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Glial cells in Amyotrophic Lateral Sclerosis

T Philips and **JD Rothstein**
Johns Hopkins University, Baltimore, MD USA

Amyotrophic Lateral Sclerosis is a motor neuron disease affecting upper and lower motor neurons in motor cortex, brain stem and spinal cord. Currently, there is no effective cure available to halt or delay disease from progressing. Over the past 20 years multiple genes that cause disease have been identified, most notably mutations in the Super Oxide Dismutase 1 (SOD1) gene and repeat expansions in the first intron of the C9ORF72 gene. Moreover, pathology associated with a very rare genetic form of ALS, TDP-43, is thought to be involved in disease pathogenesis in the majority of ALS patients (Neumann et al., 2006). The discovery of different disease causing mutations has led to the development of ALS animal models, which provide researchers a tool to better understand disease mechanisms. For the past 20 years, the mSOD1 transgenic overexpression mouse and rat has been the most widely studied animal model to study disease mechanisms and therapeutics (Gurney et al., 1994). More recently, some TDP-43 animal models have been generated, but their true potential for studying disease pathogenesis and drug discovery remains uncertain.

Studies using ALS animal models have taught us that the well being of neurons in general and specifically motor neurons is highly dependent on a whole range of other cell types, commonly named glial cells, which surround motor neurons and provide nutritional and trophic support to them. The study of glial cells in the past has led to the conclusion that ALS is a non-cell autonomous multifactorial disease in which many cell types as well as divergent disease mechanisms all converge to the focal death of interneurons and motor neurons. This review will introduce the different glial cell types in the CNS and provide an overview of the role of glial cells in motor neuron degeneration. Several potential relevant disease mechanisms for each specific glial subtype will be mentioned. These have led to the development of therapeutics specifically targeting the glial compartment.

Glial cellular reaction in ALS

Microglia and Immune reactivity

Microglial cells are of mesodermal origin and the main immune-competent cells of the central nervous system. Microglia release a whole range of pro-inflammatory versus anti-inflammatory cytokines and chemokines when encountering any damaging hazard. They will respond through the release of pro-inflammatory (so-called ‘classically activated’ or

M1, with release of e.g. tumor necrosis factor alpha (TNFα), interferon 1beta (IL-1β), nitric oxide (NO), O_2, interferon gamma (IFN-γ) factors which clear and/or limit biological hazards followed by release of anti-inflammatory (so-called 'alternatively activated' M2, with release of e.g. interleukin 4(IL-4), IL-10, insulin growth factor 1 (IGF-1) factors which repair and mediate restoration. Microglial cells function in close interaction with inflammatory T-cells as well as astrocytes in mediating this inflammatory response. Microglia do not exclusively express either M1 versus M2 cytokines and can release different combinations of cytokines and chemokines depending on the environment they are exposed to and their direct interaction with other cells, and so may present a different phenotype at different time points and locations in the CNS.

In ALS patients as well as ALS rodent models, there is a clear microglial reaction characterized by the upregulation of a whole range of markers used to identify microglia like CD11b and Iba1 as well as an upregulation of markers associated with antigen presentation like CD11c, intracellular adhesion molecule 1 (ICAM-1) and CD86, suggesting that microglial cells closely interact with concomitant infiltrates of inflammatory T-cells (Alexianu et al., 2001; Henkel et al., 2004; Turner et al., 2004; Henkel et al., 2006; Corcia et al., 2012). Similarly, microglia increase in the release of pro-inflammatory cytokines as well as chemokines (Poloni et al., 2000; Nguyen et al., 2001; Hensley et al., 2003; Henkel et al., 2004; Henkel et al., 2006; Meissner et al., 2010). Analysis of microgliosis in ALS patient CNS largely depends on studies of post mortem tissue, which demonstrate increased microgliosis in motor cortex, motor nuclei of the brain stem, the corticospinal tract and the ventral horn of the spinal cord (Kawamata et al., 1992). Live in vivo PET scanning with ^{11}C-PK11195, a ligand that highly binds to CNS microglia, as well as other CNS cells to a lesser extent, reveals apparent microgliosis in ALS patients, suggesting a correlation between the extent of microgliosis and damage to upper motor neurons, but not lower motor neurons (Turner et al., 2004). Another microglial imaging approach uses a radioligand of the translocator protein (TSPO), upregulated by microglia upon microglial activation (Corcia et al., 2012). Significant microgliosis was detected in primary motor cortex, supplementary motor cortex and temporal cortex (Corcia et al., 2012).

In mSOD1 animal models, there is an increase in microglial reactivity in the ventral horn of the spinal cord, which follows closely the nerve de-innervation at the neuromuscular junction, one of the first pathological signs of motor neuron degeneration (Alexianu et al., 2001; Saxena et al., 2009). This microglial reaction in the CNS is thought to be mainly mediated by local proliferation, rather than infiltration and microglial differentiation of myeloid cells from the blood stream through the blood brain barrier (BBB)(Ajami et al., 2007; Gowing et al., 2008).

Interestingly other myeloid (cells from myeloid origin like monocytes, macrophages and microglia) subtypes might contribute to the overall 'microglial' reaction and motor neuron degeneration. A recent study indicated that Ly6C+ spleen derived monocytes are recruited to the spinal cord of mSOD1 mice by CCL2 expressing resident microglia (Butovsky et al., 2012). Concomitantly with this infiltration of Ly6C+ monocytes, CD39+ resident microglial cells are degenerating, suggesting against a so-called 'microgliosis' but rather a decrease in resident microglia and increase in spleen derived monocytes. These Ly6C+ macrophages in

mSOD1 mice seem to accelerate the loss of motor neurons as anti-Ly6C antibody treatment lead to a significant prolongation of life span in mSOD1 mice. Interestingly, similar to what is seen in the mSOD1 mice, M1-primed monocytes accumulate in the spinal cord of ALS patients. It has not been proven however if the resident microglia were truly dying in contrast to losing immunoreactivity for the marker used to identify these cells. In addition, technical limitations in identifying the origin of specific myeloid subsets prevail, as irradiation and transplantation of bone marrow cells causes non-physiological damage to the BBB and a non-physiological influx of progenitor cells and might impair the conclusions drawn from this study (Ajami et al., 2007). Another recent study performing transcriptional profiling of microglial cells in the spinal cord of mSOD1 mice failed to confirm the presence of Ly6C expressing infiltrating macrophages in the spinal cord (Chiu et al., 2013). The authors suggested that resident microglial cells increased in the spinal cord of mSOD1 mice whereas monocytes did not (Chiu et al., 2013). Given the discrepancies of this study as compared to others, it remains to be fully elucidated whether infiltrating monocytes truly contribute to motor neuron disease. Importantly the translation of the numerous transgenic mouse studies has not be equally carried out in human ALS tissue-making difficult comprehensive assumptions regarding relevance to human disease.

Astroglia

Astrocytes are ectodermal cells involved in ion homeostasis, neurotransmitter recycling and metabolic support to surrounding neurons. One of the most important and extensively studied supportive functions of astrocytes is their involvement in the glutamate-glutamine cycle (Danbolt, 2001). Glutamate is one of the most important neurotransmitters in the CNS mediating excitatory synaptic communication between neurons. Uptake of glutamate from the synaptic cleft between presynaptic and postsynaptic neuron through glutamate transporters EAAT2 (GLT-1 in rodents) and EAAT1 (GLAST in rodents) expressed by astrocytes will prevent excessive postsynaptic stimulation of glutamate receptors and motor neuron cell death, a process which is called glutamate mediated excitotoxicity. Another important function for astrocytes is their involvement in the metabolic support of neurons. Astrocytes are tightly coupled to the blood stream and strongly interconnected through gap-junctions through which they provide metabolic substrates over long distances. Under conditions of increased neuronal activity and metabolic substrate demand, astrocytes increase their glycolytical activity, converting glucose to lactate (Pellerin and Magistretti, 1994). Both glucose and lactate are hypothesized to be distributed by astrocytes and gap-junction connected oligodendrocytes throughout the parenchyma and used as an energy substrate by neurons (Funfschilling et al., 2012; Lee et al., 2012). Like microglia, the astrocyte function is highly influenced by T-cells, (motor)-neurons and possibly oligodendrocytes. Astrocytes are usually identified by the expression of several astroglia specific (or enriched) proteins including: glial fibrillary acidic protein (GFAP), aldehyde dehydrogenase 1L1 (ALDH1L1), EAAT2/GLT1, EAAT1/GLAST and aquaporin4 (AQP4). When astrocytes encounter any biological hazard in their immediate surroundings, astrocytes become reactive and increase the expression of some astrocyte markers (e.g. GFAP and ALDH1L1) in a process called astrogliosis. Typically, with neuronal or dendritic injury, astroglial GLT1 actually decreases expression (Rothstein et al., 1992; Bruijn et al., 1997; Tawfik et al., 2008).

In the brain of ALS patients, astrogliosis is seen in both the grey as well as white matter and not limited to the motor cortex (Kushner et al., 1991; Nagy et al., 1994). In the spinal cord of ALS patients, there is an enhanced astrocytic reactivity in the ventral horn, dorsal horn and the regions where the corticospinal tract fibers enter the grey matter (Schiffer et al., 1996). Similarly in rodents, astrogliosis has been observed in the spinal cord, though with varying intensity depending on the mouse line studied (Levine et al., 1999). Using a luciferase based approach to live in vivo analyze the astroglial activation, it was found that astrogliosis occurs in waves, becoming more prominent with disease progression (Keller et al., 2009). This astrogliosis is, unlike for microgliosis, not mediated by proliferation of resident astroglia, but rather by activation of pre-existing resident astrocytes and a change in their expression of proteins (e.g. increased GFAP, decreased GLT1) and altered morphology of their fine processes (Bruijn et al., 1997; Gowing et al., 2008; Lepore et al., 2008a). Not much is known about the existence of a so-called astrocyte precursor cell that could differentiate into reactive astrocytes in response to motor neuron degeneration; and there is little strong evidence for such activity in vivo. Another source for astrocytes could be the ependymal cells lining the central canal of the spinal cord and ventricles of the brain (Chi et al., 2006; Barnabe-Heider et al., 2010). These cells differentiate in astrocytes in other models of neurodegeneration like stroke and dorsal funiculus lesion (cf. below) but it remains to be explored whether these cells contribute to astrogliosis in models of motor neuron loss (Carlen et al., 2009; Barnabe-Heider et al., 2010). Although abundant evidence teaches us that astroglia are pathologically and functionally abnormal, from post mortem studies, little is known about physiological alteration of astrocytes in living human patients. New PET ligands, specific for astroglia are under development and could greatly aid in the understanding of temporal and regional dysfunction of astroglia in human disease (unpublished observations).

NG2 – Oligodendroglial Progenitor cells

NG2 glia or oligodendrocyte progenitor cells (OPCs) are widely distributed throughout the CNS and comprise around 5% of total cell number. Besides their role as progenitor cells of oligodendrocytes, replacing oligodendrocytes upon any demyelinating insult, there are ongoing studies to determine whether these cells have specific functions on their own. NG2 cells are coupled to neurons and receive synaptic contact from neurons presumably monitoring the neuronal firing activity (Bergles et al., 2000). The exact role for this synaptic monitor is unclear, as it may be tied to some way to their role as progenitors- or other modulators of CNS activity. Their role as progenitor cells has been more thoroughly explored. Until recently, NG2 cells were thought to be able to differentiate into other cells than oligodendrocyte lineage cells, for example astrocytes, since in vitro these cell readily can differentiate into either mature forms of either astroglia or oligodendroglia (Kondo and Raff, 2000). Several independent rodent fate-tracing studies however limit their differentiation in vivo to only oligodendroglia under normal conditions (Rivers et al., 2008; Kang et al., 2010). In fact, even in neuro injury and disease conditions these progenitors remain committed to an oligodendroglial lineage in vivo (Kang et al., 2010; Tripathi et al., 2010; Zawadzka et al., 2010).

Oligodendroglia

The progeny of the OPC's, the oligodendrocyte, is the cell important for generating and maintaining the myelin sheath around the axon enabling fast salutatory conduction of action potentials as well as providing motor neurons with metabolic support. Some motor neurons can be significantly long (>3 feet) and are particularly dependent on metabolic substrates provided by oligodendrocytes. It has recently been established that oligodendrocytes express the monocarboxylate transporter 1 (MCT1) through which metabolic substrates like lactate and ketone bodies together with hydrogen ions are being exported and subsequently provided to neurons, especially at regions of close neuronal contact like at the paranodal region as well as at the Schmidt-Lanterman incisures (Lee et al., 2012). After release of lactate through MCT1, lactate can be taken up by neurons through another MCT, MCT2(Rafiki et al., 2003). Neurons themselves are highly dependent on this oligodendrocyte-derived lactate as mice with reduced MCT1 mediated transport develop widespread axonal degeneration (Lee et al., 2012). Whether Schwann Cells, who myelinate the axons in the peripheral nervous system, are similarly providing metabolic support to neurons still remains to be established. In addition, mice lacking the expression of specific oligodendrocyte markers like PLP and CNPase develop axonal degeneration independent of widespread demyelination, again suggesting that oligodendrocytes (and Schwann Cells in the periphery), independent of their function as generating the myelin sheath, are instrumental in providing widespread support for axons (Griffiths et al., 1998; Lappe-Siefke et al., 2003).

Interestingly, in ALS patients post mortem tissue, OPCs in brain and spinal cord appear to be damaged and patches of demyelination have been identified, indicating oligodendrocyte injury and abnormal remyelination (Kang et al., 2013). In addition, MCT1 expression levels are reduced, suggesting an impairment of oligodendrocyte metabolic support to neurons (Lee et al., 2012). In transgenic ALS mice, expressing mutant SOD1 (G93 SOD1), oligodendrocytes become injured, degenerate and die and are replaced by proliferating and differentiating OPCs which ensure oligodendrocyte cell number is restored (Kang et al., 2013; Philips et al., 2013). This oligodendrocyte degeneration is an early event, well before motor neuron loss becomes apparent. In spite of this replacement of dying oligodendrocytes and the lack of loss of oligodendrocyte cell number, there is clear loss of oligodendrocyte function as the expression of many markers associated with oligodendrocyte biology like MBP, a component of the myelin sheath, as well as MCT1 expression, are significantly reduced (Lee et al., 2012; Kang et al., 2013; Philips et al., 2013). This reduction is concomitant with a reduction in lactate release in the spinal cord of mSOD1 mice (Ferraiuolo et al., 2011). It is still uncertain why newly generated oligodendrocytes fail to function properly, but their demise contributes to the demise of motor neuron, as evidence by the fact that partial prevention of oligodendroglial injury greatly delays disease in the ALS rodent model (Kang et al., 2013). Importantly, the injury to oligodendroglia was not just seen in an ALS mouse model, but human ALS tissue as well (Kang et al., 2013). Aside from providing evidence of injury of these cells in ALS—these studies unexpectedly revealed a major role for this glial cell in ALS pathophysiology (Kang et al., 2013; Philips et al., 2013).

Pericytes

Pericytes in the CNS are cells who contribute to the generation of the blood brain barrier as well as being important for ion homeostasis and angiogenesis. They are usually identified by the expression of platelet derived growth factor β receptor, to which PDGFβ, released by endothelial cells, binds and communicates with pericytes. Pericytes are also involved in glial scar formation upon a CNS insult (Goritz et al., 2011). In ALS rodent models and to a lessor extend in ALS patients, the BBB is disrupted, characterized by upregulation of adhesion molecules and downregulation of tight junction proteins (Zhong et al., 2008; Winkler et al., 2013). In mSOD1 mice, this disruption occurs well before the onset of motor neuron loss and is characterized by extravasation of erythrocytes and plasma proteins (Zhong et al., 2008). In ALS patients spinal cord, pericyte number is significantly affected, and plasma derived immunoglobulin G, fibrin and thrombin is accumulating in the parenchyma (Winkler et al., 2013). Perivascular hemoglobin deposits are present, their presence negatively correlating with pericyte cell number.

Ependymal Glia

The CNS contains ependymal cells who line the ventricles in the brain and central canal in the spinal cord. These cells produce cerebrospinal fluid and circulate CSF by means of their cilia expressed at their apical membrane. They are also suggested to have stem cell properties and generate astrocytes and oligodendrocytes in response to forebrain ischemic insults as well as a spinal cord dorsal funiculus lesion (Carlen et al., 2009; Barnabe-Heider et al., 2010). Using Nestin-lacZ animals crossbred to mSOD1 mice, it has been shown that ependymal cells proliferate and migrate to the dorsal and later also ventral horn of the spinal cord where they generate new neurons suggesting attempts are being made to restore neuronal cell loss in the spinal cord of mSOD1 mice (Chi et al., 2006). Whether these neural progenitor cells truly migrate from the ependymal zone and contribute to the generation of newly derived fully functional neuronal units has not been established. Given the extensive motor neuron and interneuron neurodegeneration in the ALS rodent models, this process, if it occurs, is certainly not robust.

Glial cells contribute to motor neuron degeneration

The first evidence of glial dysfunction in ALS, in patients and in animal models, came from studies in the mid-1990's examining astroglial glutamate transporters. These studies documented that astroglia had markedly diminished expression of EAAT2 GLT1 in motor cortex and spinal cord, in both sporadic ALS, familial ALS (mutant SOD1 and C9orf72) as well as mSOD1 rodent models (Rothstein et al., 1992; Bruijn et al., 1997). Similarly, early in vivo and in vitro studies documented that specific molecular knockdown of this astroglial protein could produce neuronal, including motor neuronal degeneration and paralysis (Rothstein et al., 1996). These studies thus demonstrated that astroglia themselves could be significant contributors to motor neuron degeneration through comprehensive studies of human tissue, animal models and in vitro models.

Further investigations that markedly strengthened the concept that non-neuronal cells could contribute to ALS came from studies a decade later, using mice chimeric for mSOD1. These

mice which are a mixture of mSOD1 expressing cells and wild type cells, had a disease onset and progression highly affected by the level of the mSOD1 chimerism, with a higher contribution of mSOD1 expressing cells to total cell number accelerating the phenotype (Clement et al., 2003). For example, some of the animals developed with spinal motor neurons bilaterally expressing mSOD1. However, the loss of motor neurons was more prominent when surrounded by mSOD1 expressing non-neuronal cells as compared to wild type non-neuronal cells. Wild type motor neurons could also show signs of injury e.g. intracellular ubiquitination, when they were found to be surrounded by mSOD1 expressing non-neuronal cells.

When mSOD1 was selectively expressed in motor neurons alone it was insufficient to cause full blown motor neuron disease when compared to the ubiquitous expressing mSOD1 expressing animals (Pramatarova et al., 2001; Jaarsma et al., 2008). On the other hand, selective depletion of mSOD1 in motor neurons of mSOD1 G37R mice affects disease onset, does not affect disease progression but significantly affects survival (Boillee et al., 2006; Yamanaka et al., 2008b). These studies indicate that mSOD1 in the surrounding glial cells strongly influences the degeneration of the surrounding motor neurons. Similar to what is shown with motor neurons, selective mSOD1 expression in CD11b+ microglia, GFAP+ astrocytes or P0+ Schwann cells does not lead to motor neuron degeneration whereas mSOD1 expression targeted to skeletal muscle induces mild muscle weakness and mitochondrial dysfunction, but not death (Gong et al., 2000; Beers et al., 2006; Dobrowolny et al., 2008; Turner et al., 2010). On the other hand cervical transplantation of mSOD1 glial restricted precursor (GRP) and subsequent engraftment of progenitor cell derived mSOD1 expressing GFAP+ astrocytes in an otherwise wild type environment did induce motor neuron loss and forelimb paralysis (Papadeas et al., 2011).

A more specific cellular contribution of non-neuronal cells was addressed using mice in which mSOD1 was depleted in a cell specific manner. Loss of mSOD1 G37R or mSOD1 G85R from microglia did not delay disease onset but delayed disease progression and increased survival (Boillee et al., 2006; Wang et al., 2009). This was further validated in another mSOD1 G93A line in which upon wild type bone marrow cells transplantation into mSOD1 G93A/PU1−/− animals (which do not have microglia), life span was significantly delayed as compared to ubiquitous expressing mSOD1 G93A mice, again suggesting that wild type microglia are more neuroprotective than mSOD1 microglia (Beers et al., 2006). Interestingly, this extension of survival might also be mediated in part by mSOD1 deletion from peripheral myeloid cells, like the Ly6C+ monocytes infiltrating the spinal cord (cf. above) or macrophages infiltrating the sciatic nerve of mSOD1 mice (Chiu et al., 2009; Butovsky et al., 2012). On the other hand, depletion of proliferating mSOD1 expressing microglial cells in mSOD1 G93A mice, although reducing the number of activated microglia, did not affect motor neuron degeneration (Gowing et al., 2008). Therefore, depletion of mSOD1 expression in microglia, generating wild type microglial cells, seems to be more neuroprotective as compared to depleting microglial cell number.

Similar to microglia, deletion of mSOD1 G37R from astrocytes delayed disease progression and extended survival (Yamanaka et al., 2008a). These results in astrocytes were mimicked by transplantation of wild type glial progenitor cells into the spinal cord of mSOD1 G93A

transgenic rats (Lepore et al., 2008b). Upon transplantation these GRPs differentiate into wild type GFAP and GLT-1 expressing astrocytes in an otherwise mSOD1 expressing environment, delaying disease progression and improving survival. In addition, AAV9 vectors carrying shRNA directed against mSOD1 and mainly targeting CNS astrocytes upon tail vein injection were able to delay disease progression in different mSOD1 lines (Foust et al., 2013). Interestingly, the same study showed that intrathecal delivery of these shRNA's targeting SOD1 successfully depleted SOD1 in motor neurons of non-human primates, setting the stage for AAV9 mediated therapies in clinical trials.

Although these in vivo studies provide compelling evidence for a role of microglia and astrocytes in ALS, they provide little in the way of a molecular or biochemical mechanism for this toxicity. For astrocytes, other than the well replicated loss of GLT1/EAAT2 in ALS patients and in rodent models (see above), it remains unclear how astroglia in vivo can directly lead to neurodegeneration, for example through the release of unknown toxic molecules and/or the loss of expression of other trophic factors. Importantly, the role for astroglia and microglia in disease pathogenesis almost certainly must be partial in that selective mSOD1 expression in one particular cell type causes subtle pathological changes leading to minor motor neuron degeneration, but more widespread mSOD1 expression is necessary to see motor neuron degeneration to the degree reminiscent of that seen in the ubiquitous expressing mSOD1 mouse.

More recently, depletion of mSOD1 expression from PDGFRa+ oligodendrocyte progenitor cells significantly delayed disease onset and increased survival (Kang et al., 2013). In these mice, depletion of mSOD1 is not restricted to OPCs but also includes OPC derived oligodendrocytes. Early studies suggested that this protection was mediated, at least in part by the prevention of loss of MCT1 expression by oligodendroglia (Kang et al., 2013). Interestingly, peripheral deletion of mutant SOD1 from Schwann cells was actually neurotoxic rather than becoming more neuroprotective (Lobsiger et al., 2009). The mechanism for this unexpected outcome was unclear, but hypothesized to be the result of residual neuroprotective dismutase activity specifically in the Schwann cells. Lastly, selective depletion of mSOD1 from endothelial cells or skeletal muscle did not affect disease (Miller et al., 2006; Zhong et al., 2009).

These studies addressing cell-specific depletion of mSOD1 originally have lead to the suggestion that selective deletion of mSOD1 from glial cells affect disease progression whereas mSOD1 deletion from motor neurons affects disease onset. As attractive as that hypothesis might seem, this was not confirmed in another study though in which deletion of another mSOD1, mSOD1 G85R, from astrocytes lead to similar results as with mSOD1 G37R, but in this study disease onset was also affected upon deletion of mSOD1 from astrocytes (Wang et al., 2011). The different nature of the mutation might underlie this discrepancy. Attributing changes in onset and progression to one particular cell type in fact may be erroneous and rather ambiguous. Does the dramatic alteration in onset attributed to oligodendroglial injury imply that these cell initiate disease (Kang et al., 2013)? In chimeric mice in which all motor neurons and oligodendrocytes express mSOD1 G37R but all other glial cells are chimeric for mSOD1 expression, disease onset is rather determined by the

chimerism of the non-neuronal (excluding oligodendrocyte) cells than the mSOD1 expression in motor neurons (Yamanaka et al., 2008a).

Besides mSOD1, more recent studies have tried to understand glial cell toxicity using newly generated TDP-43 overexpressing animals, another candidate model for ALS. Unlike for mSOD1, mTDP-43 transgenic mice overexpressing the mutant protein specifically in motor neurons do develop severe signs of motor neuron degeneration with the extent of motor neuron loss dependent on the level of mTDP-43 overexpression (Shan et al., 2010; Wils et al., 2010). Would this suggest a cell autonomous mechanism of motor neuron degeneration in contrast to what is seen in mSOD1 mouse models? That would be a premature conclusion. Models based on TDP overexpression (mutant or wildtype) are more complicated for studying cell-specific pathophysiology. As studies have now reliability revealed, merely alerting the expression of the native protein in cells (not just CNS cells) can be quite cytotoxic (Wegorzewska et al., 2009; Wils et al., 2010). Thus interpretations of cell specific expression are difficult to interpret when on considers TDP-43 pathophysiology in ALS. It now seems that both cell autonomous as well as non-cell autonomous mechanisms are involved in TDP-43 mediated motor neuron degeneration. In rats, selective mutant TDP-43 (mTDP) (M337V mutant) overexpression in astrocytes does lead to progressive motor neuron degeneration, likely because direct astroglia injury/ degeneration may occur in that model as reflect by loss of GFAP and GLT-1 expression seen in those studies (Tong et al., 2013). Injury to astroglia, in vivo, is known to cause neurotoxicity as a consequence of losing normal astroglia functions such as glutamate transport, altered potassium buffering and metabolic support. In contrast, transplantation of mTDP-43 (A315T mutant) transgenic or TDP-43 depleted mouse derived GRPs does not induce motor neuron degeneration and forelimb paralysis upon transplantation in the cervical spinal cord of wild type rats (Haidet-Phillips et al., 2013). Another study used human iPS derived astrocytes from a patient carrying a mTDP-43-M337V mutation and found that these cells were not neurotoxic to co-cultured iPS derived wild type or mTDP-43 M337V expressing motor neurons, although mutant astrocytes degenerated faster as compared to control derived astrocytes (Serio et al., 2013). Differences in transgene expression level and differences in the nature of the mutation and species being studied almost certainly underlie the discrepancies in these studies, and make comparison difficult. In addition, a recent study reported that muscle specific knockdown or overexpression of the Drosophila TDP-43 orthologue TBPH, causes motor abnormalities versus motor deficits with early lethality respectively (Diaper et al., 2013). The authors suggested that changes in the Drosophila orthologues for EAAT1/EAAT2 which are involved in glutamate transport, might underlie the phenotype in both knockdown as well as overexpression models. Although motor neuron specific mTDP-43 expression in Drosophila seems to confer different effects on the cellular level as compared to mTDP-43 expression in glial cells, its effects on the phenotypical level is very similar (Estes et al., 2013).

Another cell type involved in motor neuron degeneration is the inflammatory T-cell. In ALS patient post mortem tissue, T-cell infiltrates were seen in both brain and spinal cord (Engelhardt et al., 1993). In mSOD1 mice, T-cells are readily seen infiltrating the spinal cord throughout disease progression (Beers et al., 2008; Chiu et al., 2008). To explore the role of T-cells in ALS, mSOD1 G93A mice were crossbred with RAG2−/− animals (who do

not develop T and B-cells), CD4 knockout mice or T-cell receptor knockout mice (TCR−/−) (Beers et al., 2008; Chiu et al., 2008). T-cell depletion or functional depletion of T-cells led to a significantly reduction in life span as compared to mSOD1 mice (Beers et al., 2008; Chiu et al., 2008). Microglial immunoreactivity for markers like Iba1 and CD11b is significantly reduced, but there is a concomitant upregulation of inflammatory markers associated with a M1 microglial phenotype and a reduction in M2 inflammatory markers (Beers et al., 2008; Chiu et al., 2008). Upon T-cell depletion, astrocytes also show lower expression levels for GLT-1 and GLAST, promoting glutamate-mediated excitotoxicity (Beers et al., 2008). Postnatal transplantation of either mSOD1 or wild type bone marrow cells restored T-cell number, the microglial and astroglial phenotype and restored life span (Beers et al., 2008). The presence of T-cells therefore seem to be essential to induce a neuroprotective M2 phenotype in microglial cells which seems to ultimately fail as disease is still progressing. This has been further elucidated in vitro using adult microglial cultures comprising of adult microglia derived from mSOD1 mice at disease onset and at end stage of disease. Microglial cells derived at disease onset had a M2 phenotype and were more neuroprotective to co-cultured motor neurons as compared to microglia derived at end stage of disease, which had a M1 inflammatory phenotype and induced cell death in co-cultured motor neurons (Liao et al., 2012).

A thorough evaluation of different T-cell subsets in the CNS of mSOD1 G93A mice was performed to assess the different contribution of different T-helper (Th2, Th1) and CD4+FoxP3+ expressing regulatory T-cells (T-reg)(Beers et al., 2008; Beers et al., 2011). mSOD1 expressing T-reg cells at the early disease stages mediate the M2 microglial neuroprotective effect through release of IL-4 rather than Th2 cells whose presence does not change during disease progression (Beers et al., 2011; Zhao et al., 2013). Secreted IL-4 is known to inhibit the toxic properties of mSOD1 expressing microglia in vitro and is associated with neuroprotection in vivo (Beers et al., 2008; Zhao et al., 2012). Interestingly, these mSOD1 transgenic T-reg cells from early disease stages were found to be more neuroprotective than wild type T-reg cells. On the other hand, at later disease stages mSOD1 expressing T-regs have lost their ability to provide neuroprotection, cytotoxic Th1 cells become more prominent and microglia seem to become more neurotoxic (Beers et al., 2008; Beers et al., 2011). The mechanism(s) behind this conversion of neuroprotective to neurotoxic activity is still poorly understood and needs further investigation. This importance of FoxP3 expressing T-reg cells was further suggested in humans, as an early reduction in expression of the T-reg transcription factor FoxP3 was found to be predictive of a rapid disease progression and attenuated survival (Henkel et al., 2013). As Treg cells are very abundant outside the CNS, an alternative explanation for the neuroprotective action of T-reg cells could be the release of protective mediators in the peripheral circulation who subsequently reach the CNS. In addition, natural killer (NK) T-cells are known to infiltrate the spinal cord and inhibit neuroprotective T-cell responses (Finkelstein et al., 2011). Inhibition of NK T-cell activity led to a delay in disease onset and increased survival (Finkelstein et al., 2011).

A recent study performing transcriptional analysis of a whole range of microglia expressed chemokines and cytokines rebuffed the idea that microglia would become more polarized toward an M1 phenotype at later disease stages as no significant bias towards either

phenotype was seen at any disease stage and microglia express a whole range of both pro and anti-inflammatory chemokines and cytokines at any given disease stage (Chiu et al., 2013). The microglial reaction is therefore not beneficial or detrimental per definition, but rather a double-edged sword with different immune modulatory properties at different time points and probably different CNS regions. This could explain why the majority of immune modulatory therapeutic interventions in the past have failed (see below): they could target both detrimental as well as beneficial immune reactivity equally with no net outcome on disease progression.

Mechanisms of glial toxicity and therapeutics

A change in the expression of both proinflammatory as well as anti-inflammatory mediators has been found in the mSOD1 mouse model as well as ALS patients (Poloni et al., 2000; Nguyen et al., 2001; Hensley et al., 2003; Henkel et al., 2004; Henkel et al., 2006; Meissner et al., 2010). There is a concomitant increase in the microglial production of reactive oxygen species and reactive nitrogen species due to upregulation of the NADPH oxidase and NO synthase 2 (NOS2) respectively, as well as an increased activity of cyclo-oxygenase 2, involved in the pro-inflammatory prostanoid synthesis pathway (Almer et al., 2001; Beers et al., 2006; Wu et al., 2006). A recent report suggested that microglial exert neurotoxicity through activation of NF-kB signaling, as microglial reduction of NF-kB signaling lead to significant prolongation of disease progression and life span of mSOD1 mice (Frakes et al., 2014). A significant reduction in the expression of proinflammatory iNOS, CD86 and CD68 could underlie the neuro protective effects observed (Frakes et al., 2014). Interestingly, depletion of NF-kB signaling from microglial cells in otherwise wild type animals was sufficient to cause late onset motor neuron degeneration and loss of hind limb grip strength{Frakes, 2014 #858}. Another study also reported a significant change in the miRNA expression profile of Ly6C+ monocytes that had infiltrated the spinal cord, but not brain of mSOD1 transgenic mice (see above)(Butovsky et al., 2012). This change in miRNA profile occurred very early in disease and might contribute to the toxic action these monocytes confer upon infiltration. Interestingly, both mSOD1 fALS as well as sALS patients showed a similar miRNA profile as compared to mSOD1 mice.

Several studies have tried to explore whether the inhibition of the release of these pro-inflammatory factors is beneficial in the mSOD1 mouse model as well as in ALS patients (reviewed in (Philips and Robberecht, 2011)). The general conclusions of these studies which more or less specifically target specific inflammatory cytokines or chemokines, is that anti-inflammatory therapeutics have without exception proven to be highly unsuccessful, and if successful only moderately enhanced survival. The results from these studies in mSOD1 mice have shown to be highly dependent on the specific mSOD1 mouse model studied, the strain of the mSOD1 mice being used, potential compensation of other inflammatory factors and the non-specific targeting of inflammatory pathways. In fact, as outlined above, most of these therapeutical applications lack the spatial and temporal specificity and might overall target both beneficial as well as detrimental outcomes of the inflammatory reaction equally with no net outcome on disease. When applied to humans, it is not very surprising that none of the clinical trials based on the limited efficacy in mice were eventually successful.

Astrocytes are another cell involved in motor neuron degeneration. Just like microglia, mouse primary astrocytes derived from mSOD1 ALS mouse models are toxic to co-cultured embryonic stem cell derived or primary motor neurons (Di Giorgio et al., 2007; Nagai et al., 2007). One might question the relevance of these in vitro astroglial motor neuron co-cultures for in vivo biology but a recent study interestingly found a remarkable concordance between the expression profile of mSOD1 expressing in vitro astrocyte motor neuron co-cultures and the expression profile of spinal cords of mSOD1 mice in the progressive stage of disease (Phatnani et al., 2013). Interestingly, astrocytes derived from human spinal cord stem cells derived from both fALS and sALS patients were shown to be toxic to co-cultured mouse motor neurons (Haidet-Phillips et al., 2011). Although astrocytes are not immune cells by definition, they do contribute to the inflammatory response. In fact human fALS and sALS derived astrocytes upregulated 22 different, complement factors, chemokines and cytokines as compared to control astrocytes (Haidet-Phillips et al., 2011). Puzzingly, the toxicity derived from sALS astrocytes was dependent on the expression of the wild type SOD1 protein, supporting recent publications that oxidized wild type SOD1 as found in spinal cord of sALS patients could be a contributing factor in the pathogenesis of this non-mSOD1 mediated ALS(Haidet-Phillips et al., 2011). Importantly, these studies have all been performed in vitro and no strong data has emerged identifying the molecular or biochemical pathway for this toxicity or and perhaps most importantly, whether these events are active in human ALS patients. To date, only the loss of astroglial function, EAAT2, remains a confirmed pathway in humans as well as animal models for toxicity.

Thus, the identification of the whole range of factors causative for astrocyte mediated motor neuron degeneration still awaits identification and validation in human ALS. An interesting target could be a dysfunction in astrocyte metabolic support. In vitro, lactate release by astrocytes through the astrocyte Slc16a4 (MCT4) lactate carrier is significantly reduced (Ferraiuolo et al., 2011). Its in vivo relevance is unsure, as lactate release by oligodendrocytes, which express another lactate carrier SLC16a1 (MCT1), seems to be more substantial in providing metabolic support to motor neurons (Lee et al., 2012). In fact, heterozygous MCT1 null mice do develop widespread axonal degeneration and specific lentiviral mediated depletion of MCT1 in oligodendrocytes led to a significant reduction in the amount of motor neurons (Lee et al., 2012). Such a phenotype was not seen in MCT4 null animals (our unpublished observation).

Another supportive function mediated by astrocytes is the release of a whole range of neurotrophic factors like GDNF, BDNF, CNTF and VEGF. Adeno-viral (AAV) expression of IGF-1 or VEGF in mSOD1 mice has been shown to be beneficial in mSOD1 mice, delaying disease progression and increasing survival (Kaspar et al., 2003; Azzouz et al., 2004). Engraftment of GDNF or IGF-1 expressing human progenitor cells in the CNS of mSOD1 mice attenuated the loss of motor neurons but did not affect survival (Park et al., 2009). Muscle delivery of GDNF expressing mesenchymal stem cells did significantly affect survival in mSOD1 transgenic rats (Suzuki et al., 2008). Past clinical trials exploring the benefit of treating ALS patients with IGF1 and BDNF were repeatedly found to be ineffective (Kalra et al., 2003; Sorenson et al., 2008). Intrathecal delivery of GDNF trials were once initiated—but halted due to toxicity with no clear clinical efficacy reported. Clinical trials exploring the efficacy of VEGF are currently under way.

Astrocytes are instrumental in that they take-up excessive glutamate from the synaptic cleft upon release from the presynaptic function. Loss of GLT-1 might underlie the increased calcium inward current in the postsynaptic neuron, enhanced cytochrome-c release from the mitochondria and cell death due to excitotoxicity. GLT-1 levels in mSOD1 animals as well as the human EAAT2 homologue in ALS patients are significantly reduced as disease progresses contributing to motor neuron degeneration (Rothstein et al., 1992; Bruijn et al., 1997; Howland et al., 2002). These astrocytic GLT-1 levels are regulated by KBBP in astrocytes which in turn is regulated by an unknown factor released by motor neurons (Yang et al., 2009). Interestingly, exosomal release of miR124a from neurons is able to increase astrocyte GLT-1 expression levels in vitro as well as in vivo and could be an interesting target for therapeutic intervention (Morel et al., 2013). Enhancement of GLT-1 levels by transgenic overexpression (Guo et al., 2003) or by treatment of mSOD1 mice with ceftriaxone increases mSOD1 mice life span (Rothstein et al., 2005). A clinical trial using ceftriaxone to treat ALS patients has recently completed. An initial subgroup of patients, as part of the Phase 2 component of the trial, appeared to greatly benefit with disease slowing, but when the trial was expanded to a larger cohort of the phase 3 component, this clinical benefit was largely lost. Whether EAAT2 expression was actually altered by the drug in ALS patients was not determined (unpublished observations) and thus whether astroglial EAAT2 expression or function was actually augmented is not known. Future studies should require the use of pharmacodynamic markers for drug action and a PET ligand for EAAT2/astroglia is now in development (unpublished observations).

Motor neuron death mediated by oligodendrocytes and potentially their NG2 glial progenitor cells has only recently been addressed (cf, above). Loss of oligodendrocyte function both through an impairment of myelination and impairment in sustaining trophic support of surrounding motor neurons, underlies this neurotoxicity (Kang et al., 2013; Philips et al., 2013). These functions might become impaired very early onward, before motor neuron loss is apparent, when an increased amount of newly generated CC1+ oligodendrocytes is being observed in the spinal cord of mSOD1 mice (Kang et al., 2013; Philips et al., 2013). Why oligodendrocytes are dying, and whether this is cell autonomous death or rather stimulated by surrounding microglial cells and astrocytes, still remains to be elucidated, as is the factor(s) which regulate oligodendrocyte death and survival. Any signaling pathways that mediate oligodendrocyte survival and demise, like the Notch, Wnt, Nogo, and Id signaling pathways, could be implicated in oligodendrocyte mediated motor neuron degeneration (Richardson et al., 2011). Understanding these events could prove fruitful for ALS, as pharmaceutical modification of oligodendroglial injury is a highly investigated area in multiple sclerosis, which has seen great success in disease modifying agents.

Future perspectives

Although targeting glial cells in therapeutical trials has so far been largely unsuccesfull, one should not forget that many caveats are associated with these trials. First of all, many therapeutical trials are founded on results obtained using the mSOD1 rodent model and very few of the mouse studies have provided human evidence for target involvement. The recent discoveries of a wide range of new ALS causing genes, especially C9ORF72, will soon lead to the development of new, possibly better animal models to study disease more relevant to

a wider range of subjects as well as human biomarkers. This will enable one to explore whether disease mechanisms are generally shared among the different ALS causing mutations or not, the results of which are of extreme relevance for future therapeutical trials. Secondly, the generation of iPS derived neurons, oligodendrocytes and astrocytes might enable us to better model human disease. These cells have the added advantage of identifying human biomarkers that can be more readily relevant to patient selection and validation of drug activity. These models are also highly applicable for large therapeutical screenings and open the door for personalized therapy as iPS cell derived progeny are easily obtained from each individual ALS patient. Lastly, new diagnostic tools will provide us with better information on whether a certain therapeutical avenue will be beneficial or not. This is of essential interest as the majority of previous therapeutical trials lack any indication about whether the therapeutic was effective in reaching and/or lowering the expression of its target. As we look to the future of preclinical drug development it will be essential to have programs in pharmacodynamic markers for drug action or molecular tools for identifying subsets of patients. An ongoing study in primates is currently exploring the efficacy of using PET ligands for EAAT2 astroglial transporters. This will enable us to obtain a measurement of the density of EAAT2 protein levels, identify subset of patients that have astroglia abnormities and will give us insight into the neuropharmacological dynamics of therapeutical interventions. The development of similar in vivo human glial markers will be important.

Conclusions

ALS is a non-cell autonomous disease in which several glial cells are contributing to the death of motor neurons. Microglial cells and astroglial cell involvement in ALS has been widely investigated, and recently a significant contribution of NG2 glial progenitor cells as oligodendrocytes has been revealed. Most of these studies have focused on the study of mSOD1 and although mSOD1 is only involved in 1% of all ALS cases, clear correlates with sALS cases was found, like the loss of GLT-1 mediated glutamate transport as well as the release of a whole range of inflammatory factors. Unfortunately, the identification of glial mechanisms in disease has not lead to effective therapeutical targeting. The recent identification of new ALS genes, like the repeat expansion in the C9ORF72 gene as being causative for the majority of all familial ALS as well as 10% of sporadic ALS cases, will lead to the development of new, potentially better ALS disease models. In addition, the use of iPS derived glial cells to study the disease will enable us to study human disease more specifically, potentially leading to a better identification of disease mechanisms more relevant for human disease.

References

Ajami B, Bennett JL, Krieger C, Tetzlaff W, Rossi FM. Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. Nat Neurosci. 2007; 10:1538–1543. [PubMed: 18026097]

Alexianu ME, Kozovska M, Appel SH. Immune reactivity in a mouse model of familial ALS correlates with disease progression. Neurology. 2001; 57:1282–1289. [PubMed: 11591849]

Almer G, Guegan C, Teismann P, Naini A, Rosoklija G, Hays AP, Chen C, Przedborski S. Increased expression of the pro-inflammatory enzyme cyclooxygenase-2 in amyotrophic lateral sclerosis. Ann Neurol. 2001; 49:176–185. [PubMed: 11220737]

Azzouz M, Ralph GS, Storkebaum E, Walmsley LE, Mitrophanous KA, Kingsman SM, Carmeliet P, Mazarakis ND. VEGF delivery with retrogradely transported lentivector prolongs survival in a mouse ALS model. Nature. 2004; 429:413–417. [PubMed: 15164063]

Barnabe-Heider F, Goritz C, Sabelstrom H, Takebayashi H, Pfrieger FW, Meletis K, Frisen J. Origin of new glial cells in intact and injured adult spinal cord. Cell Stem Cell. 2010; 7:470–482. [PubMed: 20887953]

Beers DR, Henkel JS, Zhao W, Wang J, Appel SH. CD4+ T cells support glial neuroprotection, slow disease progression, and modify glial morphology in an animal model of inherited ALS. Proc Natl Acad Sci U S A. 2008; 105:15558–15563. [PubMed: 18809917]

Beers DR, Henkel JS, Zhao W, Wang J, Huang A, Wen S, Liao B, Appel SH. Endogenous regulatory T lymphocytes ameliorate amyotrophic lateral sclerosis in mice and correlate with disease progression in patients with amyotrophic lateral sclerosis. Brain. 2011; 134:1293–1314. [PubMed: 21596768]

Beers DR, Henkel JS, Xiao Q, Zhao W, Wang J, Yen AA, Siklos L, McKercher SR, Appel SH. Wild-type microglia extend survival in PU.1 knockout mice with familial amyotrophic lateral sclerosis. Proc Natl Acad Sci U S A. 2006; 103:16021–16026. [PubMed: 17043238]

Bergles DE, Roberts JD, Somogyi P, Jahr CE. Glutamatergic synapses on oligodendrocyte precursor cells in the hippocampus. Nature. 2000; 405:187–191. [PubMed: 10821275]

Boillee S, Yamanaka K, Lobsiger CS, Copeland NG, Jenkins NA, Kassiotis G, Kollias G, Cleveland DW. Onset and progression in inherited ALS determined by motor neurons and microglia. Science. 2006; 312:1389–1392. [PubMed: 16741123]

Bruijn LI, Becher MW, Lee MK, Anderson KL, Jenkins NA, Copeland NG, Sisodia SS, Rothstein JD, Borchelt DR, Price DL, Cleveland DW. ALS-linked SOD1 mutant G85R mediates damage to astrocytes and promotes rapidly progressive disease with SOD1-containing inclusions. Neuron. 1997; 18:327–338. [PubMed: 9052802]

Butovsky O, Siddiqui S, Gabriely G, Lanser AJ, Dake B, Murugaiyan G, Doykan CE, Wu PM, Gali RR, Iyer LK, Lawson R, Berry J, Krichevsky AM, Cudkowicz ME, Weiner HL. Modulating inflammatory monocytes with a unique microRNA gene signature ameliorates murine ALS. J Clin Invest. 2012; 122:3063–3087. [PubMed: 22863620]

Carlen M, Meletis K, Goritz C, Darsalia V, Evergren E, Tanigaki K, Amendola M, Barnabe-Heider F, Yeung MS, Naldini L, Honjo T, Kokaia Z, Shupliakov O, Cassidy RM, Lindvall O, Frisen J. Forebrain ependymal cells are Notch-dependent and generate neuroblasts and astrocytes after stroke. Nat Neurosci. 2009; 12:259–267. [PubMed: 19234458]

Chi L, Ke Y, Luo C, Li B, Gozal D, Kalyanaraman B, Liu R. Motor neuron degeneration promotes neural progenitor cell proliferation, migration, and neurogenesis in the spinal cords of amyotrophic lateral sclerosis mice. Stem Cells. 2006; 24:34–43. [PubMed: 16099995]

Chiu IM, Chen A, Zheng Y, Kosaras B, Tsiftsoglou SA, Vartanian TK, Brown RH Jr, Carroll MC. T lymphocytes potentiate endogenous neuroprotective inflammation in a mouse model of ALS. Proc Natl Acad Sci U S A. 2008; 105:17913–17918. [PubMed: 18997009]

Chiu IM, Phatnani H, Kuligowski M, Tapia JC, Carrasco MA, Zhang M, Maniatis T, Carroll MC. Activation of innate and humoral immunity in the peripheral nervous system of ALS transgenic mice. Proc Natl Acad Sci U S A. 2009; 106:20960–20965. [PubMed: 19933335]

Chiu IM, Morimoto ET, Goodarzi H, Liao JT, O'Keeffe S, Phatnani HP, Muratet M, Carroll MC, Levy S, Tavazoie S, Myers RM, Maniatis T. A neurodegeneration-specific gene-expression signature of acutely isolated microglia from an amyotrophic lateral sclerosis mouse model. Cell Rep. 2013; 4:385–401. [PubMed: 23850290]

Clement AM, Nguyen MD, Roberts EA, Garcia ML, Boillee S, Rule M, McMahon AP, Doucette W, Siwek D, Ferrante RJ, Brown RH Jr, Julien JP, Goldstein LS, Cleveland DW. Wild-type nonneuronal cells extend survival of SOD1 mutant motor neurons in ALS mice. Science. 2003; 302:113–117. [PubMed: 14526083]

Corcia P, Tauber C, Vercoullie J, Arlicot N, Prunier C, Praline J, Nicolas G, Venel Y, Hommet C, Baulieu JL, Cottier JP, Roussel C, Kassiou M, Guilloteau D, Ribeiro MJ. Molecular imaging of microglial activation in amyotrophic lateral sclerosis. PLoS One. 2012; 7:e52941. [PubMed: 23300829]

Danbolt NC. Glutamate uptake. Prog Neurobiol. 2001; 65:1–105. [PubMed: 11369436]

Di Giorgio FP, Carrasco MA, Siao MC, Maniatis T, Eggan K. Non-cell autonomous effect of glia on motor neurons in an embryonic stem cell-based ALS model. Nat Neurosci. 2007; 10:608–614. [PubMed: 17435754]

Diaper DC, Adachi Y, Lazarou L, Greenstein M, Simoes FA, Di Domenico A, Solomon DA, Lowe S, Alsubaie R, Cheng D, Buckley S, Humphrey DM, Shaw CE, Hirth F. Drosophila TDP-43 dysfunction in glia and muscle cells cause cytological and behavioural phenotypes that characterize ALS and FTLD. Hum Mol Genet. 2013; 22:3883–3893. [PubMed: 23727833]

Dobrowolny G, Aucello M, Rizzuto E, Beccafico S, Mammucari C, Boncompagni S, Belia S, Wannenes F, Nicoletti C, Del Prete Z, Rosenthal N, Molinaro M, Protasi F, Fano G, Sandri M, Musaro A. Skeletal muscle is a primary target of SOD1G93A-mediated toxicity. Cell Metab. 2008; 8:425–436. [PubMed: 19046573]

Engelhardt JI, Tajti J, Appel SH. Lymphocytic infiltrates in the spinal cord in amyotrophic lateral sclerosis. Arch Neurol. 1993; 50:30–36. [PubMed: 8093428]

Estes PS, Daniel SG, McCallum AP, Boehringer AV, Sukhina AS, Zwick RA, Zarnescu DC. Motor neurons and glia exhibit specific individualized responses to TDP-43 expression in a Drosophila model of amyotrophic lateral sclerosis. Dis Model Mech. 2013; 6:721–733. [PubMed: 23471911]

Ferraiuolo L, Higginbottom A, Heath PR, Barber S, Greenald D, Kirby J, Shaw PJ. Dysregulation of astrocyte-motoneuron cross-talk in mutant superoxide dismutase 1-related amyotrophic lateral sclerosis. Brain. 2011; 134:2627–2641. [PubMed: 21908873]

Finkelstein A, Kunis G, Seksenyan A, Ronen A, Berkutzki T, Azoulay D, Koronyo-Hamaoui M, Schwartz M. Abnormal changes in NKT cells, the IGF-1 axis, and liver pathology in an animal model of ALS. PLoS One. 2011; 6:e22374. [PubMed: 21829620]

Foust KD, Salazar DL, Likhite S, Ferraiuolo L, Ditsworth D, Ilieva H, Meyer K, Schmelzer L, Braun L, Cleveland DW, Kaspar BK. Therapeutic AAV9-mediated Suppression of Mutant SOD1 Slows Disease Progression and Extends Survival in Models of Inherited ALS. Mol Ther. 2013; 21:2148–2159. [PubMed: 24008656]

Frakes AE, Ferraiuolo L, Haidet-Phillips AM, Schmelzer L, Braun L, Miranda CJ, Ladner KJ, Bevan AK, Foust KD, Godbout JP, Popovich PG, Guttridge DC, Kaspar BK. Microglia induce motor neuron death via the classical NF-kappaB pathway in amyotrophic lateral sclerosis. Neuron. 2014; 81:1009–1023. [PubMed: 24607225]

Funfschilling U, Supplie LM, Mahad D, Boretius S, Saab AS, Edgar J, Brinkmann BG, Kassmann CM, Tzvetanova ID, Mobius W, Diaz F, Meijer D, Suter U, Hamprecht B, Sereda MW, Moraes CT, Frahm J, Goebbels S, Nave KA. Glycolytic oligodendrocytes maintain myelin and long-term axonal integrity. Nature. 2012; 485:517–521. [PubMed: 22622581]

Gong YH, Parsadanian AS, Andreeva A, Snider WD, Elliott JL. Restricted expression of G86R Cu/Zn superoxide dismutase in astrocytes results in astrocytosis but does not cause motoneuron degeneration. J Neurosci. 2000; 20:660–665. [PubMed: 10632595]

Goritz C, Dias DO, Tomilin N, Barbacid M, Shupliakov O, Frisen J. A pericyte origin of spinal cord scar tissue. Science. 2011; 333:238–242. [PubMed: 21737741]

Gowing G, Philips T, Van Wijmeersch B, Audet JN, Dewil M, Van Den Bosch L, Billiau AD, Robberecht W, Julien JP. Ablation of proliferating microglia does not affect motor neuron degeneration in amyotrophic lateral sclerosis caused by mutant superoxide dismutase. J Neurosci. 2008; 28:10234–10244. [PubMed: 18842883]

Griffiths I, Klugmann M, Anderson T, Yool D, Thomson C, Schwab MH, Schneider A, Zimmermann F, McCulloch M, Nadon N, Nave KA. Axonal swellings and degeneration in mice lacking the major proteolipid of myelin. Science. 1998; 280:1610–1613. [PubMed: 9616125]

Guo H, Lai L, Butchbach ME, Stockinger MP, Shan X, Bishop GA, Lin CL. Increased expression of the glial glutamate transporter EAAT2 modulates excitotoxicity and delays the onset but not the outcome of ALS in mice. Hum Mol Genet. 2003; 12:2519–2532. [PubMed: 12915461]

Gurney ME, Pu H, Chiu AY, Dal Canto MC, Polchow CY, Alexander DD, Caliendo J, Hentati A, Kwon YW, Deng HX, et al. Motor neuron degeneration in mice that express a human Cu, Zn superoxide dismutase mutation. Science. 1994; 264:1772–1775. [PubMed: 8209258]

Haidet-Phillips AM, Gross SK, Williams T, Tuteja A, Sherman A, Ko M, Jeong YH, Wong PC, Maragakis NJ. Altered astrocytic expression of TDP-43 does not influence motor neuron survival. Exp Neurol. 2013; 250:250–259. [PubMed: 24120466]

Haidet-Phillips AM, Hester ME, Miranda CJ, Meyer K, Braun L, Frakes A, Song S, Likhite S, Murtha MJ, Foust KD, Rao M, Eagle A, Kammesheidt A, Christensen A, Mendell JR, Burghes AH, Kaspar BK. Astrocytes from familial and sporadic ALS patients are toxic to motor neurons. Nat Biotechnol. 2011; 29:824–828. [PubMed: 21832997]

Henkel JS, Beers DR, Siklos L, Appel SH. The chemokine MCP-1 and the dendritic and myeloid cells it attracts are increased in the mSOD1 mouse model of ALS. Mol Cell Neurosci. 2006; 31:427–437. [PubMed: 16337133]

Henkel JS, Engelhardt JI, Siklos L, Simpson EP, Kim SH, Pan T, Goodman JC, Siddique T, Beers DR, Appel SH. Presence of dendritic cells, MCP-1, and activated microglia/macrophages in amyotrophic lateral sclerosis spinal cord tissue. Ann Neurol. 2004; 55:221–235. [PubMed: 14755726]

Henkel JS, Beers DR, Wen S, Rivera AL, Toennis KM, Appel JE, Zhao W, Moore DH, Powell SZ, Appel SH. Regulatory T-lymphocytes mediate amyotrophic lateral sclerosis progression and survival. EMBO Mol Med. 2013; 5:64–79. [PubMed: 23143995]

Hensley K, Fedynyshyn J, Ferrell S, Floyd RA, Gordon B, Grammas P, Hamdheydari L, Mhatre M, Mou S, Pye QN, Stewart C, West M, West S, Williamson KS. Message and protein-level elevation of tumor necrosis factor alpha (TNF alpha) and TNF alpha-modulating cytokines in spinal cords of the G93A-SOD1 mouse model for amyotrophic lateral sclerosis. Neurobiol Dis. 2003; 14:74–80. [PubMed: 13678668]

Howland DS, Liu J, She Y, Goad B, Maragakis NJ, Kim B, Erickson J, Kulik J, DeVito L, Psaltis G, DeGennaro LJ, Cleveland DW, Rothstein JD. Focal loss of the glutamate transporter EAAT2 in a transgenic rat model of SOD1 mutant-mediated amyotrophic lateral sclerosis (ALS). Proc Natl Acad Sci U S A. 2002; 99:1604–1609. [PubMed: 11818550]

Jaarsma D, Teuling E, Haasdijk ED, De Zeeuw CI, Hoogenraad CC. Neuron-specific expression of mutant superoxide dismutase is sufficient to induce amyotrophic lateral sclerosis in transgenic mice. J Neurosci. 2008; 28:2075–2088. [PubMed: 18305242]

Kalra S, Genge A, Arnold DL. A prospective, randomized, placebo-controlled evaluation of corticoneuronal response to intrathecal BDNF therapy in ALS using magnetic resonance spectroscopy: feasibility and results. Amyotroph Lateral Scler Other Motor Neuron Disord. 2003; 4:22–26. [PubMed: 12745614]

Kang SH, Fukaya M, Yang JK, Rothstein JD, Bergles DE. NG2+ CNS glial progenitors remain committed to the oligodendrocyte lineage in postnatal life and following neurodegeneration. Neuron. 2010; 68:668–681. [PubMed: 21092857]

Kang SH, Li Y, Fukaya M, Lorenzini I, Cleveland DW, Ostrow LW, Rothstein JD, Bergles DE. Degeneration and impaired regeneration of gray matter oligodendrocytes in amyotrophic lateral sclerosis. Nat Neurosci. 2013; 16:571–579. [PubMed: 23542689]

Kaspar BK, Llado J, Sherkat N, Rothstein JD, Gage FH. Retrograde viral delivery of IGF-1 prolongs survival in a mouse ALS model. Science. 2003; 301:839–842. [PubMed: 12907804]

Kawamata T, Akiyama H, Yamada T, McGeer PL. Immunologic reactions in amyotrophic lateral sclerosis brain and spinal cord tissue. Am J Pathol. 1992; 140:691–707. [PubMed: 1347673]

Keller AF, Gravel M, Kriz J. Live imaging of amyotrophic lateral sclerosis pathogenesis: disease onset is characterized by marked induction of GFAP in Schwann cells. Glia. 2009; 57:1130–1142. [PubMed: 19115383]

Kondo T, Raff M. Oligodendrocyte precursor cells reprogrammed to become multipotential CNS stem cells. Science. 2000; 289:1754–1757. [PubMed: 10976069]

Kushner PD, Stephenson DT, Wright S. Reactive astrogliosis is widespread in the subcortical white matter of amyotrophic lateral sclerosis brain. J Neuropathol Exp Neurol. 1991; 50:263–277. [PubMed: 2022968]

Lappe-Siefke C, Goebbels S, Gravel M, Nicksch E, Lee J, Braun PE, Griffiths IR, Nave KA. Disruption of Cnp1 uncouples oligodendroglial functions in axonal support and myelination. Nat Genet. 2003; 33:366–374. [PubMed: 12590258]

Lee Y, Morrison BM, Li Y, Lengacher S, Farah MH, Hoffman PN, Liu Y, Tsingalia A, Jin L, Zhang PW, Pellerin L, Magistretti PJ, Rothstein JD. Oligodendroglia metabolically support axons and contribute to neurodegeneration. Nature. 2012; 487:443–448. [PubMed: 22801498]

Lepore AC, Dejea C, Carmen J, Rauck B, Kerr DA, Sofroniew MV, Maragakis NJ. Selective ablation of proliferating astrocytes does not affect disease outcome in either acute or chronic models of motor neuron degeneration. Exp Neurol. 2008a; 211:423–432. [PubMed: 18410928]

Lepore AC, Rauck B, Dejea C, Pardo AC, Rao MS, Rothstein JD, Maragakis NJ. Focal transplantation-based astrocyte replacement is neuroprotective in a model of motor neuron disease. Nat Neurosci. 2008b; 11:1294–1301. [PubMed: 18931666]

Levine JB, Kong J, Nadler M, Xu Z. Astrocytes interact intimately with degenerating motor neurons in mouse amyotrophic lateral sclerosis (ALS). Glia. 1999; 28:215–224. [PubMed: 10559780]

Liao B, Zhao W, Beers DR, Henkel JS, Appel SH. Transformation from a neuroprotective to a neurotoxic microglial phenotype in a mouse model of ALS. Exp Neurol. 2012; 237:147–152. [PubMed: 22735487]

Lobsiger CS, Boillee S, McAlonis-Downes M, Khan AM, Feltri ML, Yamanaka K, Cleveland DW. Schwann cells expressing dismutase active mutant SOD1 unexpectedly slow disease progression in ALS mice. Proc Natl Acad Sci U S A. 2009; 106:4465–4470. [PubMed: 19251638]

Meissner F, Molawi K, Zychlinsky A. Mutant superoxide dismutase 1-induced IL-1beta accelerates ALS pathogenesis. Proc Natl Acad Sci U S A. 2010; 107:13046–13050. [PubMed: 20616033]

Miller TM, Kim SH, Yamanaka K, Hester M, Umapathi P, Arnson H, Rizo L, Mendell JR, Gage FH, Cleveland DW, Kaspar BK. Gene transfer demonstrates that muscle is not a primary target for non-cell-autonomous toxicity in familial amyotrophic lateral sclerosis. Proc Natl Acad Sci U S A. 2006; 103:19546–19551. [PubMed: 17164329]

Morel L, Regan M, Higashimori H, Ng SK, Esau C, Vidensky S, Rothstein J, Yang Y. Neuronal exosomal miRNA-dependent translational regulation of astroglial glutamate transporter GLT1. J Biol Chem. 2013; 288:7105–7116. [PubMed: 23364798]

Nagai M, Re DB, Nagata T, Chalazonitis A, Jessell TM, Wichterle H, Przedborski S. Astrocytes expressing ALS-linked mutated SOD1 release factors selectively toxic to motor neurons. Nat Neurosci. 2007; 10:615–622. [PubMed: 17435755]

Nagy D, Kato T, Kushner PD. Reactive astrocytes are widespread in the cortical gray matter of amyotrophic lateral sclerosis. J Neurosci Res. 1994; 38:336–347. [PubMed: 7523689]

Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT, Bruce J, Schuck T, Grossman M, Clark CM, McCluskey LF, Miller BL, Masliah E, Mackenzie IR, Feldman H, Feiden W, Kretzschmar HA, Trojanowski JQ, Lee VM. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science. 2006; 314:130–133. [PubMed: 17023659]

Nguyen MD, Julien JP, Rivest S. Induction of proinflammatory molecules in mice with amyotrophic lateral sclerosis: no requirement for proapoptotic interleukin-1beta in neurodegeneration. Ann Neurol. 2001; 50:630–639. [PubMed: 11706969]

Papadeas ST, Kraig SE, O'Banion C, Lepore AC, Maragakis NJ. Astrocytes carrying the superoxide dismutase 1 (SOD1G93A) mutation induce wild-type motor neuron degeneration in vivo. Proc Natl Acad Sci U S A. 2011; 108:17803–17808. [PubMed: 21969586]

Park S, Kim HT, Yun S, Kim IS, Lee J, Lee IS, Park KI. Growth factor-expressing human neural progenitor cell grafts protect motor neurons but do not ameliorate motor performance and survival in ALS mice. Exp Mol Med. 2009; 41:487–500. [PubMed: 19322031]

Pellerin L, Magistretti PJ. Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. Proc Natl Acad Sci U S A. 1994; 91:10625–10629. [PubMed: 7938003]

Phatnani HP, Guarnieri P, Friedman BA, Carrasco MA, Muratet M, O'Keeffe S, Nwakeze C, Pauli-Behn F, Newberry KM, Meadows SK, Tapia JC, Myers RM, Maniatis T. Intricate interplay

between astrocytes and motor neurons in ALS. Proc Natl Acad Sci U S A. 2013; 110:E756–765. [PubMed: 23388633]

Philips T, Robberecht W. Neuroinflammation in amyotrophic lateral sclerosis: role of glial activation in motor neuron disease. Lancet Neurol. 2011; 10:253–263. [PubMed: 21349440]

Philips T, Bento-Abreu A, Nonneman A, Haeck W, Staats K, Geelen V, Hersmus N, Kusters B, Van Den Bosch L, Van Damme P, Richardson WD, Robberecht W. Oligodendrocyte dysfunction in the pathogenesis of amyotrophic lateral sclerosis. Brain. 2013; 136:471–482. [PubMed: 23378219]

Poloni M, Facchetti D, Mai R, Micheli A, Agnoletti L, Francolini G, Mora G, Camana C, Mazzini L, Bachetti T. Circulating levels of tumour necrosis factor-alpha and its soluble receptors are increased in the blood of patients with amyotrophic lateral sclerosis. Neurosci Lett. 2000; 287:211–214. [PubMed: 10863032]

Pramatarova A, Laganiere J, Roussel J, Brisebois K, Rouleau GA. Neuron-specific expression of mutant superoxide dismutase 1 in transgenic mice does not lead to motor impairment. J Neurosci. 2001; 21:3369–3374. [PubMed: 11331366]

Rafiki A, Boulland JL, Halestrap AP, Ottersen OP, Bergersen L. Highly differential expression of the monocarboxylate transporters MCT2 and MCT4 in the developing rat brain. Neuroscience. 2003; 122:677–688. [PubMed: 14622911]

Richardson WD, Young KM, Tripathi RB, McKenzie I. NG2-glia as multipotent neural stem cells: fact or fantasy? Neuron. 2011; 70:661–673. [PubMed: 21609823]

Rivers LE, Young KM, Rizzi M, Jamen F, Psachoulia K, Wade A, Kessaris N, Richardson WD. PDGFRA/NG2 glia generate myelinating oligodendrocytes and piriform projection neurons in adult mice. Nat Neurosci. 2008; 11:1392–1401. [PubMed: 18849983]

Rothstein JD, Martin LJ, Kuncl RW. Decreased glutamate transport by the brain and spinal cord in amyotrophic lateral sclerosis. N Engl J Med. 1992; 326:1464–1468. [PubMed: 1349424]

Rothstein JD, Dykes-Hoberg M, Pardo CA, Bristol LA, Jin L, Kuncl RW, Kanai Y, Hediger MA, Wang Y, Schielke JP, Welty DF. Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. Neuron. 1996; 16:675–686. [PubMed: 8785064]

Rothstein JD, Patel S, Regan MR, Haenggeli C, Huang YH, Bergles DE, Jin L, Dykes Hoberg M, Vidensky S, Chung DS, Toan SV, Bruijn LI, Su ZZ, Gupta P, Fisher PB. Beta-lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. Nature. 2005; 433:73–77. [PubMed: 15635412]

Saxena S, Cabuy E, Caroni P. A role for motoneuron subtype-selective ER stress in disease manifestations of FALS mice. Nat Neurosci. 2009; 12:627–636. [PubMed: 19330001]

Schiffer D, Cordera S, Cavalla P, Migheli A. Reactive astrogliosis of the spinal cord in amyotrophic lateral sclerosis. J Neurol Sci. 1996; 139(Suppl):27–33. [PubMed: 8899654]

Serio A, Bilican B, Barmada SJ, Ando DM, Zhao C, Siller R, Burr K, Haghi G, Story D, Nishimura AL, Carrasco MA, Phatnani HP, Shum C, Wilmut I, Maniatis T, Shaw CE, Finkbeiner S, Chandran S. Astrocyte pathology and the absence of non-cell autonomy in an induced pluripotent stem cell model of TDP-43 proteinopathy. Proc Natl Acad Sci U S A. 2013; 110:4697–4702. [PubMed: 23401527]

Shan X, Chiang PM, Price DL, Wong PC. Altered distributions of Gemini of coiled bodies and mitochondria in motor neurons of TDP-43 transgenic mice. Proc Natl Acad Sci U S A. 2010; 107:16325–16330. [PubMed: 20736350]

Sorenson EJ, et al. Subcutaneous IGF-1 is not beneficial in 2-year ALS trial. Neurology. 2008; 71:1770–1775. [PubMed: 19029516]

Suzuki M, McHugh J, Tork C, Shelley B, Hayes A, Bellantuono I, Aebischer P, Svendsen CN. Direct muscle delivery of GDNF with human mesenchymal stem cells improves motor neuron survival and function in a rat model of familial ALS. Mol Ther. 2008; 16:2002–2010. [PubMed: 18797452]

Tawfik VL, Regan MR, Haenggeli C, Lacroix-Fralish ML, Nutile-McMenemy N, Perez N, Rothstein JD, DeLeo JA. Propentofylline-induced astrocyte modulation leads to alterations in glial glutamate promoter activation following spinal nerve transection. Neuroscience. 2008; 152:1086–1092. [PubMed: 18358622]

Tong J, Huang C, Bi F, Wu Q, Huang B, Liu X, Li F, Zhou H, Xia XG. Expression of ALS-linked TDP-43 mutant in astrocytes causes non-cell-autonomous motor neuron death in rats. EMBO J. 2013; 32:1917–1926. [PubMed: 23714777]

Tripathi RB, Rivers LE, Young KM, Jamen F, Richardson WD. NG2 glia generate new oligodendrocytes but few astrocytes in a murine experimental autoimmune encephalomyelitis model of demyelinating disease. J Neurosci. 2010; 30:16383–16390. [PubMed: 21123584]

Turner BJ, Ackerley S, Davies KE, Talbot K. Dismutase-competent SOD1 mutant accumulation in myelinating Schwann cells is not detrimental to normal or transgenic ALS model mice. Hum Mol Genet. 2010; 19:815–824. [PubMed: 20008901]

Turner MR, Cagnin A, Turkheimer FE, Miller CC, Shaw CE, Brooks DJ, Leigh PN, Banati RB. Evidence of widespread cerebral microglial activation in amyotrophic lateral sclerosis: an [11C] (R)-PK11195 positron emission tomography study. Neurobiol Dis. 2004; 15:601–609. [PubMed: 15056468]

Wang L, Gutmann DH, Roos RP. Astrocyte loss of mutant SOD1 delays ALS disease onset and progression in G85R transgenic mice. Hum Mol Genet. 2011; 20:286–293. [PubMed: 20962037]

Wang L, Sharma K, Grisotti G, Roos RP. The effect of mutant SOD1 dismutase activity on non-cell autonomous degeneration in familial amyotrophic lateral sclerosis. Neurobiol Dis. 2009; 35:234–240. [PubMed: 19442735]

Wegorzewska I, Bell S, Cairns NJ, Miller TM, Baloh RH. TDP-43 mutant transgenic mice develop features of ALS and frontotemporal lobar degeneration. Proc Natl Acad Sci U S A. 2009; 106:18809–18814. [PubMed: 19833869]

Wils H, Kleinberger G, Janssens J, Pereson S, Joris G, Cuijt I, Smits V, Ceuterick-de Groote C, Van Broeckhoven C, Kumar-Singh S. TDP-43 transgenic mice develop spastic paralysis and neuronal inclusions characteristic of ALS and frontotemporal lobar degeneration. Proc Natl Acad Sci U S A. 2010; 107:3858–3863. [PubMed: 20133711]

Winkler EA, Sengillo JD, Sullivan JS, Henkel JS, Appel SH, Zlokovic BV. Blood-spinal cord barrier breakdown and pericyte reductions in amyotrophic lateral sclerosis. Acta Neuropathol. 2013; 125:111–120. [PubMed: 22941226]

Wu DC, Re DB, Nagai M, Ischiropoulos H, Przedborski S. The inflammatory NADPH oxidase enzyme modulates motor neuron degeneration in amyotrophic lateral sclerosis mice. Proc Natl Acad Sci U S A. 2006; 103:12132–12137. [PubMed: 16877542]

Yamanaka K, Boillee S, Roberts EA, Garcia ML, McAlonis-Downes M, Mikse OR, Cleveland DW, Goldstein LS. 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. 2008a; 105:7594–7599. [PubMed: 18492803]

Yamanaka K, Chun SJ, Boillee S, Fujimori-Tonou N, Yamashita H, Gutmann DH, Takahashi R, Misawa H, Cleveland DW. Astrocytes as determinants of disease progression in inherited amyotrophic lateral sclerosis. Nat Neurosci. 2008b; 11:251–253. [PubMed: 18246065]

Yang Y, Gozen O, Watkins A, Lorenzini I, Lepore A, Gao Y, Vidensky S, Brennan J, Poulsen D, Won Park J, Li Jeon N, Robinson MB, Rothstein JD. Presynaptic regulation of astroglial excitatory neurotransmitter transporter GLT1. Neuron. 2009; 61:880–894. [PubMed: 19323997]

Zawadzka M, Rivers LE, Fancy SP, Zhao C, Tripathi R, Jamen F, Young K, Goncharevich A, Pohl H, Rizzi M, Rowitch DH, Kessaris N, Suter U, Richardson WD, Franklin RJ. CNS-resident glial progenitor/stem cells produce Schwann cells as well as oligodendrocytes during repair of CNS demyelination. Cell Stem Cell. 2010; 6:578–590. [PubMed: 20569695]

Zhao W, Beers DR, Appel SH. Immune-mediated mechanisms in the pathoprogression of amyotrophic lateral sclerosis. J Neuroimmune Pharmacol. 2013; 8:888–899. [PubMed: 23881705]

Zhao W, Beers DR, Liao B, Henkel JS, Appel SH. Regulatory T lymphocytes from ALS mice suppress microglia and effector T lymphocytes through different cytokine-mediated mechanisms. Neurobiol Dis. 2012; 48:418–428. [PubMed: 22820142]

Zhong Z, Deane R, Ali Z, Parisi M, Shapovalov Y, O'Banion MK, Stojanovic K, Sagare A, Boillee S, Cleveland DW, Zlokovic BV. ALS-causing SOD1 mutants generate vascular changes prior to motor neuron degeneration. Nat Neurosci. 2008; 11:420–422. [PubMed: 18344992]

Zhong Z, Ilieva H, Hallagan L, Bell R, Singh I, Paquette N, Thiyagarajan M, Deane R, Fernandez JA, Lane S, Zlokovic AB, Liu T, Griffin JH, Chow N, Castellino FJ, Stojanovic K, Cleveland DW, Zlokovic BV. Activated protein C therapy slows ALS-like disease in mice by transcriptionally inhibiting SOD1 in motor neurons and microglia cells. J Clin Invest. 2009; 119:3437–3449. [PubMed: 19841542]

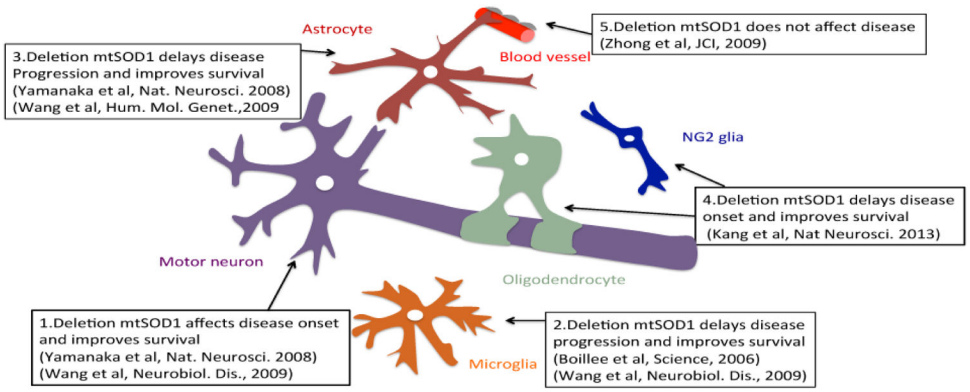

Figure 1.