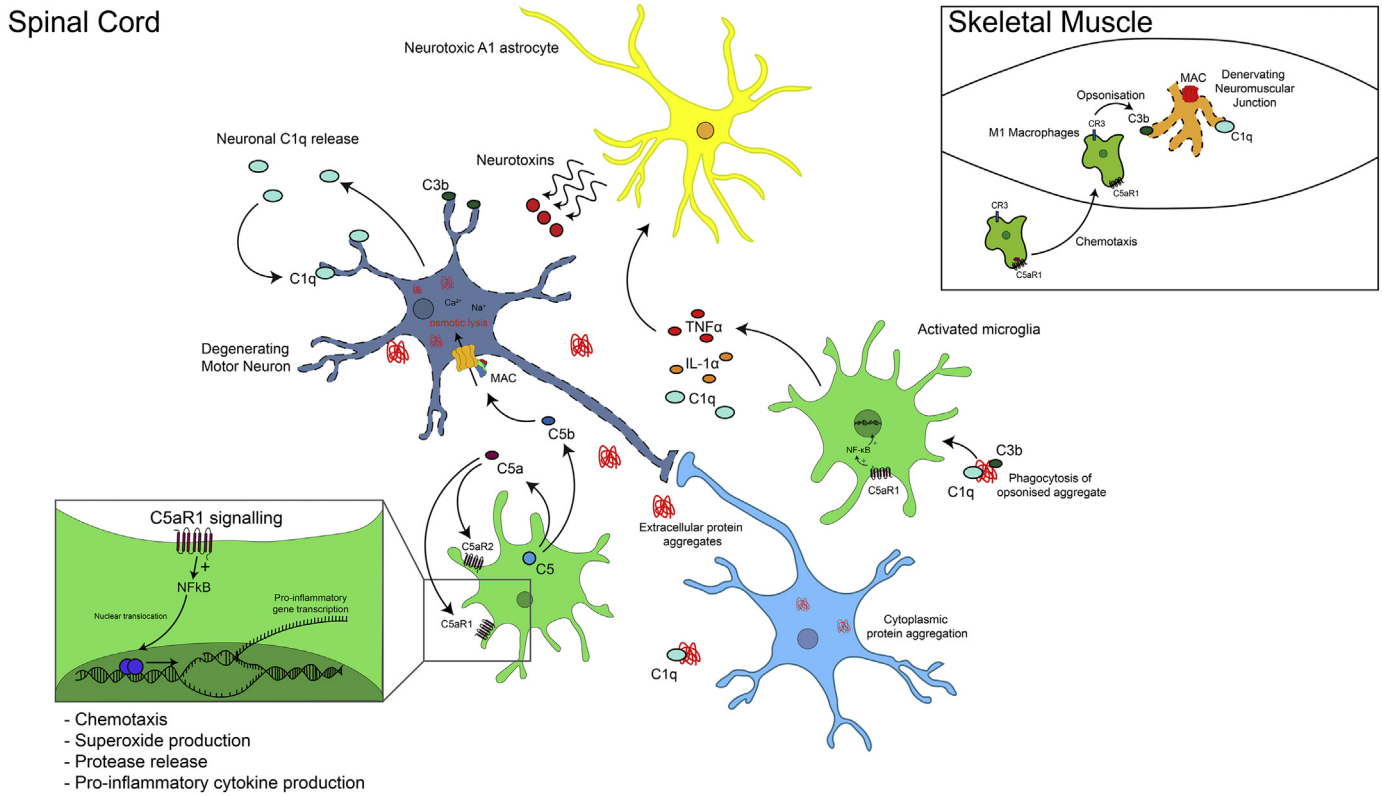

Fig. 3. Proposed mechanism of complement activation in the spinal cord and skeletal muscle during ALS. Complement is activated by cytoplasmic inclusions or aggregates like TDP43 in degenerating motor neurons. This leads to deposition and opsonization of C1q and C3b on motor neuron surfaces and protein aggregates for clearance by activated microglia through phagocytosis. This also leads to the production of anaphylatoxin C5a and subsequent activation of C5aR1 and C5aR2. C5aR1 activation on microglia recruits the cell to sites of motor neuron death (chemotaxis) and induces superoxide production, protease release and pro-inflammatory cytokines including TNFα and IL-1α. Release of these astrocyte activating signals from activated microglia (TNFα, IL-1α and C1q) will also cause these reactive astrocytes (A1 astrocytes) to release neurotoxins that will kill the motor neurons. Unlike C5aR1, the functional role of the second C5a receptor C5aR2 in ALS is still unknown. Complement activation also lead to the formation of membrane attack complex (MAC) on degenerating motor neurons, which will ultimately lyse these cell. In this proposed model, overactivation of complement following initial disease onset, propagates inflammation and cause motor neuron death, leading to further complement activation and contributing to disease pathology (self-perpetuating neurotoxicity). In skeletal muscle, denervation of neuromuscular synapses promotes infiltration of peripheral macrophages through C5a– C5aR1 signalling, to phagocytose the degenerating neuromuscular synapses through opsonin C1q and C3b. Deposition of MAC also contribute to the loss of degenerating neuromuscular synapses during ALS.

development of novel, disease-modifying therapeutics. Research into the innate immune complement system and its pathogenic role in ALS progression has revealed promising findings since its commencement around two decades ago. Within the complement cascade, there have been numerous proteins implicated in the progressive and selective neurodegeneration seen in ALS, not only in animal models but also in human patients. Given that complement system plays such a dynamic role in innate and adaptive immune responses, these factors remain a viable target that could lead to the amelioration of motor symptoms, and ultimately a greatly improved life expectancy for ALS patients.

Several complement inhibitors that target different components of the cascade are in clinical development that may be potential therapies to treat ALS patients (for review see (Ricklin et al., 2017)). Such compounds include eculizumab, compstatin and PMX205 or Avacopan, which together can block key proteins of the system (i.e. C3, C5 and C5a), neutralising the effects of excessive complement activation. Although previous studies showed no effect on gene deletion of upstream molecules C1q, C3 and C4 in SOD1 transgenic mice (Chiu et al., 2009; Lobsiger et al., 2013), investigation of inhibitors of upstream complement molecules in animal models of ALS is warranted, due to the limitations of gene knockout studies, including downstream compensation by terminal components. Unlike the upstream complement components, the terminal complement pathway has been clearly shown to play an important role in driving ALS pathophysiology in rodent models. The cyclic peptide C5a receptor antagonist PMX205 is a promising treatment for ALS, that is currently undergoing clinical development by Alsonex Pty Ltd. This compound, originally derived from the linear C5aR1 antagonist Me-FKPdChaWR (Konteatis et al., 1994) and later cyclised for structural and metabolic stability (Finch et al., 1999; March et al., 2004), has been shown to display therapeutic efficacy in numerous rodent models of inflammatory disease. These include rheumatoid arthritis (Woodruff et al., 2002), ischemic reperfusion injuries (Arumugam et al., 2002), inflammatory bowel disease (Jain et al., 2013; Woodruff et al., 2003), as well as toxin-induced neurodegeneration (Woodruff et al., 2006). PMX205 is more lipophilic than the original C5aR1 antagonist PMX53, which increases its CNS penetrance and makes it more suitable for CNS disorders (Kumar et al., 2018). Hence it has been extensively used to reduce disease severity and prolong survival in animal models of neurodegenerative disorders including Alzheimer's disease (Ager et al., 2010; Fonseca et al., 2009). Furthermore, pre-clinical investigation in rodent models of ALS in our laboratory demonstrated that PMX205 could effectively enter the brain and spinal cord of $SOD1^{G93A}$ mice at levels substantially above the expected IC_{50} for C5aR1 inhibition (Lee et al., 2017). Combined with its documented beneficial effects on survival, motor function and disease progression in rat and mouse models of ALS (Lee et al., 2017; Woodruff et al., 2008), this promotes the use of the compound for future clinical trials in ALS. It will be important to validate the efficacy seen with PMX205 in $SOD1^{G93A}$ rodents, in other familial mouse models of ALS, such as $TDP43^{Q331K}$ mice where C5aR1 expression was also shown to be

increased in the spinal cord and skeletal muscle (Lee et al., 2018). This will bolster the confidence of using PMX205 in future clinical trials for ALS.

There are remaining components of the terminal complement pathway that have yet to be interrogated for their involvement in ALS. While much interest has been directed towards C5a and its modulation of chronic inflammation in neurodegeneration, few resources have explored the potential for its fragment counterpart, C5b and the multimeric MAC in ALS (Bahia El Idrissi et al., 2016b). Although the C5 antibody eculizumab is clinically available, and would block the production of both C5a and C5b, it is unlikely this antibody would be able to enter the CNS to prevent local complement activation. Regardless, targeting C5 cleavage itself may be another potential therapeutic target to block all terminal pathways in ALS patients, which may have even greater beneficial effects on the disease progression. Finally, the potential for the non-classical C5a receptor, C5aR2, in regulating aberrant processes in ALS neurodegeneration provides another line of research for the future. Despite these potential therapeutic targets within the complement cascade, the multifactorial nature of ALS will likely require combined therapies targeting other key systems to effectively treat its pathologies. Anti-complement inhibitors may provide one much needed step towards ALS treatment, to ease and ameliorate motor symptoms for patients with this debilitating disease.

8. Conclusions

Although the precise pathological mechanisms behind ALS are yet to be determined, there is now increasing evidence implicating components of the complement system in the onset and progression of its motor phenotypes. Specifically, the aberrant activation or regulation of the complement system may be acting as a driving force for non-cell autonomous damage, wherein immune cell recruitment and pro-inflammatory activation causes an accelerated degeneration of motor neurons. Clinical evidence comparing human ALS patients to healthy controls has confirmed upregulated complement components in the blood, CSF, spinal cord and brain. Supporting findings include studies of complement system involvement in the $SOD1^{G93A}$ mouse, a well-documented model of the human condition, as well as other transgenic animals such as $TDP43^{Q331K}$ and $NFL^{-/-}$ mice. A prominent breakthrough in the complement/ALS field has come from investigations into its terminal pathways; most notably, from genetic and pharmacological blockade of C5aR1 and C6. Although the full myriad of complement proteins is yet to be examined for their potential efficacy in ALS research, numerous studies have inextricably linked complement pathways to the harmful disease processes. Therefore, a continued research effort into complement-induced neuroinflammation is warranted, as there exists a potential for important therapeutic targets to be identified to treat this debilitating disease.

Acknowledgements

The authors acknowledge support from the Motor Neuron Disease Research Institute of Australia (MNDRIA), the National Health and Medical Research Council (NHMRC; project grant 1082271), FightMND, Wesley Medical Research, and an Advance Queensland Innovation Partnership grant. TMW holds a NHMRC Career Development Fellowship (1105420) and JDL is also supported by a MNDRIA Postdoctoral Fellowship (PDF1604).

References

Ager, R.R., et al., 2010. Microglial C5aR (CD88) expression correlates with amyloid-beta deposition in murine models of Alzheimer's disease. J. Neurochem. 113, 389–401.
Akira, S., et al., 2006. Pathogen recognition and innate immunity. Cell 124, 783–801.
Apostolski, S., et al., 1991. Serum and CSF immunological findings in ALS. Acta Neurol. Scand. 83, 96–98.
Arthur, K.C., et al., 2016. Projected increase in amyotrophic lateral sclerosis from 2015 to 2040. Nat. Commun. 7, 12408.
Arumugam, T.V., et al., 2002. Protective effect of a new C5a receptor antagonist against ischemia-reperfusion injury in the rat small intestine. J. Surg. Res. 103, 260–267.
Baalasubramanian, S., et al., 2004. CD59a is the primary regulator of membrane attack complex assembly in the mouse. J. Immunol. 173, 3684–3692.
Bahia El Idrissi, N., et al., 2016a. Complement activation at the motor end-plates in amyotrophic lateral sclerosis. J. Neuroinflammation 13, 72.
Bahia El Idrissi, N., et al., 2016b. Complement component C6 inhibition decreases neurological disability in female transgenic SOD1G93A mouse model of amyotrophic lateral sclerosis. Ann Neurodegener Dis. 1, 1015.
Bamberg, C.E., et al., 2010. The C5a receptor (C5aR) C5L2 is a modulator of C5aR-mediated signal transduction. J. Biol. Chem. 285, 7633–7644.
Barnum, S.R., 1995. Complement biosynthesis in the central nervous system. Crit. Rev. Oral Biol. Med. 6, 132–146.
Boillee, S., et al., 2006. Onset and progression in inherited ALS determined by motor neurons and microglia. Science 312, 1389–1392.
Brennan, F.H., et al., 2016. Therapeutic targeting of complement to modify disease course and improve outcomes in neurological conditions. Semin. Immunol. 28, 292–308.
Brown, R.H., Al-Chalabi, A., 2017. Amyotrophic lateral sclerosis. N. Engl. J. Med. 377, 162–172.
Bruijn, L.I., Cleveland, D.W., 1996. Mechanisms of selective motor neuron death in ALS: insights from transgenic mouse models of motor neuron disease. Neuropathol. Appl. Neurobiol. 22, 373–387.
Chen, N.J., et al., 2007. C5L2 is critical for the biological activities of the anaphylatoxins C5a and C3a. Nature 446, 203–207.
Chiu, I.M., et al., 2009. Activation of innate and humoral immunity in the peripheral nervous system of ALS transgenic mice. Proc. Natl. Acad. Sci. U. S. A. 106, 20960–20965.
Clement, A.M., et al., 2003. Wild-type nonneuronal cells extend survival of SOD1 mutant motor neurons in ALS mice. Science 302, 113–117.
Coulthard, L.G., Woodruff, T.M., 2015. Is the complement activation product C3a a proinflammatory molecule? Re-evaluating the evidence and the myth. J. Immunol. 194, 3542–3548.
Coulthard, L.G., et al., 2017. Complement C5aR1 Signaling promotes polarization and proliferation of embryonic neural progenitor cells through PKCzeta. J. Neurosci. 37, 5395–5407.
Coulthard, L.G., et al., 2018a. Complement C3a receptor modulates embryonic neural progenitor cell proliferation and cognitive performance. Mol. Immunol. 101, 176–181.
Coulthard, L.G., et al., 2018b. Complement: the emerging architect of the developing brain. Trends Neurosci. 41, 373–384.
Croker, D.E., et al., 2016. Discovery of functionally selective C5aR2 ligands: novel modulators of C5a signalling. Immunol. Cell Biol. 94, 787–795.
Daha, M.R., et al., 1976. C3 requirements for formation of alternative pathway C5 convertase. J. Immunol. 117, 630–634.
DeJesus-Hernandez, M., et al., 2011. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron 72, 245–256.
Dharmadasa, T., Kiernan, M.C., 2018. Riluzole, disease stage and survival in ALS. Lancet Neurol. 17, 385–386.
Donnenfeld, H., et al., 1984. Deposits of IgG and C3 in the spinal cord and motor cortex of ALS patients. J. Neuroimmunol. 6, 51–57.
Dunkelberger, J.R., Song, W.C., 2010. Role and mechanism of action of complement in regulating T cell immunity. Mol. Immunol. 47, 2176–2186.
Engelhardt, J.I., et al., 1993. Lymphocytic infiltrates in the spinal cord in amyotrophic lateral sclerosis. Arch. Neurol. 50, 30–36.
Ferraiuolo, L., et al., 2007. Microarray analysis of the cellular pathways involved in the adaptation to and progression of motor neuron injury in the SOD1 G93A mouse model of familial ALS. J. Neurosci. 27, 9201–9219.
Finch, A.M., et al., 1999. Low-molecular-weight peptidic and cyclic antagonists of the receptor for the complement factor C5a. J. Med. Chem. 42, 1965–1974.
Fluiter, K., et al., 2014. Inhibition of the membrane attack complex of the complement system reduces secondary neuroaxonal loss and promotes neurologic recovery after traumatic brain injury in mice. J. Immunol. 192, 2339–2348.
Fonseca, M.I., et al., 2009. Treatment with a C5aR antagonist decreases pathology and enhances behavioral performance in murine models of Alzheimer's disease. J. Immunol. 183, 1375–1383.
Fraser, D.A., et al., 2010. C1q enhances microglial clearance of apoptotic neurons and neuronal blebs, and modulates subsequent inflammatory cytokine production. J. Neurochem. 112, 733–743.
Fukada, Y., et al., 2007. Gene expression analysis of the murine model of amyotrophic lateral sclerosis: studies of the Leu126delTT mutation in SOD1. Brain Res. 1160, 1–10.
Galvan, M.D., et al., 2012. C1q and phagocytosis: the perfect complement to a good meal. J. Leukoc. Biol. 92, 489–497.
Ganesalingam, J., et al., 2011. Combination of neurofilament heavy chain and complement C3 as CSF biomarkers for ALS. J. Neurochem. 117, 528–537.
Gasque, P., et al., 1997. Expression of the receptor for complement C5a (CD88) is up-regulated on reactive astrocytes, microglia, and endothelial cells in the inflamed human central nervous system. Am. J. Pathol. 150, 31–41.
Goldknopf, I.L., et al., 2006. Complement C3c and related protein biomarkers in amyotrophic lateral sclerosis and Parkinson's disease. Biochem. Biophys. Res. Commun. 342, 1034–1039.
Gomez-Cambronero, J., et al., 2007. Understanding phospholipase D (PLD) using leukocytes: PLD involvement in cell adhesion and chemotaxis. J. Leukoc. Biol. 82, 272–281.

Gong, Y.H., et al., 2000. Restricted expression of G86R Cu/Zn superoxide dismutase in astrocytes results in astrocytosis but does not cause motoneuron degeneration. J. Neurosci. 20, 660–665.
Greter, M., et al., 2005. Dendritic cells permit immune invasion of the CNS in an animal model of multiple sclerosis. Nat. Med. 11, 328–334.
Hadders, M.A., et al., 2012. Assembly and regulation of the membrane attack complex based on structures of C5b6 and sC5b9. Cell Rep. 1, 200–207.
Hanisch, U.K., Kettenmann, H., 2007. Microglia: active sensor and versatile effector cells in the normal and pathologic brain. Nat. Neurosci. 10, 1387–1394.
Hardiman, O., van den Berg, L.H., 2017. Edaravone: a new treatment for ALS on the horizon? Lancet Neurol. 16, 490–491.
Hardiman, O., et al., 2017. Amyotrophic lateral sclerosis. Nat Rev Dis Primers. 3, 17085.
Harhausen, D., et al., 2010. Membrane attack complex inhibitor CD59a protects against focal cerebral ischemia in mice. J. Neuroinflammation 7, 15.
Hawksworth, O.A., et al., 2017. Complement in the fundamental processes of the cell. Mol. Immunol. 84, 17–25.
Hess, C., Kemper, C., 2016. Complement-mediated regulation of metabolism and basic cellular processes. Immunity. 45, 240–254.
Heurich, B., et al., 2011. Complement upregulation and activation on motor neurons and neuromuscular junction in the SOD1 G93A mouse model of familial amyotrophic lateral sclerosis. J. Neuroimmunol. 235, 104–109.
Hsu, W.C., et al., 2014. C5L2 is required for C5a-triggered receptor internalization and ERK signaling. Cell. Signal. 26, 1409–1419.
Huber-Lang, M., et al., 2006. Generation of C5a in the absence of C3: a new complement activation pathway. Nat. Med. 12, 682–687.
Humayun, S., et al., 2009. The complement factor C5a receptor is upregulated in NFL−/− mouse motor neurons. J. Neuroimmunol. 210, 52–62.
Hwang, Y.Y., McKenzie, A.N., 2013. Innate lymphoid cells in immunity and disease. Adv. Exp. Med. Biol. 785, 9–26.
Ilieva, H., et al., 2009. Non-cell autonomous toxicity in neurodegenerative disorders: ALS and beyond. J. Cell Biol. 187, 761–772.
Ingram, G., et al., 2009. Complement in multiple sclerosis: its role in disease and potential as a biomarker. Clin. Exp. Immunol. 155, 128–139.
Ingram, G., et al., 2014. Complement activation in multiple sclerosis plaques: an immunohistochemical analysis. Acta Neuropathol Commun. 2, 53.
Jain, U., et al., 2013. The C5a receptor antagonist PMX205 ameliorates experimentally induced colitis associated with increased IL-4 and IL-10. Br. J. Pharmacol. 168, 488–501.
Jiang, H., et al., 1994. Beta-amyloid activates complement by binding to a specific region of the collagen-like domain of the C1q a chain. J. Immunol. 152, 5050–5059.
Julier, Z., et al., 2017. Promoting tissue regeneration by modulating the immune system. Acta Biomater. 53, 13–28.
Kang, S.H., et al., 2013. Degeneration and impaired regeneration of gray matter oligodendrocytes in amyotrophic lateral sclerosis. Nat. Neurosci. 16, 571–579.
Karsten, C.M., et al., 2015. Monitoring and cell-specific deletion of C5aR1 using a novel floxed GFP-C5aR1 reporter knock-in mouse. J. Immunol. 194, 1841–1855.
Kierdorf, K., et al., 2013. Bone marrow cell recruitment to the brain in the absence of irradiation or parabiosis bias. PLoS One 8, e58544.
Kildsgaard, J., et al., 2000. Cutting edge: targeted disruption of the C3a receptor gene demonstrates a novel protective anti-inflammatory role for C3a in endotoxin-shock. J. Immunol. 165, 5406–5409.
Kjaeldgaard, A.L., et al., 2018. Amyotrophic lateral sclerosis: the complement and inflammatory hypothesis. Mol. Immunol. 102, 14–25.
Kolev, M., et al., 2014. Complement—tapping into new sites and effector systems. Nat. Rev. Immunol. 14, 811–820.
Konteatis, Z.D., et al., 1994. Development of C5a receptor antagonists. Differential loss of functional responses. J. Immunol. 153, 4200–4205.
Koppers, M., et al., 2012. VCP mutations in familial and sporadic amyotrophic lateral sclerosis. Neurobiol. Aging 33 (837 e7-13).
Kumar, V., et al., 2018. Development and validation of a LC-MS/MS assay for pharmacokinetic studies of complement C5a receptor antagonists PMX53 and PMX205 in mice. Sci. Rep. 8, 8101.
Kwiatkowski Jr., T.J., et al., 2009. Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. Science. 323, 1205–1208.
Lee, J.D., et al., 2012. Innate immunity in ALS. In: Maurer, M.H. (Ed.), Amyotrophic Lateral Sclerosis. InTech, Croatia.
Lee, J.D., et al., 2013. Dysregulation of the complement cascade in the hSOD1G93A transgenic mouse model of amyotrophic lateral sclerosis. J. Neuroinflammation 10, 119.
Lee, J.D., et al., 2017. Pharmacological inhibition of complement C5a-C5a1 receptor signalling ameliorates disease pathology in the hSOD1G93A mouse model of amyotrophic lateral sclerosis. Br. J. Pharmacol. 174, 689–699.
Lee, J.D., et al., 2018. Complement components are upregulated and correlate with disease progression in the TDP-43(Q331K) mouse model of amyotrophic lateral sclerosis. J. Neuroinflammation 15, 171.
Li, R., et al., 2013. C5L2: a controversial receptor of complement anaphylatoxin, C5a. FASEB J. 27, 855–864.
Liddelow, S.A., et al., 2017. Neurotoxic reactive astrocytes are induced by activated microglia. Nature. 541, 481–487.
Lino, M.M., et al., 2002. Accumulation of SOD1 mutants in postnatal motoneurons does not cause motoneuron pathology or motoneuron disease. J. Neurosci. 22, 4825–4832.
Liszewski, M.K., et al., 2013. Intracellular complement activation sustains T cell homeostasis and mediates effector differentiation. Immunity. 39, 1143–1157.
Lobsiger, C.S., et al., 2007. Toxicity from different SOD1 mutants dysregulates the complement system and the neuronal regenerative response in ALS motor neurons. Proc. Natl. Acad. Sci. U. S. A. 104, 7319–7326.
Lobsiger, C.S., et al., 2013. C1q induction and global complement pathway activation do not contribute to ALS toxicity in mutant SOD1 mice. Proc. Natl. Acad. Sci. U. S. A. 110, E4385–E4392.
Loeffler, D.A., et al., 2006. Complement activation in the Parkinson's disease substantia nigra: an immunocytochemical study. J. Neuroinflammation 3, 29.
Louveau, A., et al., 2015. Revisiting the mechanisms of CNS immune privilege. Trends Immunol. 36, 569–577.
Manthey, H.D., et al., 2009. Complement component 5a (C5a). Int. J. Biochem. Cell Biol. 41, 2114–2117.
Mantovani, S., et al., 2014. Elevation of the terminal complement activation products C5a and C5b-9 in ALS patient blood. J. Neuroimmunol. 276, 213–218.
March, D.R., et al., 2004. Potent cyclic antagonists of the complement C5a receptor on human polymorphonuclear leukocytes. Relationships between structures and activity. Mol. Pharmacol. 65, 868–879.
Melendez, A.J., Ibrahim, F.B., 2004. Antisense knockdown of sphingosine kinase 1 in human macrophages inhibits C5a receptor-dependent signal transduction, Ca2+ signals, enzyme release, cytokine production, and chemotaxis. J. Immunol. 173, 1596–1603.
Michailidou, I., et al., 2018. Systemic inhibition of the membrane attack complex impedes neuroinflammation in chronic relapsing experimental autoimmune encephalomyelitis. Acta Neuropathol Commun. 6, 36.
Morgan, B.P., 2018. Complement in the pathogenesis of Alzheimer's disease. Semin. Immunopathol. 40, 113–124.
Nagai, M., et al., 2007. Astrocytes expressing ALS-linked mutated SOD1 release factors selectively toxic to motor neurons. Nat. Neurosci. 10, 615–622.
O'Barr, S.A., et al., 2001. Neuronal expression of a functional receptor for the C5a complement activation fragment. J. Immunol. 166, 4154–4162.
Okinaga, S., et al., 2003. C5L2, a nonsignaling C5A binding protein. Biochemistry. 42, 9406–9415.
Olsen, M.K., et al., 2001. Disease mechanisms revealed by transcription profiling in SOD1-G93A transgenic mouse spinal cord. Ann. Neurol. 50, 730–740.
Paidassi, H., et al., 2008. C1q binds phosphatidylserine and likely acts as a multiligand-bridging molecule in apoptotic cell recognition. J. Immunol. 180, 2329–2338.
Perrin, F.E., et al., 2005. No widespread induction of cell death genes occurs in pure motoneurons in an amyotrophic lateral sclerosis mouse model. Hum. Mol. Genet. 14, 3309–3320.
Pramatarova, A., et al., 2001. Neuron-specific expression of mutant superoxide dismutase 1 in transgenic mice does not lead to motor impairment. J. Neurosci. 21, 3369–3374.
Quell, K.M., et al., 2017. Monitoring C3aR expression using a Floxed tdTomato-C3aR reporter Knock-in mouse. J. Immunol. 199, 688–706.
Renton, A.E., et al., 2011. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron. 72, 257–268.
Ricklin, D., et al., 2010. Complement: a key system for immune surveillance and homeostasis. Nat. Immunol. 11, 785–797.
Ricklin, D., et al., 2016. Complement in disease: a defence system turning offensive. Nat. Rev. Nephrol. 12, 383–401.
Ricklin, D., et al., 2017. Complement in clinical medicine: clinical trials, case reports and therapy monitoring. Mol. Immunol. 89, 10–21.
Rittirsch, D., et al., 2008. Functional roles for C5a receptors in sepsis. Nat. Med. 14, 551–557.
Rosen, D.R., et al., 1993. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature. 362, 59–62.
Serna, M., et al., 2016. Structural basis of complement membrane attack complex formation. Nat. Commun. 7, 10587.
Shastri, A., et al., 2013. Innate immunity and neuroinflammation. Mediat. Inflamm. 2013, 342931.
Siddique, N., Siddique, T., 2008. Genetics of amyotrophic lateral sclerosis. Phys. Med. Rehabil. Clin. N. Am. 19, 429–439 (vii).
Singhrao, S.K., et al., 1999. Increased complement biosynthesis by microglia and complement activation on neurons in Huntington's disease. Exp. Neurol. 159, 362–376.
Sochocka, M., et al., 2017. Inflammatory response in the CNS: friend or foe? Mol. Neurobiol. 54, 8071–8089.
Sreedharan, J., et al., 2008. TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. Science 319, 1668–1672.
Sta, M., et al., 2011. Innate and adaptive immunity in amyotrophic lateral sclerosis: evidence of complement activation. Neurobiol. Dis. 42, 211–220.
Stevens, B., et al., 2007. The classical complement cascade mediates CNS synapse elimination. Cell. 131, 1164–1178.
Tenner, A.J., et al., 1995. Mannose binding protein (MBP) enhances mononuclear phagocyte function via a receptor that contains the 126,000 M(r) component of the C1q receptor. Immunity 3, 485–493.
Tenner, A.J., et al., 2018. New tricks for an ancient system: physiological and pathological roles of complement in the CNS. Mol. Immunol. 102, 3–13.
Togo, T., et al., 2002. Occurrence of T cells in the brain of Alzheimer's disease and other neurological diseases. J. Neuroimmunol. 124, 83–92.
Torres, M., Forman, H.J., 1999. Activation of several MAP kinases upon stimulation of rat alveolar macrophages: role of the NADPH oxidase. Arch. Biochem. Biophys. 366, 231–239.
Trbojevic-Cepe, M., et al., 1998. Cerebrospinal fluid complement activation in neurological diseases. J. Neurol. Sci. 154, 173–181.
Troost, D., et al., 1990. Immunohistochemical characterization of the inflammatory infiltrate in amyotrophic lateral sclerosis. Neuropathol. Appl. Neurobiol. 16, 401–410.
Tsuboi, Y., Yamada, T., 1994. Increased concentration of C4d complement protein in CSF in amyotrophic lateral sclerosis. J. Neurol. Neurosurg. Psychiatry 57, 859–861.
Vogt, W., et al., 1978. A new function of the activated third component of complement: binding to C5, an essential step for C5 activation. Immunology. 34, 29–40.

Wang, L., et al., 2009. The effect of mutant SOD1 dismutase activity on non-cell autonomous degeneration in familial amyotrophic lateral sclerosis. Neurobiol. Dis. 35, 234–240.
Wang, R., et al., 2013. Disruption of the complement anaphylatoxin receptor C5L2 exacerbates inflammation in allergic contact dermatitis. J. Immunol. 191, 4001–4009.
Wang, H.A., et al., 2017. Complement C5a-C5aR1 signalling drives skeletal muscle macrophage recruitment in the hSOD1G93A mouse model of amyotrophic lateral sclerosis. Skelet. Muscle 7, 10.
Woodruff, T.M., et al., 2002. Antiarthritic activity of an orally active C5a receptor antagonist against antigen-induced monarticular arthritis in the rat. Arthritis Rheum. 46, 2476–2485.
Woodruff, T.M., et al., 2003. A potent human C5a receptor antagonist protects against disease pathology in a rat model of inflammatory bowel disease. J. Immunol. 171, 5514–5520.
Woodruff, T.M., et al., 2006. Therapeutic activity of C5a receptor antagonists in a rat model of neurodegeneration. FASEB J. 20, 1407–1417.
Woodruff, T.M., et al., 2008. The complement factor C5a contributes to pathology in a rat model of amyotrophic lateral sclerosis. J. Immunol. 181, 8727–8734.
Woodruff, T.M., et al., 2010. The role of the complement system and the activation fragment C5a in the central nervous system. NeuroMolecular Med. 12, 179–192.
Woodruff, T.M., et al., 2014. Role for terminal complement activation in amyotrophic lateral sclerosis disease progression. Proc. Natl. Acad. Sci. U. S. A. 111, E3–E4.
Writing, G., Edaravone, A.L.S.S.G., 2017. Safety and efficacy of edaravone in well defined patients with amyotrophic lateral sclerosis: a randomised, double-blind, placebo-controlled trial. Lancet Neurol. 16, 505–512.
Wu, M.C., et al., 2013. The receptor for complement component C3a mediates protection from intestinal ischemia-reperfusion injuries by inhibiting neutrophil mobilization. Proc. Natl. Acad. Sci. U. S. A. 110, 9439–9444.
Xu, Z., et al., 2018. Mass spectrometry analysis of plasma from amyotrophic lateral sclerosis and control subjects. Amyotroph Lateral Scler Frontotemporal Degener. 19, 362–376.
Yamada, T., et al., 1994. Increased concentration of C4d complement protein in the cerebrospinal fluids in progressive supranuclear palsy. Acta Neurol. Scand. 89, 42–46.
Yamanaka, K., et al., 2008a. Mutant SOD1 in cell types other than motor neurons and oligodendrocytes accelerates onset of disease in ALS mice. Proc. Natl. Acad. Sci. U. S. A. 105, 7594–7599.
Yamanaka, K., et al., 2008b. Astrocytes as determinants of disease progression in inherited amyotrophic lateral sclerosis. Nat. Neurosci. 11, 251–253.
Yin, C., et al., 2019. ApoE attenuates unresolvable inflammation by complex formation with activated C1q. Nat. Med.
Zarei, S., et al., 2015. A comprehensive review of amyotrophic lateral sclerosis. Surg. Neurol. Int. 6, 171.
Zhang, T., et al., 2017. The controversial C5a receptor C5aR2: its role in health and disease. J Immunol Res 2017, 8193932.