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Review

# Innate immunity in amyotrophic lateral sclerosis

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**Abstract**

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative condition in which motor neurons are selectively targeted. Although the underlying cause remains unclear, evidence suggests a role for innate immunity in disease pathogenesis. Neuroinflammation in areas of motor neuron loss is evident in presymptomatic mouse models of ALS and in human patients. Efforts aimed at attenuating the inflammatory response in ALS animal models have delayed symptom onset and extended survival. Seemingly conversely, attempts to sensitize cells of the innate immune system and modulate their phenotype have also shown efficacy. Effectors of innate immunity in the CNS appear to have ambivalent potential to promote either repair or injury. Because ALS is a syndromic disease in which glutamate excitotoxicity, altered cytoskeletal protein metabolism, oxidative injury, mitochondrial dysfunction and neuroinflammation all contribute to motor neuron degeneration, targeting inflammation via modulation of microglial function therefore holds significant potential as one aspect of therapeutic intervention and could provide insight into the exclusive vulnerability of motor neurons.


*Keywords:* Inflammation; Microglia; Apoptosis; Cytokine

## 1. Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the selective loss of motor neurons. It is perhaps the most devastating neurodegenerative condition because of its insidious onset, rapid progression, and inevitable endpoint. It is also the most frequently diagnosed adult-onset motor neuron disease [1] with a world-wide prevalence of approximately 8 per 100,000. Patients present with symptoms of both upper and lower motor neuron degeneration which leads to failure of respiratory muscles and death on average within 3–5 years [2]. While between 5 and 8% of ALS cases are familial (fALS), of which 20% harbour missense mutations in the copper–zinc superoxide dismutase (SOD1) gene [3], the majority of ALS cases are sporadic (sALS). Although the etiology of ALS remains to be defined, the contemporary understanding of ALS is that it is likely syndromic as opposed to a discrete disease entity [4]. Key components of the pathological process of ALS include glutamate excitotoxicity, disturbances in cytoskeletal protein metabolism (e.g., neurofilament, peripherin), oxidative injury, altered mitochondrial function and neuroinflammation [5,6]. Although these processes seem disparate, there is increasingly an understanding that these processes are tightly integrated. This review will focus on the potential role of neuroinflammation, mediated predominantly through microglial cells, as a key determinant of the disease process.

## 2. Inflammation in the CNS

Inflammation is one aspect of the innate immune response. Innate immunity is naturally present and is not stimulated by antigens or mediated by antibodies. It is therefore generally non-specific and is executed by a variety of phagocytic cells including circulating neutrophils and monocytes and macrophages residing in tissues. The resident macrophage of the CNS is the microglial cell. Throughout development microglia are immunologically active and capable of responding to events associated with CNS organization and formation of the

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neuronal–glial environment. In the healthy adult brain, they exist in a resting state providing surveillance and acting as the first line of immunological defense. They are highly mobile and responsive to cues from surrounding cells [7]. In response to a variety of insults, microglia transform into active, phagocytic cells that release several products governing the inflammatory response. This transformation is seen as a morphological transition from a resting, ramified state to an active amoeboid state through an intermediate, early activated or "primed" [8] state. Microglia become primed in response to primary stimuli from neurons or astrocytes including interferon γ (IFNγ), tumour necrosis factor α (TNFα), macrophage colony stimulating factor (M-CSF), and granulocyte macrophage colony stimulating factor (GM-CSF). This involves a thickening of the cell body and proximal processes, and a resulting intensification of local surveillance. In this state, they express major histocompatibility (MHC) class II molecules and present antigens. In response to secondary stimuli such as interleukin-1 (IL-1), IL-6, and TNF-α, microglia exert maximal activity through secretion of inflammatory mediators (Fig. 1).

It is critical to note that the net effect of microglial activation can be either to induce repair and regeneration, or to further neuronal injury. Through the release of anti-inflammatory cytokines, neurotrophins and growth factors, and via controlled phagocytosis with the removal of presynaptic axon terminals from damaged neurons (synaptic stripping), microglia can enhance neuronal repair. Conversely, through the release of pro-inflammatory cytokines, lysosomal proteases, neurotoxic reactive oxygen radicals and nitrating species, and through astrocytic activation and chronic phagocytosis, microglia can enhance neuronal injury [9,10]. Understanding the mechanisms by which this delicate balance between neuronal repair and neuronal injury is modulated is of critical importance to understanding the role of the innate immune response in ALS. This key point is illustrated by the observation that the extent of neuronal injury in a chimeric model of motor neuron degeneration, in which the fALS associated G93A SOD1 mutation is expressed, is dependant on the extent of non-neuronal cell pathology [11].

Although microglia are the resident macrophage of the CNS, CNS macrophages can also originate from recently blood-derived precursors or from resident perivascular macrophages and related cells [12]. Evidence from peripheral nerve injury models suggests that microglia are the principal executors of the inflammatory response although hematogenous macrophages [13] and lymphocytes [14] are also recruited to the injury site.

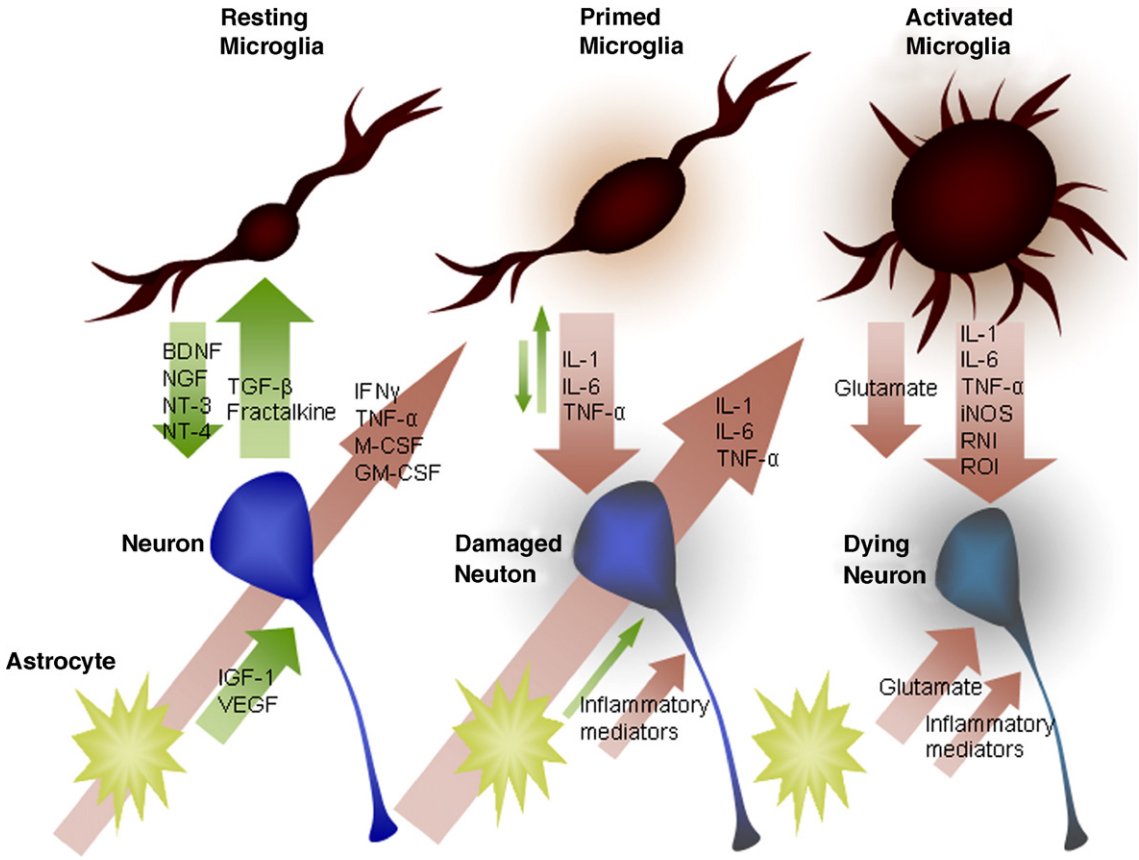


Fig. 1. Neuroinflammatory interaction of microglia, neurons, and astrocytes. In the healthy CNS (left) microglia reside in a ramified morphology providing immune surveillance in the neuronal milieu. In response to primary stimuli from neurons and astrocytes (initiating mechanism unknown in ALS), microglia become primed producing pro-inflammatory cytokines and downregulating expression of growth factors. In response to secondary stimuli microglia become fully activated amoeboid cells with full phagocytic potential. These cells produce increased quantities of pro-inflammatory cytokines, reactive nitrating intermediates (RNI), reactive oxygenating intermediates (ROI), and glutamate. Astrocytes decrease their growth factor contribution and release glutamate and inflammatory mediators. Neurons residing in the inflammatory microenvironment degenerate via apoptotic mechanisms.

Injuries within the CNS however do not result in such significant microglial activation and hematogenous macrophage entry unless the injury itself involves a breakdown in the blood–brain barrier [13]. In the context of neurodegeneration, recent work suggests the robust inflammatory response observed in symptomatic $SOD1^{G93A}$ transgenic mice is mostly attributable to proliferation of resident microglia with only a small contribution from infiltrating bone marrow-derived microglia [15].

## 3. Microglia in ALS

There is considerable morphological and neurochemical evidence for the proliferation and activation of microglia in ALS [15,15–25]. These findings are observed in areas of significant motor neuron loss, including the motor cortex, motor nuclei of the brainstem, the corticospinal tract and the ventral horn of the spinal cord [19,26]. Relevant to observations derived from animal models (discussed below), microglial activation is found even in areas of only mild degeneration [26]. While constitutively expressed in the human brain, macrophage-colony stimulating factor (M-CSF) receptor expression is upregulated in ALS precentral gyrus. Both TNF-α and the soluble extracellular domains of its receptors TNFRI and TNFRII are increased in ALS serum [27,28]. An increased expression of pro-inflammatory cytokines, COX-2 [29–31] and of microglia-mediated protein oxidative pathology [32] is also observed in ALS.

The important issue as to when in the course of degeneration in ALS that microglial activation becomes prominent is being addressed through neuroimaging studies. Using positron emission tomography (PET) imaging with in vivo radio-labeled ligand ([(11)C](R)-PK11195 ) binding to the peripheral benzodiazepine binding site (as a marker of microglia), microglial activation can be observed in patients with ALS [33]. In a variety of models of motor neuron degeneration, microglial priming occurs either prior to or concomitantly with the onset of clinical disease. In these in vivo models, microglial activation and proliferation increases throughout the disease course [34], raising the possibility that microglial activation occurs early in the pathogenesis of ALS (Fig. 2).

In vitro experiments have shown that products released by activated microglia can lead to motor neuron death via TNF-α-mediated apoptotic mechanisms [35] and by Fas ligand or NO-induced apoptotic pathways [36]. It has also been demonstrated that microglia derived from mutant SOD1 transgenic mice have increased cytotoxic potential in culture [37]. Using a chimeric mutant SOD1 model in which only a proportion of cells express the mutant transgene, it has been observed that the extent of motor neuron injury varies in severity proportionate to the extent of non-neuronal cells expressing the mutant transgene [11]. In this latter model, non-transgenic motor neurons surrounded by glia expressing the mutant SOD1 transgene degenerated while transgenic motor neurons surrounded by non-transgenic glia remained healthy. This study suggests that SOD1 mutations, once thought to selectively confer motor neuron susceptibility, can result in glial-mediated neurotoxicity. A recent review cited work from Don Cleveland's group suggesting that genetic knock-down of mutant SOD1 in cells of the macrophage lineage can significantly slow the progression of ALS [38]. Note, however, that other non-neuronal cells interacting with motor neurons, such as the skeletal muscle will also express mutant SOD1 and could contribute to the health of motor neurons. These experiments have been interpreted to support the concept that non-neuronal cells, and in this case microglia specifically, are key determinants of both the timing of induction and the rate of propagation of motor neuron degeneration in ALS. In contrast to these studies, transgenic mice in which $SOD1^{G37R}$ expression is driven by the mouse prion promoter, resulting in levels of mutant SOD1 expression highest in neurons and astrocytes in the CNS and in muscle, develop motor neuron disease despite a lack of expression of the mutant protein in cells of the macrophage lineage including microglia [39].

## 4. The role of the inflammatory response in experimental motor neuron degeneration

To date, the most direct evidence of a temporal association between inflammation and the progression of motor neuron disease in ALS arises from the study of murine models of motor neuron degeneration, including the wobbler mouse (*wr/wr*) [40], the *mnd* mouse [41,42], and the low-molecular weight neurofilament knockout mouse (NFL−/−) [43]. In both the

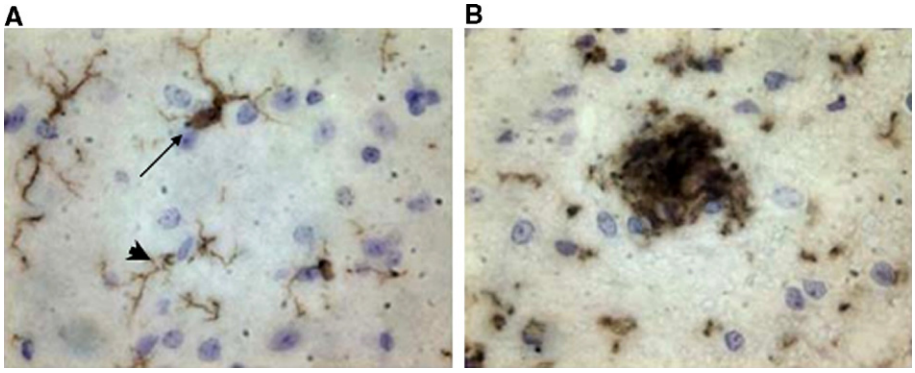


Fig. 2. Microglial morphology: (A) resting/ramified (arrow head), primed/ramified (arrow); (B) a cluster of highly activated/amoeboid microglia. Macrophages immunolabeled using Iba-1 antibody (Wako). Magnification 400× prior to reproduction.

mutant SOD1 transgenic mice and rat, an alteration in the expression and up-regulation of pro-inflammatory factors in the presymptomatic phase with a prominent and sustained microglial response is observed throughout the active phase of the disease progression [44–50]. Specifically, both TGF-β1 and M-CSF expression are upregulated in presymptomatic mice, with TNF-α expression being increased well in advance of the appearance of motor deficits. A significant increase in microglia numbers is observed in early symptomatic mice [45]. This process is associated with an increased level of COX-2 mRNA and protein, and an increase in $PGE_2$ content limited to the regions associated with motor neuron pathology, further confirming a role for microglial activation [31,51]. The latter observation is consistent with in vitro observations that the motor neuron death induced by chronic glutamate excitotoxicity in organotypic spinal cord cultures can be suppressed by COX-2 inhibition [52]. Motor neuron disease is accelerated by chronic stimulation of inflammation using LPS in the $SOD1^{G37R}$ mouse model of ALS, with increasing levels of pro-inflammatory cytokines and increased expression of toll-like receptor 2 (TLR-2) [53].

We have also observed an association between microglial activation and neuronal viability in a model of chronic motor neuron degeneration induced by monthly intracisternal inoculums of aluminum chloride. In this, the presence of a microglial proliferative response was a critical determinant of the extent of recovery upon cessation of aluminum exposure [54–56]. In vitro, both organic and inorganic aluminum compounds were found to inhibit microglial activation in the absence of microglial death and to inhibit microglial-mediated motor neuron death [57]. This suggested that the inhibition of microglial activation by aluminum chloride in vivo allowed for a neuronal milieu permissive to motor neuron recovery. We confirmed this using an immortalized murine motor neuron hybridoma (NSC-34) [58] to demonstrate that activated microglia can kill motor neurons by a mechanism that is dependant on the microglial release of factor(s) that act synergistically with TNF-α. This process can be fully inhibited by inhibiting microglial activation. Floden and colleagues reported that conditioned media obtained from β-amyloid-stimulated primary mouse microglia induces neurotoxicity in primary cortical neurons via coincident stimulation of TNF-α and NMDA receptors. Stimulation of either TNF-α or NMDA receptors alone does not initiate cell death. Memantine and 2-amino-5-phosphopetanoic acid, NMDA receptor antagonists, and soluble TNF-α receptor protect neurons from microglial-conditioned media-dependent death which is thought to result from oxidative damage resulting from inducible nitric oxide synthase (iNOS) activity [59]. NSC-34 cell death can be triggered by oxidative injury mediated through hydroxynonenal (HNE) formation and the triggering of apoptosis [60], suggesting that another potential pathway of microglial-mediated motor neuron injury could be through the release of reactive oxygen species.

While these studies speak to the toxicity of microglia to cultured neurons, including primary motor and cortical neurons and a motor neuron cell line, an important consideration in the context of ALS is the selective vulnerability of motor neurons in vivo. Motor neurons derived from mutant SOD1 transgenic mice exhibit an increased susceptibility to activation of a Fas-triggered cell death pathway that is unique to motor neurons and which is dependent on transcriptional upregulation of neuronal NOS [36]. This work may offer an explanation for the sensitivity of motor neurons in particular to Fas ligand and NO-triggered cell death mediated at least in part by neighbouring microglia, as trophic deprivation and excitotoxic stimulation did not have similar effects.

While these in vitro and in vivo data suggest that inflammatory products released by microglia can act to mediate motor neuron injury, the mechanism of induction of the microglial response is unknown. In models of direct axonal injury, such as a proximal axotomy, a prominent proliferation and migration of microglia to the perineuronal region occurs within 24 h of the lesion—implying that the injured neuron possesses the inherent capacity to induce a microglial response following injury [61,62]. This suggests a capacity for the motor neuron itself to "summon" a microglial response, a postulate supported to some degree by the observation that granulocyte/macrophage colony stimulating factor (GM-CSF) receptors are up-regulated on microglial cells adjacent to axotomized facial motor neurons [63]. Colony stimulating factor-1 (CSF-1) promotes the proliferation and differentiation of both monocytes [64] and microglia [65]. In mice lacking this factor ($Csf1^{op/op}$ mutant mice), only a limited microglial response to peripheral nerve injury occurs [66]. Microglia do not proliferate or migrate toward neurons in response to injury, but do undergo minor morphological changes. Following facial axotomy, a similar loss of the early stages of microglial activation is observed in mice in which macrophage colony stimulating factor (M-CSF) is absent [67]. In this latter model, microglia fail to migrate across the surface of the axotomized motor neuron and there is a lack of microglial proliferation. IL-6 knockout mice not only fail to demonstrate the early microglial response to motor neuron injury, but also show a reduced astrocytic response, in keeping with a dual role of IL-6 in both mediating motor neuron/microglial and microglial/astrocytic interactions [68]. It is of interest that microglia derived from adult mutant SOD1 transgenic mice show decreased IL-6 production in response to LPS-stimulated activation compared to controls [37]. This may be a function of the method of microglial activation.

The chemokine fractalkine has been identified as a chemoattractant in signaling the microglial response by injured motor neurons [69–71]. The fractalkine receptor, CX3CR1, is expressed on both microglia and neurons [72]. Fractalkine induces proliferation of human microglia in vitro [73]. In addition to a role in the migration and proliferation of microglia, fractalkine may also function as a microglial activator as receptor expression is increased on highly activated, phagocytic microglia [74]. Through activation of the phosphatidylinositol-3 kinase/protein kinase B pathway, fractalkine will inhibit Fas ligand-induced microglial apoptosis through down-regulation of the pro-apoptotic function of BAD and up-regulation of the anti-apoptotic activity of $Bcl_{XL}$ [71] thus promoting microglial cell survival. A similar effect of fractalkine upon hippocampal neurons exposed to the HIV envelop protein gp120 has been

observed and attributed to activation of the protein kinase Akt and the nuclear translocation of NF-κB [72,75]. In murine cell culture experiments fractalkine suppressed the production of nitric oxide (NO), IL-6 and TNF-α by activated microglia and suppressed neuronal cell death induced by microglia activated with LPS and interferon-gamma (IFN-γ), in a dose-dependent manner. Thus, fractalkine might function as an intrinsic inhibitor against activated microglial-induced neurotoxicity [76] and may therefore represent a viable target for pharmacological manipulation in ALS.

Potential candidates for mediating microglia/motor neuron interactions include a number of pro-inflammatory cytokines (e.g., IL-1, IL-6 and TNF-α) [77–79] and neurotrophic factors (e.g., plasminogen, TGF-β, bFGF, BDNF, NGF, NT-3 and NT-4) [80–82]. IL-1 and TNF-α have similar biological properties in that at higher concentrations both mimic the cytotoxic effects of LPS [83]. IL-1 mediates a general inflammatory response that recruits the further secretion of pro-inflammatory cytokines (e.g., IL-6, IL-8, colony stimulating factors (CSFs), IFN-α/β) and can also have a trophic effect. The use of a recombinant IL-1 receptor antagonist (r-Hu-met Il-1ra) significantly reduces the volume of damage following brain injury [84]. When IL-1ra-expressing cells are implanted into the wound of spinal cord injured rats, a significant suppression of the extent of both microglial proliferation and NGF up-regulation is observed [85]. When IL-1 is added to mixed astrocytic/neuronal cultures, a 5- to 7-fold increase in astrocytes is observed [86].

Ascribing an unambiguous role to TNF-α is more difficult [87]. When signaling through the TNFR1, TNFR1 recruits a TNF receptor associated death domain (TRADD) that can then interact with the Fas-associated death domain to activate caspase 8, leading to downstream activation of effector caspases. TNFR1 and TRADD activation can also lead to NFκB dependent reporter gene expression that in turn drives expression of *anti*apoptotic gene products including survivin, inhibitor of apoptosis protein-1 (IAP1), IAP2, X-chromosome-linked IAP, Bcl-2, Bcl-$_{XL}$, Bfl-1/A1, and FLIP [88]. The interaction of TNF-α or TNF-β with TNFR2 leads to the activation of NF-κB and the transcription of a number of possible protective cytokines or neurotrophic factors. Of interest, knock out mice lacking TNFR2 show a failure to limit the immune response in experimental autoimmune encephalitis and the use of p74 TNF receptor (TNFR2) antisense oligonucleotides will increase the extent of a hypoxic injury [89,90]. Following an ischemic injury, the severity and extent of brain injury is increased in transgenic mice lacking the TNF-α receptor [91]. Microglia can also inhibit sodium nitroprusside (an NO-donor)-induced neuronal apoptosis in vitro through a TNF-α dependant mechanism whereas IL-3, IL-6, bFGF and M-CSF are ineffective in the same experimental paradigm [92]. Hence, the role of TNF-α as a mediator of microglial/motor neuron interactions is complex, and not a simple effect of inducing cell death.

## 5. Deciphering the ambivalence

While the evidence for microglial neurotoxicity in vitro is extensive, it is important to acknowledge that the function of glia is to sustain normal neuronal function. In ALS, neuronal function is compromised. Is it not logical to expect microglia to react by removing cells damaged beyond the point of repair, constituting a neuroprotective response? This point is alluded to by Streit and referred to as "cellular euthanasia" [93]. Although in vivo evidence for such a phenomenon is lacking, it is an attractive hypothesis to consider that microglia may accelerate the death of dying neurons in order to maintain an environment permissive to the normal function of healthy neurons. However, this is more likely a process involved in acute rather than chronic neurodegeneration. Streit proposes that indeed microglial neurotoxicity may contribute to the pathogenesis of neurodegenerative disease, but this may result more primarily from microglial senescence rather than aggression [93]. Similarly Wyss-Coray and Mucke postulate that the inflammatory response is inherently beneficial and that direction and instruction of inflammatory mediators may be more therapeutic than suppression of them [94].

An important addition is that the beneficial and harmful effects of microglia may be a function of their phenotype and are thus grossly dependent on the nature of the insult. Hence it is an incorrect assumption to extrapolate the behaviour of LPS-activated microglia to that of microglia neighbouring degenerating motor neurons in vivo. This is supported by the observation that glutamate uptake by microglia, (a beneficial behaviour in the context of neurodegenerative disease), and the cytotoxic potential of microglia varied greatly with the nature of microglial activation [95]. Similarly, microglia encountering regulatory T cells adopt a protective role against β-amyloid-induced cytotoxicity [96]. Thus microglia are cells with potentially different or even opposing phenotypes depending on the environment. Perhaps driving the expression of a more neurotrophic phenotype would prove effective in promoting neuronal survival. This concept is reviewed in the discussion of adaptive immunity in ALS.

## 6. Adaptive and acquired immunity in ALS?

Microglia and astrocytes are executors of innate immunity within the CNS. This process begins with the recognition of abnormality within the neuronal environment by surrounding glia, which leads to the production of inflammatory mediating substances and usually phagocytosis of the abnormal cell(s) recognized via opsonization. However, microglia can proceed to present antigens to a distinct subset of helper T-cells (Th1 cells) capable of producing additional cytokines in order to amplify and generalize the inflammatory response. A study investigating systemic immune system changes in sporadic ALS revealed that increased levels of circulating monocytes and macrophages were observed in all sALS patients and that the degree of activation was directly related to the rate of disease progression [97]. Increased levels of monocyte chemoattractant protein-1 (MCP-1) are seen in the cerebrospinal fluid (CSF) from ALS patients [98,99]. Similarly, increased monocyte and dendritic cell (both antigen presenting cells) transcripts are seen in tissue from sALS and fALS patients [25]. These cells present antigen to Th1 cells which can in turn produce IL-1, IFN-γ, TNF-β, and further activate

macrophages. T-cell infiltration has been observed in several studies using human tissue [19,97,100]. However, the presence of cells mediating adaptive immunity in the CNS such as Th1 infiltrates is limited to disease end stage time points in mouse models of ALS [34]. Of interest, driving the adaptive immune response is protective against mutant SOD1 induced neurodegeneration. Vaccination of $SOD1^{G93A}$ mice with Copaxone (copolymer-1) led to an increase in lifespan of almost 25%. This efficacy is thought to result from the sensitization of Th1 cells to self-antigens. This theoretically leads to an increase in Th1 migration to injured sites, activation, and production of cytokines and neurotrophins [101]. Protection against β-amyloid and glutamate excitotoxicity has been achieved through control of microglial phenotype via the presence or absence of CD4+CD25+ helper T cells [96]. A series of experiments demonstrated that the neuroprotection resulting from vaccination with CNS-specific self-antigens may arise from stimulation of microglia with IFN-γ, which is produced by reactive helper T cells. This stimulation results in increased glutamate uptake and decreased NO production as well as decreased mRNA expression of inflammatory products including COX-2, iNOS, and IκBα (an inhibitor of NF-κB) by microglia compared to LPS-treated microglia. In addition, IFN-γ-stimulated microglia had greater antigen-presenting capability and were therefore able to more efficiently recruit Th1 cells and increase glutamate clearance [95]. The dual role of microglia as protectors and destructors demands strict regulation, which is likely performed by the adaptive immune system and could be intensified by therapies such as vaccination. Although one downfall to such therapies is the risk of possible autoimmune disease, in vivo immunization studies using Copaxone and myelin-associated antigens have proven successful in increasing neuronal survival and attenuating behavioural deficits in both acute and chronic models of neurodegeneration without evoking autoimmune disease [96,102].

Helper T cells also aid B-cells, effectors of acquired immunity, in immunoglobulin (Ig)G production. While there has been some controversy regarding the role of IgG in inducing altered calcium permeability in ALS motor neurons, IgG isolated from ALS patient serum injected intraperitoneally into mice has been shown to localize within lower motor neuron axons [103]. Several in vivo studies have demonstrated the ability of passively transferred ALS IgG to cause recruitment of activated microglia [104] and motor neuron degeneration in recipient animals via increased calcium permeability and excitotoxicity [105,106]. An in vitro experiment demonstrated in mixed primary spinal cord cultures that IgG from patients with ALS induces apoptosis via the caspase-3 pathway selectively in motor neurons [107]. One experiment demonstrated the ability of ALS IgG to shift the activation curve of calcium current, indicating an alteration in the voltage dependence of calcium channels in IgG-treated rat motor neurons [108]. The controversy arises, however, in that others have failed to demonstrate such effects in rat cerebral synaptosomes, thus concluding that ALS IgG is ineffective in enhancing presynaptic calcium influx [109]. Perhaps some specificity exists which makes presynaptic voltage-sensitive calcium channels on motor neurons exclusively antigenic targets.

## 7. The mediating role of astrocytes

Astrocytes represent a different class of supportive glial cells that also become reactive in areas of motor neuron degeneration in ALS. A central role of these cells is to support and sustain proper neuronal function by regulating extracellular glutamate levels. Their function as executors of glutamate clearance may be compromised in ALS. Astrocytes isolated from a mutant SOD1 transgenic rat show a 3-fold higher expression of mGluR5 over controls, activation of which fails to lead to increased glutamate uptake [110]. Although the evidence is controversial, astrocytes in ALS have been observed to have decreased expression of the glutamate transporter EAAT2, potentially leading to decreased glutamate transport and subsequent increases in extracellular glutamate [111]. However, this decrease in expression resulting from alternately spliced variants of EAAT2 has been demonstrated in normal controls in addition to ALS patients [112,113].

Monocyte chemoattractant protein-1 (MCP-1) is critical for migration of monoctyes to areas of injury. As discussed in relation to adaptive and acquired immunity in ALS, MCP-1 concentrations are significantly increased in both serum and cerebrospinal fluid (CSF) from ALS patients. MCP-1 immunoreactivity is highest in astrocytes in ALS spinal cord, suggesting that astrocytes have an important role in mediating the inflammatory response to injury in ALS [98].

Astrocytes can also participate directly in inflammatory reactions. Like microglia, reactive astrocytes express inflammatory markers including iNOS and COX-2 [114] and can produce proinflammatory mediators including prostaglandins [115], IL-6 [116] and TNF-α [114]. There is evidence that reactive astrocytes can even directly induce motor neuron death. Fibroblast growth factor-1 (FGF-1) released from motor neurons in response to injury or oxidative stress leads to accumulation of FGF receptor-1 (FGFR1) in astrocytic nuclei and stimulates nerve growth factor (NGF) expression and secretion by astrocytes [117]. This NGF production has been previously shown to induce motor neuron apoptosis via a p75-dependent mechanism [118,119]. Fas ligand and TNF-α produced by reactive astrocytes can also activate death receptors in injured motor neurons. Returning to the work of Raoul et al., ALS motor neurons are particularly susceptible to Fas signaling, and the apoptotic pathway implicated is motor neuron-specific [36]. Therefore astrocytes, like microglia, appear to suppress their trophic activity and adopt a more cytotoxic phenotype with disease progression. Their loss of glutamate clearing activity likely contributes significantly to persistent microglial activation.

## 8. Anti-inflammatory treatment of ALS

The identification of microglial activation and proliferation as hallmarks of ALS in vivo and as contributors to motor neuron death in vitro has led to anti-inflammatory treatment efforts in mouse models of motor neuron degeneration.

Support for the role of microglia in motor neuron degeneration is also gained from the observation that the administration

of minocycline, a tetracycline derivative, prolongs the symptom free interval prior to the onset of motor dysfunction by roughly 9–20% in various mutant SOD1 transgenic mice and extends survival by 13–25% (in multidrug cocktails in which minocycline is included ) [120–122]. It has also been shown to reduce the extent of motor neuron microvacuolar degeneration at 120 days [123]. Minocycline is a second-generation tetracycline with distinct antibiotic and anti-inflammatory properties [124–126]. The site of action of minocycline appears to be at both the level of the microglia in which the up-regulation of iNOS is inhibited, and at the target cell where the release of mitochondrial cytochrome *c* (and thus the initiation of a pro-apoptotic pathway) is inhibited [127–130]. The neuroprotective effects of minocycline are thought to result from inhibition of microglial activation [131,132] and possibly proliferation [133]. These results led to phase I/II studies of minocycline in ALS patients [134]. Although no difference was observed between treated and untreated groups in these studies, pivotal phase III trials are ongoing. It is noteworthy that in addition to its anti-inflammatory effects, minocycline has been shown to also have anti-apoptotic [135,136], antioxidant [137,138], and anti-glutamatergic effects [136,138]. Therefore caution must be taken when attributing its efficacy in mouse models to treatment of inflammation alone.

Celecoxib (Celebrex) and rofecoxib are inhibitors of COX-2. Treatment with these COX-2 inhibitors combined with creatine increased survival by up to 30% in SOD1 mutant mice [139–141] while treatment with a non-specific COX inhibitor (sulindac) extended survival by roughly 10% [142]. It should be noted, however, that to date none of the COX inhibitors tested have shown efficacy in human ALS patients. Celecoxib, developed for the treatment of rheumatoid arthritis, continues to be tested in a variety of neurodegenerative diseases considered to have inflammatory components.

It is likely that treatment targeting microglial activation and the production of inflammatory mediators will have to be combined with cellular or molecular therapies treating neuronal abnormalities that lead to the initial recruitment of microglia.

## 9. Conclusion

Although evidence appears to suggest a role for microglia and their inflammatory products in the pathogenesis of ALS, the most significant pieces of information remain elusive. Most importantly, what causes the initial priming of microglia? Do microglia respond to primary stimuli from predisposed motor neurons and take on a protective role, or is ALS a disease with auto-immune characteristics? Wyss-Coray and Mucke suggest that abnormal protein accumulation or other signals emanated from affected motor neurons may summon microglia and trigger their activation [94]. This is supported by evidence that mutant SOD1 secreted from neurons can activate microglia and lead to neuronal death [143]. Other studies suggest, however, that in ALS, microglia can have cytotoxic potential regardless of the health status of neighbouring motor neurons [11]. Does then the role of microglia in pathogenesis depend on whether they too are predisposed as indicated by these chimeric studies? The results of minocycline treatment of mutant SOD1 mice suggest that microglial activation is concomitant with and contributes to disease progression. However, no benefit of the inhibition of microglial activation has been observed in human patients. As discussed previously, ALS is clearly a multi-factorial disease in which complex neuronal–glial interactions exist. Hence, it is important that molecular communication between neurons and glia be studied comprehensively in both healthy and diseased states. It is also important that the relationships between various pathological features of ALS intrinsic to motor neurons themselves and the glial inflammatory response are studied independently to determine which abnormalities could be triggering inflammation. Although mutant SOD1 models allow for validation of potential treatments, they do not necessarily facilitate understanding of particular underlying pathophysiological mechanisms contributing to disease initiation.

The cells of the immune system, including the tissue-specific macrophages like microglia, are capable of coordinating highly selective attacks on specific target cells based on principles of recognition. Immunity is therefore a process that is potentially altered in ALS and, as such, is an intriguing candidate for therapeutic intervention. This is not to say that ALS is a disease of cells of the macrophage lineage. Rather, the evidence suggests that ALS is a disease in which inflammation remains a *theoretically* beneficial process. It is the ambivalent nature of activated microglia that not only creates controversy as to their role in the pathogenesis of ALS, but also offers therapeutic potential as a useful tool in the modulation of factors influencing motor neuron death in ALS such as release of inflammatory mediators with pro-apoptotic effects and extracellular glutamate clearance. Perhaps, inflammation is a key determinant of the ALS disease process, but its elimination as a therapeutic objective may be less successful than its modulation.

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