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Innate Immunity in ALS

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1. Introduction

Amyotrophic Lateral Sclerosis (ALS), also known as Lou Gehrig's disease, is the most common form of motor neuron disease. It is a debilitating, late onset neurodegenerative disorder that is characterized by the progressive death of upper and α -motor neurons within the central nervous system (CNS) (Bruijn and Cleveland, 1996). This results in symptoms of muscle weakness and atrophy of skeletal muscles, leading to paralysis and eventual death due to failure of respiratory muscles (Cozzolino et al., 2008). ALS has a prevalence of approximately 1~2 per 100,000 worldwide with males being more susceptible than females (1.3 ~ 1.6: 1) (Strong, 2003, Woodruff et al., 2008b, Worms, 2001). The majority of ALS cases (~90%) are thought to be sporadic with unknown aetiology and no robust environmental risk factors, with the remaining 10% being familial ALS. Of this 10%, approximately 20% have been linked to dominant mis-sense point mutations in the Copper/Zinc superoxide dismutase 1 (SOD1) gene which results in a gain of unidentified deleterious properties (Rosen et al., 1993). The two aetiologies of ALS (i.e. sporadic and familial) are indistinguishable on the basis of their clinical and pathological features, including progressive muscle weakness, atrophy and spasticity, each of which reflects the degeneration and death of upper and α -motor neurons (Boillee et al., 2006). The mechanisms leading to ALS are still unclear but theories have suggested that glutamate excitotoxicity, oxidative stress, protein aggregation, mitochondrial dysfunction, cytoskeletal abnormalities and neuro-inflammation may all play a role (Bruijn et al., 2004). The present chapter will review the role of innate immune system, in particular the complement system, during the disease progression of ALS. It will review evidence for an involvement of the innate immune Toll-like receptor (TLR) system and receptor for advanced glycosylation end products (RAGE) in ALS patients and animal models of ALS. It will also comprehensively evaluate the role of the innate immune complement cascade in this disease. Finally, the future therapeutic possibilities for ALS, aimed at targeting components of the innate immune system will be discussed. We provide compelling evidence for specific inhibitors of complement C5a receptors as novel treatment strategies for ALS.

2. Innate immunity in neurodegenerative disease

Innate immunity is an evolutionary ancient system that provides the host with immediately available defence mechanisms. It is a rapid and coordinated cascade of reactions by host

cells to protect them against foreign pathogens and insults (Akira et al., 2001, Nguyen et al., 2004). Until recently, the CNS was considered to be immunologically privileged because of its inability to mount an immune response and process antigens. Recent studies have revealed that immune surveillance and differentiation between self and non-self does take place in the CNS, where glial cells, including microglia, astrocytes and oligodendrocytes, act as CNS immune effector cells (Hanisch et al., 2008, Lehnardt, 2010, Ricklin et al., 2010).

The role of innate immune system in the CNS is mainly to provide protection to the neurons from foreign pathogens and injurious stimuli, and to maintain CNS homeostasis. It is also required for tissue modelling during development and following injury (Benard et al., 2008, Mastellos et al., 2005, Rahpeymai et al., 2006, Stevens et al., 2007). However sustained chronic inflammation might be harmful for neuronal integrity and may result in cellular dysfunction which triggers neurodegeneration. There is increasing evidence that suggests an involvement of the innate immune system in the development of neuro-inflammation which may drive the progression of many neurodegenerative diseases including ALS. Two major constituents of innate immune system are the TLRs and the complement cascade, each of which are described below.

3. Toll-like receptors (TLRs) and receptor for advanced glycosylation end products (RAGE) in ALS

TLRs are a large family of evolutionarily conserved transmembrane glycoproteins that initiate immune responses for host defence upon activation. These receptors are pattern recognition receptors that recognise pathogen-associated molecular patterns (PAMPs) from diverse organisms including bacteria, viruses, fungi and parasites (Liew et al., 2005). TLRs are expressed in various cell types in the CNS including microglia, astrocytes, oligodendrocytes and neurons (Aravalli et al., 2007, Bowman et al., 2003, Olson and Miller, 2004, Tang et al., 2007). This pathway has recently been implicated in the pathogenesis of ALS. Increased levels of TLRs (TLR1, TLR2, TLR5, TLR7 and TLR9) have been observed in mutant SOD1 mice as compared to controls (Letiembre et al., 2009) and mutant SOD1 expression in ALS has been suggested to facilitate microglial neurotoxic inflammatory responses via TLR2 (Liu et al., 2009). In addition, it has recently been shown that mutant SOD1 binds to CD14, which is a co-receptor of TLR2 and TLR4, and that microglial activation mediated by mutant SOD1 (G93A) can be attenuated using TLR2, TLR4 and CD14 blocking antibodies (Zhao et al., 2010). The involvement of TLR signalling in the pathogenesis of ALS is also supported by up-regulation of TLR2 and TLR4 mRNA and protein in the ALS patients, compared to control spinal cords. The increased expression level of TLR2 and TLR4 was shown on microglia and reactive astrocytes respectively (Casula et al., 2011). This suggests that TLRs could play a role in the progressive degeneration of motor neurons in ALS and indicates that the innate immune system is important in sensing neuronal injury and driving the progression of this disease.

In absence of pathogens, TLR signalling can also be activated via molecules called damage associated molecular patterns (DAMPs) including the high mobility group box 1 (HMGB1) protein released by injured tissues (Bianchi and Manfredi, 2009). HMGB1 is a nearly ubiquitous chromatin component that can regulate transcription of different sets of genes, including pro-inflammatory genes (Bianchi and Manfredi, 2009, Mouri et al., 2008). It can also be released passively by necrotic cells and actively secreted by stimulated monocytes/macrophages and astrocytes, which then bind to RAGE, TLR2 and TLR4. (Andersson et al., 2008, Hreggvidsdottir

et al., 2009, Parker et al., 2004, Scaffidi et al., 2002). Therefore, HMGB1 can act as a potent pro-inflammatory cytokine-like mediator, thus contributing to amplification of the inflammatory response (Bianchi and Manfredi, 2007, Hreggvidsdottir et al., 2009). HMGB1-RAGE signalling has also been implicated in the progression of ALS where there was a significant increase in HMGB1 mRNA expression in ALS patient spinal cords when compared to normal individuals (Casula et al., 2011). The increased expression of HMGB1 was expressed by activated microglia and astrocytes in the spinal cord (Casula et al., 2011). Interestingly, there were no significant changes in RAGE mRNA expression in 12 ALS patients when compared to 6 controls. This observation could be due to the loss of motor neurons expressing RAGE in ALS patients, thus reducing the endogenous pool of RAGE mRNA. This same study also demonstrated that there is an increased expression of RAGE on astrocytes and microglia when compared to controls (Casula et al., 2011). Furthermore, serum soluble RAGE (sRAGE) levels were decreased in the serum of ALS patients when compared to normal individuals, where sRAGE is known to be a possible modulator of inflammation in several diseases (Ilzecka, 2009). Hence it is possible that low sRAGE levels may accelerate the neurodegeneration and could be a risk factor in ALS. This suggests that TLR/RAGE signalling may play a role in the disease progression of ALS, by activating microglia and astrocytes in the vicinity of motor neuron death. Targeting TLRs and RAGE may therefore be a novel therapeutic strategy to treat degenerative neuronal loss occurring in ALS.

4. The complement system in the CNS

The complement system is a key component of the innate immune system, which participates in the recognition, trafficking and elimination of pathogens and unwanted host materials. The complement system is an enzymatic cascade consisting of more than 30 plasma proteins and glycoproteins, and either soluble or membrane-bound receptors (Guo and Ward, 2005). Complement activation participates in host defence against pathogens primarily by cytotoxic and cytolytic activity through triggering formation of the membrane attack complex (MAC or C5b-9) on the target cell membrane (van Beek et al., 2003). It is activated via three major pathways: the classical, alternative, and lectin pathways; it is also activated by a recently identified fourth, extrinsic protease pathway (Huber-Lang et al., 2006, Thoman et al., 1984) (Figure 1).

The classical pathway is primarily activated in response to the recognition molecule C1q binding to antigen-antibody complexes such as immunoglobins (IgG and IgM) and pentraxins (such as C-reactive protein) bound to their targets (Ricklin et al., 2010, Woodruff et al., 2010). C1q may also bind directly to pathogen surfaces and to non-pathogen surfaces such as beta-amyloid and liposomes (Jiang et al., 1994, Marjan et al., 1994). The alternative pathway is activated by foreign surfaces which amplifies the slow spontaneous hydrolysis of C3 which leads to the formation of C3 convertases (Pangburn et al., 1981, Ricklin et al., 2010), whereas lectin pathway is initiated following the binding of mannose-binding lectin to carbohydrate groups on the surfaces of some pathogens (Woodruff et al., 2010). The activation of each of these pathways results in assembly of C3 and C5 convertase enzymes which cleave their respective inactive complement factors C3 and C5 into their active fragments C3a, C3b, C5a and C5b. This leads to the formation of MAC through the non-enzymatic assembly of C5b with complement factors C6-C9, forming C5b-9 on the cell membrane, which creates a transmembrane pore, ultimately leading to cell lysis (Podack et al., 1982). A recently identified fourth extrinsic pathway involves direct cleavage of

complement 3 (C3) and complement 5 (C5) into C3a/C3b and C5a/C5b by proteolytic enzymes (serine proteases) such as kallikrein, thrombin and cell-derived proteases (Huber-Lang et al., 2002, Huber-Lang et al., 2006). 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 cascade precursor, C3 (Huber-Lang et al., 2006). This pathway may provide a source of complement activation factors in the absence of upstream complement activation, and in a local tissue environment such as the CNS (Woodruff et al., 2010).

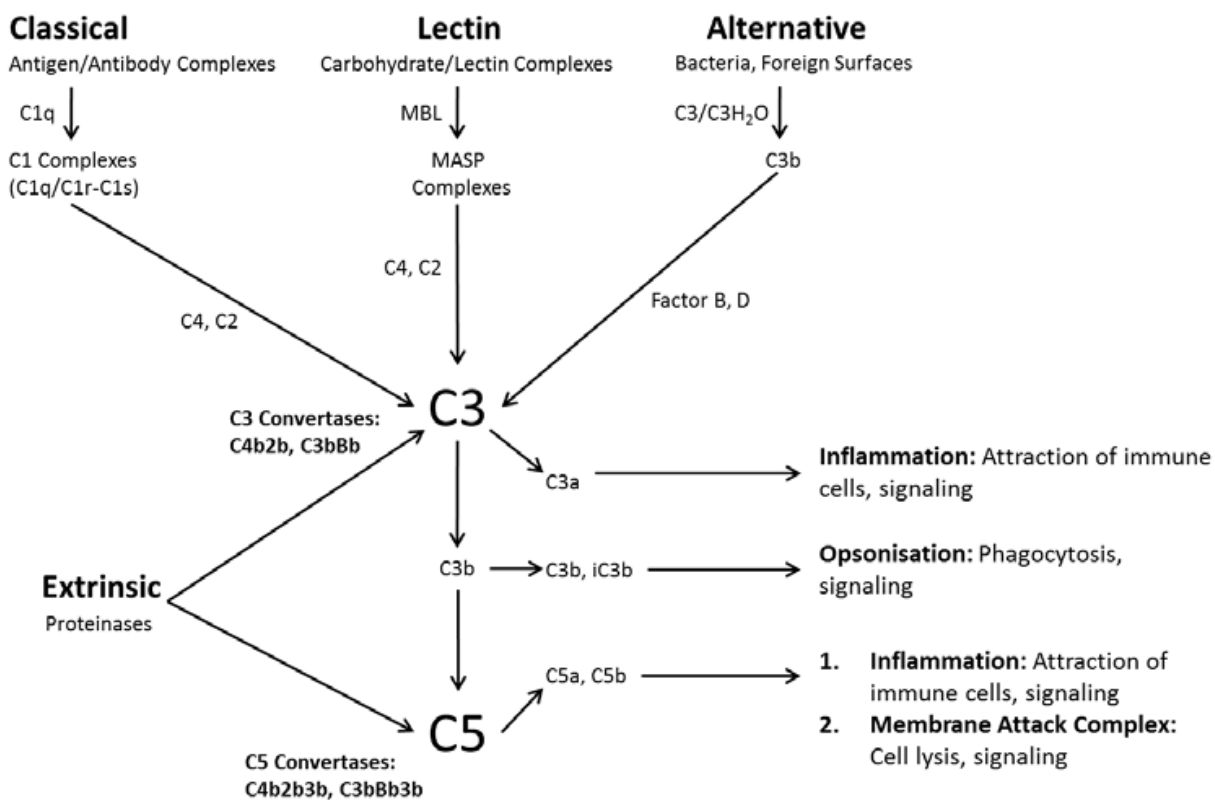


Fig. 1. Complement Cascade: Complement is part of the innate immune system and can be activated via four different pathways: the classical pathway, an antigen-antibody complex; the alternative pathway, activated by bacteria and foreign surfaces; the lectin pathway activated by mannose binding lectin; and recently discovered extrinsic protease pathway involving direct cleavage of C3 and C5. Each pathway converges at C3 and leads to a common terminal point which involves the formation of the cytolytic membrane attack complex (MAC) leading to cell lysis. Formation of pro-inflammatory anaphylatoxins C3a and C5a induces glial chemotaxis, generation of superoxide radicals and release of inflammatory mediators. C3b and iC3b facilitates phagocytosis by opsonising foreign pathogens.

The primary function of complement activation is to provide a rapid response to infection and injury by initiating the production of opsonins C1q and C3b to opsonise pathogens, the production of the pro-inflammatory anaphylatoxins C3a and C5a to recruit immune and inflammatory cells through ligand-receptor interactions with their corresponding receptors, C3aR and CD88, and the formation of cytolytic MAC, which ultimately leads to the destruction of invading organisms by cell apoptosis/necrosis (Liszewski et al., 1996).

C5a is considered to be the most potent inflammatory molecule generated upon complement activation and exhibits a broad range of functions. C5a exerts its effect through two high affinity receptors, the classical C5aR (CD88), and the C5a-like receptor 2 (C5L2/GPR77). The main C5a receptor, CD88 is a member of the rhodopsin family of seven transmembrane domain receptors coupled to the hetero-metric G proteins of the Gi subtype: pertussis toxin-sensitive G_{i2} , G_{i3} or pertussis toxin-insensitive G_{i16} (Amatruda et al., 1993, Johswich and Klos, 2007, Rollins et al., 1991). Cellular activation of CD88 involves intracellular calcium mobilization and activation of different signaling pathways including phosphatidylinositol-3-kinase/Akt (PI3K γ ; Perianayagam et al., 2002), Ras/B-Raf/mitogen-activated protein kinase (MAPK)/extracellular signal-related kinase (ERK) (Buhl et al., 1994), phospholipase A₂, phospholipase D (Cockcroft, 1992, Mullmann et al., 1990), protein kinase C (PKC; Buhl et al., 1994), p21-activated kinases, Rac GTPases (Huang et al., 1998), signal transducers and activators of transcription, sphingosine kinase (Melendez and Ibrahim, 2004) and NF- κ B (Kastl et al., 2006). It is widely expressed on variety of cells and tissues, and its activation is known to have pro-inflammatory functions such as chemotaxis, degranulation, superoxide production, and release of proteases, eicosanoids, cytokines and chemokines from inflammatory cells (Gomez-Cambronero et al., 2007, Melendez and Ibrahim, 2004, Torres and Forman, 1999, Tsai et al., 2004).

The recently discovered C5a receptor, C5L2 has the conventional G-protein coupled receptor structure but it is not coupled to intracellular G-protein activated signaling pathways (Bamberg et al., 2010, Okinaga et al., 2003). Binding of C5a to C5L2 failed to induce intracellular calcium mobilization, extracellular signal-related kinase phosphorylation or receptor internalization, by contrast to CD88 (Cain and Monk, 2002, Okinaga et al., 2003). This has led to the proposal that C5L2 may act as a decoy anaphylatoxin receptor by regulating the availability of C5a to CD88, or by forming oligomers with CD88 to interrupt C5a-CD88 signaling (Rabiet et al., 2007). Although the mechanisms underlying C5L2 activation are still unknown, several recent studies in C5L2 knockout mice have showed greater response to C5a, a greater influx of inflammatory cells and a greater release of IL-6 and TNF- α compared to the wild-type mice (Rabiet et al., 2007). This suggests that C5a signaling via C5L2 may exert anti-inflammatory functions which buffer the effects of the inflammatory C5a-CD88 signaling pathway (Rabiet et al., 2007). Furthermore, studies have shown that C5L2 may function as an intracellular receptor, which becomes activated only after ligand binds to CD88. It was suggested that C5L2 negatively modulates C5a-CD88 signaling and limits the signaling capacity of C5a via its interaction with CD88 and β -arrestins (Bamberg et al., 2010, Van Lith et al., 2009). Any role for C5L2 in neurodegenerative diseases has yet to be properly elucidated.

Although the CNS does not receive the same composition of circulating complement factors synthesised in the liver by hepatocytes, due to the blood brain barrier (BBB), many studies have revealed that the CNS contains components of complement cascade, where they are expressed by astrocytes, microglia, oligodendrocytes and neurons (Barnum, 1995, Gasque et al., 1997, Nataf et al., 2001, O'Barr et al., 2001). Similar to the peripheral system, the role of complement activation within the CNS is thought to primarily protect the neurons from foreign pathogens through activation of inflammatory and immune cascades by surrounding glial cells. In addition to their immune surveillance functions, recent studies have shown that complement molecules also have a role in adaptive immune response, nervous system development, regeneration and regulating CNS homeostasis by clearing

cellular debris and also eliminating excess synapses (i.e. synaptic pruning) (Stevens et al., 2007). Intriguingly, synaptic loss is not only a feature of neural development but is also a key pathological feature of neurodegenerative diseases (Schafer and Stevens, 2010, Woodruff et al., 2010). Hence it has been proposed that complement has multiple central roles in the CNS other than its canonical functions associated with host defence (Benard et al., 2008, Rahpeymai et al., 2006, Stevens et al., 2007). Therefore dysregulation or imbalance of the complement system in the CNS can be harmful to the neurons and may lead to, or contribute to, neurodegenerative diseases including ALS.

5. Clinical evidence of complement involvement in ALS

Several studies have been conducted on ALS patients in an attempt to identify whether complement components are up-regulated in disease progression (Table 1). It has been proposed that the classical complement system is involved in the pathophysiology of ALS, as studies have shown that activation fragments of complement components C3 and C4 are increased in the serum, cerebrospinal fluid (CSF), and neurological tissue (including spinal cord and motor cortex) of ALS patients (Annunziata and Volpi, 1985, Apostolski et al., 1991, Goldknopf et al., 2006, Kawamata et al., 1992, Trbojevic-Cepe et al., 1998). The first of these studies examined C3 immunofluorescence in spinal cord and motor cortex of 16 ALS patients and demonstrated significant C3 deposition, which appeared to be on astrocyte-like cells with no apparent neuronal staining (Donnenfeld et al., 1984). Subsequent studies measured C3c, C4, C1 inactivator and C3 activator fractions in the serum and CSF of 13 ALS patients but only detected increased levels of C3c in the CSF of ALS patients compared to normal individuals (Annunziata and Volpi, 1985). Furthermore Apostolski and colleagues (1991) measured serum C4, C3 and Factor B levels in 33 ALS patients and found an increase in C4 levels when compared to normal individuals. Increased clusters of C3d and C4d coated fibers on oligodendroglia and degenerating neurites in spinal cord and motor cortex was also found in 8 ALS patients compared to 5 normal individuals (Kawamata et al., 1992). Two separate studies also investigated C1q, C4d and C4 levels in the serum and CSF of ALS patients and found C4d levels significantly increased in 15 ALS patients which also correlated with disease severity (Tsuboi and Yamada, 1994); another study also detected upregulation of C4 in ALS patients (Trbojevic-Cepe et al., 1998). Studies by Grewal and colleagues (Grewal et al., 1999) and Jiang and colleagues (Jiang et al., 2005) have identified increased mRNA of upstream complement components (C1q and C2) in the spinal cord of ALS patients. Recently, Sta and colleagues have found increased levels of C1q, C3c, C3d and C5b-9 in the spinal cord and motor cortex of ALS patients compared to normal individuals (Sta et al., 2011). The expression of these complement components was observed in glial cells rather than neurons (Sta et al., 2011). Lastly, complement component C3 was also found to be upregulated in the CSF of 71 ALS patients when compared to 40 normal individuals (Ganesalingam et al., 2011).

These findings of upregulated complement components and activation fragments, predominantly composing the classical pathway, in the serum, CSF, and neurological tissue in ALS patients strongly suggest that the classical complement pathway is involved in the progression of disease in ALS. However it is currently unknown where these complement factors originate, and what initiates their activation. Complement factors can be produced by various cells of the CNS and thus these complement factors could be produced locally in response to disturbance in CNS homeostasis due to immunoglobulin deposits and auto-

antibodies in the CNS of ALS patients (Donnenfeld et al., 1984, Niebroj-Dobosz et al., 2006). Also the circulation could be a source of these complement factors as there is BBB breakdown in the end stages of ALS (Apostolski et al., 1991). Overall, evidence from these clinical studies helps us to propose that complement system activation occurs in ALS patients, and may play a role in the disease pathology. This is also supported by evidence of studies showing involvement of complement factors in animal models of ALS.

Complement factors	mRNA/Protein	Sample	Methods
C3	Protein	Spinal Cord, Motor cortex	Immunofluorescence
C3c	Protein	Serum, CSF	Single radial immuno-diffusion
C4	Protein	Serum	Single radial immuno-diffusion
C3d, C4d	Protein	Spinal Cord, Motor cortex	Immunohistochemistry
C4d	Protein	CSF	Sandwich ELISA
C4	Protein	CSF	Laser nephelometry
C1q	mRNA	Spinal Cord, Motor cortex	Northern blot, <i>In situ</i> hybridization
C2	mRNA	Spinal Cord	Microarray
C3c, C3dg, Factor H	Protein	Serum	2D gel electrophoresis
C1q, C3c, C3d, C5b-9	mRNA/Protein	Spinal Cord, Motor cortex	qPCR, immunohistochemistry
C3	Protein	CSF	Sandwich ELISA

Table 1. Clinical evidence of complement activation in ALS patients

6. Experimental evidence of complement involvement in ALS

Many studies in animal models of ALS have shown the involvement of the complement system during disease progression, supporting findings in ALS patients (Table 2). Although the SOD1 gene mutation only accounts for 2% of total ALS cases, mouse models carrying over-expression of mutant SOD1 enzyme are widely used, as it leads to progressive symptoms which are very similar to the human condition.

Complement factors	mRNA/Protein	Transgenic model	Reference
C1q	mRNA	Mouse SOD1 ^{G93A}	(Perrin et al., 2005)
C1q, DAF	mRNA	Mouse SOD1 ^{G37R} and SOD1 ^{G85R}	(Lobsiger et al., 2007)
C1q, C4	mRNA/Protein	Mouse SOD1 ^{G93A}	(Ferraiuolo et al., 2007)
C1q	mRNA	Mouse SOD1 ^{L126delTT}	(Fukada et al., 2007)
CD88	mRNA/Protein	Rat SOD1 ^{G93A}	(Woodruff et al., 2008a)
CD88	mRNA/Protein	Mouse NFL ^{-/-}	(Humayun et al., 2009)
C1q, C3	mRNA/Protein	Mouse SOD1 ^{G93A}	(Heurich et al., 2011)

Table 2. Experimental evidence of complement activation in animal models of ALS

The first study to demonstrate experimentally the involvement of complement factors in a SOD1 transgenic mouse model was performed by Perrin and colleagues in 2005. They isolated the ventral motor neurons from the lumbar spinal cord of SOD1^{G93A} transgenic mouse using laser-capture micro-dissection and then using microarray analysis they detected increased levels of all subcomponents of C1q in these mice at early symptomatic and end stage when compared to motor neurons from wild-type mice (~5 and ~8 fold respectively) (Perrin et al., 2005). Subsequent studies in two distinct SOD1 transgenic mouse models also used laser-capture micro-dissection to isolate lumbar motor neurons from SOD1^{G37R} and SOD1^{G85R} transgenic mice which showed upregulation of genes for all three C1q subcomponents when compared to SOD1^{WT} mice 2 months prior to clinical onset (P105) (Lobsiger et al., 2007). In addition, this group demonstrated that the complement regulatory molecule, decay accelerating factor (DAF) also decreased at this time point (Lobsiger et al., 2007). Furthermore they showed that C1q protein was expressed by motor neurons using immunohistochemistry on spinal cord sections of both SOD1^{G37R} and SOD1^{G85R} transgenic mice but absent in the age-matched control mice (Lobsiger et al., 2007).

A separate group also used laser-capture microdissection to isolate the lumbar motor neurons from SOD1^{G93A} transgenic mice. Using microarray analysis and real time quantitative PCR, they showed there were increased levels of C1q (subcomponent B) and C4 mRNA at disease onset (P90) and late-stage disease (P120) (~7 and ~8 fold respectively) (Ferraiuolo et al., 2007). A similar study also used microarray analysis in a separate SOD1 transgenic mouse model using whole lumbar spinal cord homogenate (Fukada et al., 2007). This study used SOD1^{L126delTT} transgenic mice and showed elevated levels of C1q (subcomponent B) mRNA in post-symptomatic (P154) mice compared to wild-type mice. A very recent study has shown increased levels of C1q in the neuromuscular junction of SOD1^{G93A} transgenic mice compared to wild-type mice (Heurich et al., 2011).

By contrast to the above studies, which indicates a role for the classical complement pathway in the progression of pathology of the SOD1 transgenic mouse, a recent study has demonstrated that when SOD1^{G93A} transgenic mice were bred onto a background deficient in complement C4 (a necessary component of the classical complement pathway, downstream of C1q), there was a difference in the macrophage levels and activation in the peripheral nervous system but no difference in the onset of motor symptoms and survival when compared to wild-type mice (Chiu et al., 2009). This study indicates that other molecular pathways such as the alternative or extrinsic pathway may play compensatory roles in immune activation and macrophage recruitment in the absence of the classical pathway in these mice. To support this, recent studies in SOD1^{G93A} transgenic mouse showed increases in the C3 mRNA and protein levels in the spinal cord when compared to wild-type animals at symptomatic stage (P126) (Heurich et al., 2011). They also observed upregulation of C3 at the motor end plate and nerve terminals in the SOD1^{G93A} transgenic mice at pre-symptomatic stage (P47) when compared to wild-type animal (Heurich et al., 2011).

To further validate the involvement of downstream components of the complement cascade in the disease progression of ALS, upregulation of C5a receptor CD88 mRNA and protein was observed in mice deficient in the low molecular weight neurofilament (NFL) subunit protein, a mouse model of motor neuron degeneration in which neurofilament aggregates in a similar fashion to that in ALS patients (Humayun et al., 2009). This study showed there was a 4 and 3 fold increase in CD88 mRNA expression level at 2 and 3 months respectively, a time which is early in the disease process (Humayun et al., 2009). There was also an increased immunoreactivity of CD88 in motor neurons of NFL deficient mice when compared to wild-type mice at 3, 4 and 5 months. Our own findings also support a pathogenic role for C5a in ALS (Woodruff et al., 2008a). Chronic administration of a specific C5a receptor antagonist, developed in our laboratories (Wong et al., 1998) in SOD1^{G93A} transgenic rats, markedly delayed the onset of motor symptoms and increased survival, compared to untreated animals (Woodruff et al., 2008a). We also showed upregulation of CD88 in the lumbar spinal cord of SOD1^{G93A} transgenic rats, which increased as disease progressed (Woodruff et al., 2008a).

These findings of upregulated complement components in different animal models of ALS suggest that the activation of complement system is critically linked with disease progression in ALS. Whilst inhibition of one component of the classical and lectin complement pathway, C4, failed to ameliorate disease in SOD1^{G93A} transgenic mice, inhibition of the classical receptor for C5a, CD88, reduced disease pathology in SOD1^{G93A} transgenic rats. It should be noted that C5a is expressed following activation of all

complement pathways (Figure 1). Hence inhibiting central components of the complement system, at the C3 and C5 level, may have benefits in slowing disease progression in ALS, as opposed to inhibiting an individual activation pathway. Specifically, our studies suggest that inhibiting the pro-inflammatory C5 activation fragment, C5a, which is central to, and generated by, all complement pathways, may be a novel therapeutic strategy to treat ALS.

7. Future directions: Therapeutic applications

To date, riluzole (Rilutek, Aventis Pharmaceuticals Inc) is the only approved therapeutic to treat ALS; it is known to prevent the pre-synaptic release of glutamine (Bellingham, 2011, Miller et al., 2007). In clinical trials, it has been shown to extend survival by around 2 ~ 3 months and delay the onset of ventilator dependence or tracheostomy (Bellingham, 2011, Miller et al., 2007). It is not clear that the drug improves the quality of life, however. Given this modest extension of ~2-3 months in survival there is an urgent need to develop new therapeutics which will significantly extend survival and also decrease morbidity in ALS.

Recent studies have suggested that the innate immune system is important in sensing ALS progression and its subsequent upregulation may drive the progression of this disease (Woodruff et al., 2008b). The complement system would be a logical and viable pathway to target, given the steadily accumulating clinical evidence of complement involvement in this disease. This is also supported by our findings where using specific C5a receptor antagonist improved motor symptoms and extended survival in the SOD1^{G93A} transgenic rat (Woodruff et al., 2008a).

Our laboratories have developed a series of cyclic peptide C5a receptor antagonists which are potent inhibitors of C5a receptors on human inflammatory cells (Woodruff et al., 2011). PMX53 (AcF-[OPdChaWR] and PMX205 (hydrocinnamate-[OPdChaWR]) are orally active cyclic hexapeptides, which were derived from the linear CD88 antagonist, Me-FKPdChaWR (Konteatis et al., 1994) that were cyclised to induce structural and metabolic stability (Finch et al., 1999, March et al., 2004). These drugs have been shown to display therapeutic efficacy in numerous rodent models of inflammatory disease including rheumatoid arthritis (Woodruff et al., 2002), ischemic reperfusion injuries (Arumugam et al., 2004) and inflammatory bowel disease (Woodruff et al., 2003), as well as acute neurodegeneration (Woodruff et al., 2006). PMX205 is more lipophilic than the original CD88 antagonist PMX53, which results in increased potency in certain inflammatory models (Woodruff et al., 2005) and increased CNS penetrance (Woodruff et al., 2006). Hence, it has been used to reduce disease severity and prolong survival in animal models of neural degeneration including Huntington's disease, Alzheimer's disease and ALS (Ager et al., 2010, Fonseca et al., 2009, Woodruff et al., 2006, Woodruff et al., 2008a). As a result of this work, PMX205 would be the particular PMX series compound we would promote for any future clinical trialling in ALS.

In addition to inhibiting C5a receptors, targeting other factors of the complement system may provide viable therapeutic options to treat ALS. Several complement inhibitors have been developed over the years and compounds such as sCR1, C5 antibodies, compstatin or others could be used as potential therapies for ALS. However, due to the need to chronically administer a drug in ALS, a small, orally active and BBB permeable complement inhibitor, such as PMX205, would be required. The selectivity of PMX205 towards the classical C5a receptor leaves other components of the complement system intact, allowing for the production of complement factors including the MAC, thus reducing immune suppression -

a likely side effect of other inhibitors of complement which act more upstream in the system, were they are to be used chronically. Finally, PMX53, an analogue to PMX205 has already been shown to be safe when administered to humans, successfully completing three Phase I/IIa clinical trials, thus promoting the safety of these classes of drugs in humans (Woodruff et al., 2011).

In addition to anti-complement agents, combined therapies targeting multiple and disparate pathways will most likely be needed to effectively treat ALS. Extensive controlled clinical trials will need to be conducted in order to ascertain any potential therapeutic benefit of a complement inhibitor to treat the devastating and intractable nature of ALS.

8. Conclusion

There is increasing evidence that implicates the involvement of the innate immune system in the progression of ALS. In particular, the inappropriate activation or dysregulation of the complement system may play a role in ALS pathology. Evidence for this includes elevated levels of complement activation fragments in the serum, CSF, spinal cord and motor cortex of ALS patients. This has also been supported with elevated levels of complement activation fragments in various animal models of ALS. Moreover, inhibition of the C5a receptor using a specific C5a receptor antagonist ameliorated disease symptoms in a rat model of ALS. Collectively, these studies suggest that complement activation may play a crucial role in the progression of ALS. Hence reducing complement-induced inflammation using inhibitors to target complement factors could be an important therapeutic strategy to treat ALS.

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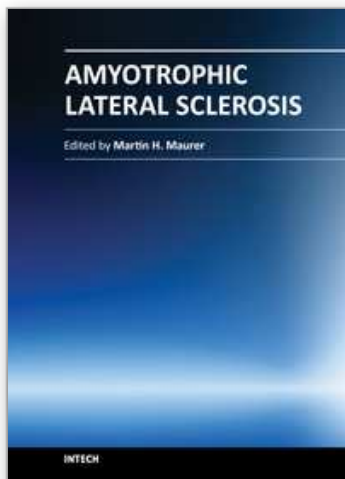
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Amyotrophic Lateral Sclerosis

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Though considerable amount of research, both pre-clinical and clinical, has been conducted during recent years, Amyotrophic Lateral Sclerosis (ALS) remains one of the mysterious diseases of the 21st century. Great efforts have been made to develop pathophysiological models and to clarify the underlying pathology, and with novel instruments in genetics and transgenic techniques, the aim for finding a durable cure comes into scope. On the other hand, most pharmacological trials failed to show a benefit for ALS patients. In this book, the reader will find a compilation of state-of-the-art reviews about the etiology, epidemiology, and pathophysiology of ALS, the molecular basis of disease progression and clinical manifestations, the genetics familial ALS, as well as novel diagnostic criteria in the field of electrophysiology. An overview over all relevant pharmacological trials in ALS patients is also included, while the book concludes with a discussion on current advances and future trends in ALS research.

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