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## **Motor neuron - immune interactions: the vicious circle of ALS**

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### **Abstract.**

Because microglial cells, the resident macrophages of the CNS, react to any lesion of the nervous system, they have for long been regarded as potential players in the pathogenesis of several neurodegenerative disorders including Amyotrophic Lateral Sclerosis, the most common motor neuron disease in the adult. In recent years, this microglial reaction to motor neuron injury, in particular, and the innate immune response, in general, has been implicated in the progression of the disease, in mouse models of ALS. The mechanisms by which microglial cells influence motor neuron death in ALS are still largely unknown. Microglial activation increases over the course of the disease and is associated with an alteration in the production of toxic factors and also neurotrophic factors. Adding to the microglial/macrophage response to motor neuron degeneration, the adaptive immune system can likewise influence the disease process. Exploring these motor neuron-immune interactions could lead to a better understanding in the physiopathology of ALS to find new pathways to slow down motor neuron degeneration.

Keywords: neuroinflammation; microglia; T lymphocytes; macrophages; cytokines; neurodegeneration; immune system.

## **Introduction**

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease of the adult characterized by degeneration of motor neurons in the spinal cord, brain stem and motor cortex. Progressive muscle atrophy and weakness are the main clinical features of the disease leading to paralysis and death of the patient in mean 3-5 years after the onset of disease, usually around the age of 45-60. Death of the patients is generally due to respiratory failure linked to denervation of the respiratory muscles and diaphragm. Although the cause of the disease is currently unknown, several hypotheses including oxidative stress, protein aggregation, mitochondrial dysfunction, glutamate excitotoxicity and neuro-immune reactions are believed to participate in the motor neuron degenerative process (Boillee et al. 2006a). The majority of ALS cases are sporadic, and 10% are inherited (familial), but sporadic and familial ALS produces similar pathological hallmarks, including an immune response in the affected tissues. This reaction includes activation of astrocytes, microglia (the macrophage of the CNS) as well as lymphocyte infiltration and, therefore, implicates both the innate and adaptive immune system. Despite the important efforts to find new therapies to ALS, including targeting the immune system, very limited options are available today. This highlights the need to better understand the processes implicated, especially in the progressive phase of the disease to slow down the course of motor neuron degeneration. We review here the different faces of the immune response in ALS including what has been learnt so far from the rodent models and verified or not in human ALS patients concerning these neuroimmune interactions, the immune factors potentially implicated in motor neuron degeneration and the involvement of microglia/macrophages and the adaptive immune system on the disease progression.

## **SOD1 mutations and rodent models of ALS**

Mutations in the Cu/Zn superoxide dismutase (or SOD1) gene are still the ones most frequently found in familial ALS (20% of inherited ALS, Rosen et al. 1993). As of today, more than 130 mutations have already been described in the human SOD1 gene involving all the regions of the 154 amino acid long protein. Beside for a few exceptions, mutations in the SOD1 gene are of dominant character and lead to motor neuron death through a gain of toxic function of the protein. Indeed, a large fraction of SOD1 mutants retain the dismutase activity of the enzyme (which functions as a radical oxygen species-superoxide scavenger) and patients carrying either a dismutase active or inactive form of the enzyme develop ALS with similar phenotypes. In addition, studies in mice showed that deleting the mouse endogenous SOD1 gene did not lead to ALS phenotypes (Reaume et al. 1996) while the transgenic expression of different forms of mutant human (or mouse) SOD1 in mice and rats recapitulated several features of the disease (Gurney et al. 1994; Ripps et al. 1995; Wong et al. 1995; Bruijn et al. 1997; Howland et al. 2002; Wang et al. 2002) that were not observed when expressing the human wild-type SOD1 at similar levels (Gurney et al. 1994; Wong et al. 1995). These mouse and rat lines expressing different forms of SOD1 represent, therefore, valuable tools and are currently the broadly used model to study ALS. However, the recent discoveries of new mutated genes involved in ALS like *TAR DNA-binding protein* (TDP-43)

and fused in sarcoma/translated in liposarcoma (FUS/TLS) (Gitcho et al. 2008; Kabashi et al. 2008; Rutherford et al. 2008; Sreedharan et al. 2008; Chow et al. 2009; Kwiatkowski et al. 2009; Vance et al. 2009), bring new comparative opportunities for designing novel animal models for studying the neurodegenerative processes occurring in ALS (Wils et al. 2010; Zhou et al. 2010; Wegorzewska et al. 2009).

### **ALS, a non-cell autonomous disease**

Although death of motor neurons is responsible for ALS, important discoveries of the last years led to the concept that motor neuron degeneration is non-cell autonomous and cells other than motor neurons, for instance glial cells, actively participate in the neurodegenerative process (Fig 1) (Boillee et al. 2006a; Ilieva et al. 2009). First transgenic mice generated to express mutant SOD1 only in motor neurons did not show evidence of neurodegeneration (Pramatarova et al. 2001; Lino et al. 2002) and a second generation of the same type of mice had to be bred homozygously to increase the level of mutant SOD1 expressed in motor neurons to show a late onset motor neuron disease (Jaarsma et al. 2008) suggesting that mutant SOD1 expressed in other cells besides motor neurons could participate to the progression of the typical ALS phenotype in mice. However, selective expression of mutant SOD1 in astrocytes alone, although leading to astrogliosis (Gong et al. 2000), or in microglial cells alone (Beers et al. 2006) was not sufficient to induce motor neuron death suggesting the need for mutant SOD1 expressed by motor neurons and a cooperation between multiple cell types to induce motor neuron degeneration. The participation of cells in the neighborhood of motor neurons to neurodegeneration in ALS mouse models was shown using chimeric mice whose motor neurons expressing mutant SOD1 survived longer when surrounded by wild-type cells (Clement et al. 2003; Yamanaka et al. 2008a). However, the type of cells participating to the disease could not be identified in the chimeric mice and a Cre/Lox approach revealed that decreasing mutant dismutase active SOD1 (SOD1<sup>G37R</sup>) levels in motor neurons (using Islet-Cre, Boillee et al. 2006b or VACHT-Cre, Yamanaka et al. 2008b mice) or decreasing dismutase inactive SOD1 in motor neurons and interneurons (SOD1<sup>G85R</sup>, using Lhx3-Cre mice, Wang et al. 2009b) led to a delayed onset of the disease. Conversely, downregulating the amount of SOD1<sup>G37R</sup> or SOD1<sup>G85R</sup> in microglial cells and macrophages (CD11b-Cre, Boillee et al. 2006b; Wang et al. 2009b) or SOD1<sup>G37R</sup> in astrocytes (Yamanaka et al. 2008b) actively participated in slowing down the progression of the disease. Looking at other cell types that surround motor neurons, downregulating the expression of the dismutase active mutant SOD1<sup>G37R</sup> in muscle cells or endothelial cells did not have an impact on the disease while in Schwann cells, the peripheral myelinating cells and the ones most in contact with motor neuron axons, it surprisingly accelerated the disease (Miller et al. 2006; Lobsiger et al. 2009; Zhong et al. 2009). Therefore, mutant SOD1 in Schwann cells does not seem to have a detrimental effect that could influence motor neuron survival; however, the dismutase activity of SOD1 in this particular cell type seems to be neuroprotective during the course of the neurodegeneration in ALS mice (Lobsiger et al. 2009; Turner et al. 2009). These studies highlight the importance of targeting therapeutic strategies using downregulation of SOD1 at the right cell type, avoiding Schwann cells.

A complementary experiment by the group of Stanley Appel used an alternative technique of bone marrow transplant to replace mutant SOD1 (dismutase active, SOD1<sup>G93A</sup>) expressing microglia with wild-type microglia. To be able to replace the entire myeloid lineage, including microglial cells, they used the PU.1 knockout mouse (McKercher et al. 1996) which, from birth, lacks myeloid cells, therefore macrophages and microglia as well as mature lymphoid B cells and reduced number of lymphoid T cells. Crossing PU.1<sup>-/-</sup> mice with SOD1<sup>G93A</sup> mice allowed them to reconstitute the myeloid lineage in ALS mice with either control or SOD1<sup>G93A</sup> cells and to compare the progression of the disease in ALS mice with microglia/macrophages (and possibly lymphocytes) expressing or not mutant SOD1. The outcome was that microglia/macrophages expressing mutant SOD1 accelerated the progression of the disease (Beers et al. 2006).

Altogether, these studies show that glial cells in the environment of motor neurons have an impact on their survival and on the disease progression (Fig 1). Knowing that the majority of ALS cases are sporadic and therefore diagnosed after showing symptoms of the disease, being able to act on the progression of the disease would provide pathways for potential therapeutic tools. Microglial cells, astrocytes and neuroinflammatory processes appear as valuable candidates to target ALS disease progression (Fig 1).

### **Implication of astrocytes in ALS disease process**

Astrocytes, the main macroglia of the CNS that are known for their major functions as synaptic glutamate uptaker and glucose/metabolites provider for neurons, have long been suspected to be implicated in ALS (Barbeito et al. 2004; Di Giorgio et al. 2007; Nagai et al. 2007; Lepore et al. 2008; Van Den Bosch and Robberecht 2008; Vargas et al. 2008; Yamanaka et al. 2008b). Astrocytes were first shown to be activated and to display a decreased expression of the glutamate transporter EAAT2 in patients and animal models of ALS (Rothstein et al. 1995; Howland et al. 2002; Pardo et al. 2006) favoring glutamate-induced excitotoxicity (Van Den Bosch et al. 2006). Further evidence for a role of astrocytes in ALS came from work on primary astrocyte cell cultures carrying mutant SOD1 and showing toxicity towards motor neurons (Vargas et al. 2006; Di Giorgio et al. 2007; Nagai et al. 2007). In addition, *in vivo* evidence for an active role of astrocytes in ALS pathology came from the CreLox experiment described above where decreasing the expression of mutant SOD1 specifically in astrocytes increases survival of LoxSOD1<sup>G37R</sup> mice (Yamanaka et al. 2008b). Reducing the mutant SOD1 expressing astrocytes as well as increasing the number of wild-type astrocytes focally in the cervical spinal cord of SOD1<sup>G93A</sup> rats by grafting wild-type lineage-restricted astrocyte precursors (that efficiently differentiated into astrocytes) could prolong lifespan (Lepore et al. 2008). These studies not only showed the implication of astrocytes in the disease process, but also that they could be potentially used to increase motor neuron survival. In this line, boosting astrocytic antioxidant defenses by overexpressing the transcription factor Nrf2 (which is downregulated in the spinal cord of ALS patients) extended the lifespan of SOD1<sup>G93A</sup> mice (Sarlette et al. 2008; Vargas et al. 2008). Although astrocytes could be taken as a part of the neuroinflammatory process, the scope of this review will remain on the innate and adaptive immune response in the nervous

system during motor neuron degeneration in ALS models and will, therefore, focus on microglia and lymphocytes.

### **Microglial cell activation as a neuroinflammatory sign in ALS**

Microglial cells are the intrinsic immune effector cells of the CNS. Under physiological conditions, they were previously considered as “resting” but after observing them with live imaging *in vitro* and *in vivo*, they had to be renamed “surveying” since they are actively screening their microenvironment most likely watching for a potential intrusion and are therefore in constant communication with surrounding cells (Kreutzberg 1996; Davalos et al. 2005; Nimmerjahn et al. 2005; Hanisch and Kettenmann 2007). Upon activation, microglial response will depend on the nature, amount and length of the stimulus and generally includes cell proliferation, phagocytosis, release of neurotransmitters, pro-inflammatory cytokines and reactive oxygen species as well as anti-inflammatory cytokines and neurotrophic factors (Fig 1) (Streit 2002; Hanisch and Kettenmann 2007). Microglial cells have, therefore, the capacities to affect neuronal survival and were shown to actively promote neuron death during development (Marin-Teva et al. 2004). Microglial cell activation occurs after any injury of the CNS including neurodegenerative disorders (McGeer et al. 1993; McGeer and McGeer 2002). In ALS patients, microglial activation has been largely documented in the early nineties from postmortem affected tissues and involves, besides proliferation and morphological changes, an up-regulation of cell surface molecules, such as complement receptor 3, major histocompatibility complex (MHC) antigens I and II, integrins and reactivity to immunoglobulins (Lampson et al. 1990; Troost et al. 1990; Kawamata et al. 1992; McGeer and McGeer 1995). The finding of increased expression of microglia cell markers in ALS tissues, however, represent a “snap-shot” at the end-stage pathology and does not give information about evolution of microglial activation during the course of the disease. Evidence from microglia activation during disease progression came from animal models where it is already apparent at early stages of the disease and increases with disease progression up to end-stage in several ALS mouse and rat lines (Hall et al. 1998; Alexianu et al. 2001; Elliott 2001; Olsen et al. 2001; Fendrick et al. 2007; Lobsiger et al. 2007; Gowing et al. 2008). More recently, PET imaging coupled to [11C](R)-PK11195, a ligand for the peripheral benzodiazepine binding site, which is expressed by activated microglia, detected a widespread microglial activation in motor (motor cortex and pons) as well as extra-motor (dorsolateral prefrontal cortex and thalamus) cerebral regions and showed evidence of increased microglial activation with the severity of the disease in ALS patients (Turner et al. 2004). Besides being able to show microglial reaction in living patients, this tool could also provide ways to define if potential therapeutic drugs act on inflammation.

In addition to activated microglia, large dendritic cells were also observed in affected areas of the CNS of ALS patients in particular in proximity to motor neuron cell bodies in the spinal cord (Fig 1) (Lampson et al. 1990; Henkel et al. 2004). Dendritic cells are antigen-presenting cells that control both innate and adaptive immunity and may infiltrate the CNS through blood vessels, choroid plexus and/or meninges. Increased dendritic cell marker transcripts (DEC205, CD1a, CD11c, CD123, CD83 and CD40) were detected both in ALS patients and in late symptomatic or end-stage mice (Henkel et al. 2004; Gowing et al. 2006;

Henkel et al. 2006). Although dendritic markers can also be expressed by activated microglial cells, dendritic cells with their stellate shape were already visible before symptoms in ALS mouse spinal cords (Henkel et al. 2004; Gowing et al. 2006; Henkel et al. 2006). However, the origin of these dendritic cells in ALS mice and patients, as well as their protective or otherwise injurious function has not yet been established. Interestingly, in ALS patients, the level of dendritic cell transcripts was higher in fast-progressing cases than in slow progressing ones supporting the involvement of the adaptive immune system in the disease process (Henkel et al. 2004). Implication of the innate immune system was also shown in ALS mice by stimulating the innate immune reactivity in SOD<sup>G37R</sup> mice through chronic i.p. injections of lipopolysaccharides (LPS), which led to a speed up of the disease (Nguyen et al. 2004). However, the mechanisms by which immune cells including microglia could favor neurodegeneration in ALS are still largely unknown and the participation of potentially infiltrating monocytes/macrophages still under debate.

### **Activated microglial cells in ALS mice are of endogenous origin**

Microglial cells originate from phagocyte progenitors generated in hematopoietic tissues including bone marrow and that enter the CNS during development, through different potential routes that are the blood vessels, the ventricles or the meninges (Perry et al. 1985; Cuadros and Navascues 1998). Several experimental designs have been used to analyze microglial turnover during physiological conditions or after lesions of the CNS. To summarize, under normal conditions in adulthood, microglial turnover seems to come from both endogenous dividing microglial cells and infiltrating monocytes but is very low and therefore monocytes entering the CNS are rare (Ling et al. 1980; Lawson et al. 1992). CNS lesions that highly compromise the blood-brain barrier (BBB) like ischemia, stab wounds or the injection of a toxin, favor blood monocytes infiltration through the damaged vessel wall (Andersson et al. 1991; Marty et al. 1991; Leong and Ling 1992). The current issue is whether similar infiltrations occur during neurodegenerative diseases such as ALS especially since the blood spinal cord barrier has been shown to be disrupted in different mutant SOD1 mice and this even before motor neuron degeneration (Fig 1) (Garbuzova-Davis et al. 2008; Zhong et al. 2008, 2009). So far, there are still no available antibodies capable of discriminating macrophages coming from outside and microglial cells because they share the same monocyte markers. To follow potential infiltration of peripheral monocytes into the CNS, the broadly used technique is irradiation of mice to deplete them from their immune cells and replenishment of the cells by bone marrow transplantation of marked (usually green fluorescent protein (GFP) positive) cells (Flugel et al. 2001; Priller et al. 2001; Simard and Rivest 2004; Ajami et al. 2007). Several authors have used this technique in SOD1<sup>G93A</sup> mice trying to understand if infiltration of monocytes was happening in the spinal cord during the course of the disease and found some GFP-positive cells, therefore cells originating from outside of the CNS, but the overall proportion of these GFP-positive microglial cells over the totality of microglial cells was very low indicating that their contribution to the local pool of microglia was limited and even if it increased over the course of the disease, the proportion of GFP versus non-GFP microglia showed that microglial expansion was rather coming from proliferation than peripheral recruitment (Solomon et al. 2006; Chiu et al. 2009). The higher

proportion of GFP-positive microglial cells in sick ALS mice as compared to controls showed that motor neuron degeneration (either directly or through increased inflammation) induced more attraction of cells from the outside. However, most of the GFP-positive cells in the spinal cord seemed to be associated with blood vessels, and therefore represented perivascular microglia (the cells located between glia limitans and endothelial cells) rather than parenchymal microglia (Lewis et al. 2009).

Recent studies, however, have revealed that this experimental paradigm of irradiation and bone marrow transplantation could lead to certain artifactual effects and actually modify what is happening in mice during the course of the disease. Indeed, first, bone marrow transplant could change the type of monocyte precursors in the blood stream and therefore the type of cells entering the CNS could be different (Mildner et al. 2007). In addition, irradiation seems to be the process provoking entrance of cells in the CNS, most probably by altering the BBB tightness (Ajami et al. 2007; Mildner et al. 2007). Mice whose brain was protected from the irradiation showed that microglial turnover from the periphery did not happen while it was the case in non-protected CNS (Mildner et al. 2007). Furthermore, a very elegant experiment used parabiosis (joining the circulatory systems of two animals leading to peripheral blood exchange to obtain 50% chimerism) between an SOD1<sup>G93A</sup> mouse and a control mouse expressing GFP in bone marrow cells and showed no evidence of microglia coming from cells recruited from the bloodstream in parabiotic chimeras (Ajami et al. 2007).

### **Bone marrow transplant to replace mutant SOD1 expressing myeloid cells by wild-type cells**

Whether bone marrow derived cells enter or not the CNS is a question that remains important for ALS especially with regard to the potential benefit to motor neuron survival of replacing mutant SOD1 expressing bone marrow by wild-type bone marrow. Grafting wild-type bone marrow cells in irradiated presymptomatic SOD1<sup>G93A</sup> mice led to conflicting outcomes of either increased survival or no benefit (Corti et al. 2004; Solomon et al. 2006). To explain the discrepancy obtained by the different groups, technical aspects could be taken into consideration including strength of irradiation, number of cells grafted, route of transplantation (i.p, i.v or intra-bone) and the rate of engraftment. Indeed, increasing the percentage of wild-type bone marrow cells in grafted SOD1<sup>G93A</sup> mice could increase the survival of ALS mice (Ohnishi et al. 2009). In this study, wild-type (GFP+) bone marrow was transplanted directly in the bone marrow cavity (intra-bone marrow-bone marrow transplant) in SOD1<sup>G93A</sup> mice at onset (defined as leg tremor in 2 consecutive days) and showed an increase in survival. It is, therefore, interesting to see that transplantation even at onset could have a positive effect on the disease (Ohnishi et al. 2009). Therefore, bone marrow transplant after irradiation could still prove to be an interesting concept to test potential therapeutic tools. Cell replacement therapies or trophic cell therapies have also been tested in mice using hematopoietic cells derived from human umbilical cord blood showing an increased survival of SOD1<sup>G93A</sup> mice (Ende et al. 2000; Garbuzova-Davis et al. 2003).

The effect of wild-type myeloid cells on motor neuron survival could also be linked to a peripheral effect because bone marrow grafting experiments replaced also the peripheral macrophages. In addition, lower expression of mutant SOD1 in peripheral macrophages was

measured in CD11bCre/LoxSOD1<sup>G37R</sup> mice as compared to LoxSOD1<sup>G37R</sup> mice and CD11bCre/LoxSOD1<sup>G37R</sup> mice survived longer (Boillee et al. 2006b). Macrophages at the periphery include the ones in the nerves, and activated macrophages are present in the peripheral nerves upon axonal degeneration in neurodegenerative conditions (Griffin et al. 1993) including in the sciatic nerves of ALS mice or rats (Graber et al. 2010; Chiu et al. 2009).

Allogeneic hematopoietic stem cell transplantation was used in a phase I trial in six ALS patients as a cell replacement therapy with the aim of slowing motor neuron degeneration and ALS disease. Two patients with 100% engraftment showed donor cells in the vicinity of affected motor neurons, but not in unaffected brain areas. Nevertheless, this trial did not show any benefit for the patients and the authors propose to transplant transduced bone marrow stem cells to deliver neurotrophic factors for therapeutic value (Appel et al. 2008).

### **Anti-inflammatory drugs in ALS clinical trials**

Inflammatory cytokines could potentiate motor neuron degeneration and some of these factors are known to be produced by microglia/macrophages or even activated astrocytes. In addition to proinflammatory factors produced around motor neurons, as the blood spinal cord barrier has been shown to be disrupted in ALS mice (Garbuzova-Davis et al. 2008; Zhong et al. 2008; Zhong et al. 2009) proinflammatory factors from outside of the CNS could also enter the spinal cord. To lower the inflammatory reaction and potentially increase motor neuron survival, anti-inflammatory drugs were tested in ALS models and some of them brought to clinical trials. The most famous one is probably minocycline, a second generation tetracycline which had been previously shown to bear anti-inflammatory properties (Yrjanheikki et al. 1999) and that had prompted a lot of hope after several groups showed delayed onset of motor neuron degeneration and increased survival of different mutant SOD1 mouse models (Kriz et al. 2002; Van Den Bosch et al. 2002; Zhu et al. 2002). Although minocycline could have a direct anti-apoptotic effect on motor neurons (Zhu et al. 2002), it induced a concomitant decrease in microglial activation (Kriz et al. 2002). However, the positive results from the mice were not visible in a phase III randomized clinical trial that showed no benefit and maybe some adverse effects, although further studies would need to address the dose of minocycline to be used and the time frame of the administration (Gordon et al. 2007; Carri 2008; Leigh et al. 2008). This outcome has hampered the relevance of using SOD1 mice for testing drugs for potential clinical trials (Schnabel 2008). However and very importantly, patients are always treated after the onset of disease, and animals are often given the drug before motor neuron degeneration has started. When considering this major difference and the divergences in experimental protocols, some guidelines have been proposed for testing potential treatments in ALS mice (Ludolph et al. 2010; Scott et al. 2008).

Other anti-inflammatory drugs focused on the cyclooxygenase (COX) 2 enzyme that can participate in the activation of inflammatory pathways in the CNS through the production of prostaglandin E2 (PGE2) (Consilvio et al. 2004). Increased COX-2 expression was measured in ALS patient spinal cords where the COX-2 immunoreactive cells corresponded to activated microglial cells/macrophages and PGE2 levels were increased in the

cerebrospinal fluid and their receptors induced in the spinal cords of ALS patients (Almer et al. 2001; Yasojima et al. 2001; Maihofner et al. 2003; Yiangou et al. 2006; Liang et al. 2008). COX-2 and PGE2 levels were also found to be increased in SOD1<sup>G93A</sup> mice (Almer et al. 2001) and PGE2 receptors deletions or COX-2 inhibitors delayed the onset and prolonged survival of ALS mice together with decreasing both astroglia and microglial reactivity (Drachman et al. 2002; Pompl et al. 2003; Liang et al. 2008). Celecoxib, the selective COX-2 inhibitor used in mouse studies, however, proved no benefit to ALS patients although PGE2 levels were not downregulated, indicating that the dose of celecoxib used could have been too low (Cudkowicz et al. 2006).

Peroxisome proliferator-activated receptor- $\gamma$  (PPAR $\gamma$ ) agonists are ligand-dependent transcription factors that are known to downregulate pro-inflammatory cytokines and inducible nitric oxide synthase (iNOS) in both macrophages and microglial cells and can also protect against glutamate excitotoxicity (Colville-Nash et al. 1998; Ricote et al. 1998; Zhao et al. 2006b). The PPAR $\gamma$  agonist, pioglitazone, is a FDA-approved drug to treat type II diabetes, because it enhances insulin sensitivity. This drug previously showed improved symptoms in experimental autoimmune encephalomyelitis mice used to model multiple sclerosis (MS) and in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated mouse model for Parkinson's disease (Braidert et al. 2002; Dehmer et al. 2004). In SOD1<sup>G93A</sup> mice, pioglitazone was given orally, starting before onset and showed increased motor performance, protection from motor neuron loss and extension of survival accompanied by a reduced gliosis and immunoreactivity to inflammatory markers (iNOS, NF $\kappa$ B, 3-nitrotyrosine) in the spinal cord of treated mice (Kiaei et al. 2005; Schutz et al. 2005). Because this drug is well tolerated in patients chronically treated for diabetes and crosses the BBB, it makes it an attractive candidate for neurodegenerative diseases and is currently given to ALS patients in an ongoing phase II clinical trial (<http://www.clinicaltrials.gov/>).

Blocking the inflammatory response could, therefore, be a strategy to slow down motor neuron degeneration; however, inflammation and microglial activation cannot be assimilated only as a negative reaction. Indeed, elimination of a significant proportion of the proliferating pool of spinal cord microglial cells in SOD1<sup>G93A</sup> mice (crossing them with CD11b-thymidin Kinase mice) had no effect on motor neuron survival (Gowing et al. 2008). However, as previously described, boosting the inflammatory reaction by chronically injecting LPS exacerbated the disease (Nguyen et al. 2004). Defining the factors secreted by activated microglial cells and that are toxic or trophic for motor neurons is therefore the strategy that several groups have employed to define the types of anti-inflammatory drugs that could be used in the future.

### **Microglia-derived pro- or anti-inflammatory factors**

The expression of several pro-inflammatory factors has been described in the spinal cord of ALS mice or patients over the course of the disease in ALS (Fig 1) and, interestingly, expression profiling of gene products modified between SOD1<sup>G93A</sup> and control mouse spinal cords show genes implicated in inflammatory processes as majorly changed in ALS mice (Yoshihara et al. 2002). Several strategies have been used to study genes involved in

neuroinflammation, including comparing isolated microglial cells (expressing or not mutant SOD1) in culture before or after adding activating factors such as LPS, but also crossing ALS mice with mice deleted for a gene implicated in neuroinflammation.

### Tumor necrosis factor-alpha

Tumor necrosis factor-alpha (TNF- $\alpha$ ) which can be released by microglial cells (Fig. 1) is a potent pro-inflammatory cytokine with direct effects on neurons, astrocytes and microglia and that can elicit toxic though sometimes also trophic responses, according to the receptor. TNF- $\alpha$  levels were shown to be increased in the blood and plasma of ALS patients (Poloni et al. 2000; Cereda et al. 2008). In SOD1<sup>G93A</sup> mice elevated TNF- $\alpha$  mRNA and protein levels are found in the spinal cord at pre-symptomatic stages which increases with the progression of the disease, suggesting that it could be related to the neurodegenerative process (Elliott 2001; Hensley et al. 2003). In addition, TNF- $\alpha$  release by microglial cell cultures was shown to be increased when adult (but not neonate) microglial cells isolated from SOD1<sup>G93A</sup> adult mouse brain were stimulated with LPS (Weydt et al. 2004). To analyze the role of TNF- $\alpha$  on motor neuron degeneration in ALS mice, SOD1<sup>G93A</sup> and SOD1<sup>G37R</sup> mice were crossed with TNF- $\alpha$  deleted mice that unexpectedly failed to decrease disease severity or even motor neuron death (Gowing et al. 2006). Neither axonal degeneration nor astrocytic or microglial activation were affected which suggests that TNF- $\alpha$  is not a key factor in motor neuron degeneration caused by SOD1 mutations. However, looking for potential compensatory processes, increased expression of transcripts for IL-1 $\beta$  (a pro-inflammatory factor) and TLR-2 (a receptor implicated in the innate immune response induction) (Fig. 1) were found in the spinal cord of SOD1<sup>G93A</sup> (but not in SOD1<sup>G37R</sup>) mice lacking TNF- $\alpha$ , which could account for the absence of effect of TNF- $\alpha$  deletion, at least in SOD1<sup>G93A</sup> mice. The drugs thalidomide and lenalidomide that are known to inhibit TNF- $\alpha$  synthesis (but also modulate the expression of other cytokines) were neuroprotective and significantly increased lifespan in SOD1<sup>G93A</sup> mice, with a slightly better performance for lenalidomide, however, the administration (oral) started way before the onset of symptoms (Kiaei et al. 2006). In a second study, where mice were treated with lenalidomide starting at symptom onset it led to a slight improvement of motor performance and a slight increase in lifespan, but far less significant than when treated long before symptom onset (Neymotin et al. 2009). Thalidomide has recently been tested in a phase II clinical trial in ALS patients and showed no improvement of the symptoms, an increased mortality and several side effects experienced by the patients (Stommel et al. 2009).

### Reactive Oxygen Species (ROS) and neuroinflammation

Oxidative stress has long been suspected to contribute to ALS pathology (Barber et al. 2006). Indeed, because SOD1 is a ROS scavenger, a loss of the dismutase activity function of the protein was first hypothesized to be part of the pathogenic process; however, it is a gain of toxic function of the different SOD1 mutants that leads to motor neuron degeneration (as described previously). Even though, downregulating the levels of dismutase active mutant

SOD1 in certain cell types, like Schwann cells, in ALS mice, still contributes to disease acceleration showing that an increase in oxidative stress most likely contributes to exacerbating motor neuron pathology (Lobsiger et al. 2009). Different markers of oxidative stress are induced during the course of ALS disease in human and mouse models. A marker of lipid peroxidation, 4-hydroxynonenal (HNE), is increased in serum, CSF and spinal cord (motor neurons and glia) of sporadic ALS patients (Smith et al. 1998; Shibata et al. 2001; Simpson et al. 2004) and expression of iNOS, mainly localized in astrocytes and microglia, is upregulated during the progression of motor neuron loss, starting at early symptomatic stages in mutant SOD1 mice (Almer et al. 1999). These observations are consistent with the fact that primary SOD1<sup>G93A</sup> microglia, in culture, produce more nitric oxide (NO) than wild-type microglia (Fig. 1), and are toxic to motor neurons which can be partially reverted with the addition of an iNOS inhibitor (Beers et al. 2006; Xiao et al. 2007). NO has been shown to be necessary for Fas-triggered death of mutant SOD1 motor neurons in culture (Raoul et al. 2002). Exogenous NO leads to increased expression of the Fas ligand itself in primary motor neurons and of the *collapsin response mediator protein 4a* (CRMP4a) that can trigger motor neuron death (Duplan et al. 2010; Raoul et al. 2006). Crossing neuronal NOS (nNOS) knockout mice with the slow progressing line of transgenic mice SOD1<sup>G93A-low</sup> (low copy number, *B6SJL-Tg(SOD1-G93A)<sup>dl1</sup>Gur/J*), did not affect motor neuron degeneration; however, these mice still produced  $\beta$  and  $\gamma$  isoforms of nNOS and of course iNOS (Facchinetti et al. 1999). Deletion of iNOS, this time, increased lifespan of the fast progressing and commonly used SOD1<sup>G93A</sup> but had no effect on the SOD1<sup>G93A-low</sup> mice (Son et al. 2001; Martin et al. 2007). Pharmacological inhibition of iNOS could also delay disease onset and extend lifespan of SOD1<sup>G93A</sup> mice (Chen et al. 2009). NO produced through iNOS seems, therefore, to have an impact on motor neuron survival in a fast-progressing ALS mouse line.

Microglial cells can also produce superoxide by the NADPH oxidase, a multiprotein complex including a transmembrane catalytic subunit, Nox, that transports electrons across biological membranes to reduce oxygen to superoxide (Bedard and Krause 2007). NADPH oxidase also modulates intracellular signaling in microglial cells since Nox2 (or phagocytic Nox), that is highly expressed by microglia, together with Nox1 are required to optimize microglial production of NO and Nox1-derived superoxide contributes to IL1 $\beta$  secretion by microglia (Cheret et al. 2008). During the course of ALS disease in mice, NADPH oxidase is upregulated in the spinal cord, increasing ROS production, that could lead to neuronal degeneration through protein oxidative damage (Fig. 1) (Wu et al. 2006; Marden et al. 2007). Crossing SOD1<sup>G93A</sup> mice with mice either deleted for Nox2 or Nox1 significantly increased survival (with a greater efficiency for Nox2, Wu et al. 2006; Marden et al. 2007). In addition, apocynin, an NADPH oxidase blocker given in the drinking water to ALS mice remarkably prolonged their survival by more than 100 days (with the highest dose tested and with the treatment started at 2 weeks of age and therefore way before disease onset) what is to date the highest effect obtained with a drug treatment in this model (Harrasz et al. 2008). However, given after onset, the outcome was less striking and this effect could well be linked to mutant SOD1 mediated ALS only (and maybe even a subpopulation of SOD1 mutants). Indeed, analyzing the mechanisms by which mutant SOD1 led to an increase in superoxide

production by Nox2, in transfected cell lines, dismutase active mutants of SOD1 but not dismutase inactive or wild-type forms hyperactivated Nox2, through binding to Rac1 (a regulatory subunit of Nox2) enhancing the production of superoxide (Harraz et al. 2008). Increased superoxide production has also been reported for microglial cells in culture expressing SOD1<sup>G93A</sup> compared with control microglia (Fig. 1) (Xiao et al. 2007); however, downregulation of overall microglial reaction by apocynin or Nox2 deletion was either not analyzed or reached divergent outcome (Wu et al. 2006; Marden et al. 2007; Harraz et al. 2008). Nox2 upregulation has also been reported in spinal cords of sporadic ALS cases (Wu et al. 2006), but considering the direct action of mutant SOD1 on Nox2 activation, it would be important to know if patients with SOD1 mutations generate similar or greater increase in Nox2 and if the NADPH oxidase pathway could be a target for potential therapeutics also in sporadic ALS (since activated microglial cells, even without expressing mutant SOD1 and can still produce superoxide through NADPH oxidase activation).

#### Anti-inflammatory and trophic factors produced by microglia.

When compared with the well studied pro-inflammatory factors, not much is known about the trophic factors potentially released by microglial cells and especially downregulated in ALS. IL-4 known to be released by T cells can also be produced by microglia (Fig. 1) and was shown to be protective to microglial-induced motor neuron injury through the reduction in microglial NO and superoxide production mediated by LPS (Zhao et al. 2006a). Microglial cells can also produce the trophic factor for motor neurons, insulin-like growth factor-1 (IGF-1) and primary SOD1<sup>G93A</sup> microglial cell cultures release less IGF-1 when compared with non-transgenic microglia (Fig. 1) and further maintains this difference upon treatment with LPS (Xiao et al. 2007). However, when microglial cells were directly isolated from adult mutant SOD1 mice, IGF-1 levels were found to be increased at pre-symptomatic stages and further increase later in disease when compared with non-transgenic or transgenic mice expressing wild-type SOD1 (Chiu et al. 2008). IGF-1 also showed anti-inflammatory properties, reducing the release of TNF- $\alpha$  and NO by BV2 microglial cell lines expressing mutant SOD1 and neuroprotective capacities against mutant SOD1 induced toxicity of astrocytes towards motor neurons in culture (Dodge et al. 2008).

Importantly, several groups have shown the motor neuron protective effect of IGF-1 in animal models of ALS, however, the site of effectiveness of the treatment is debated. Kaspar et al. (2003) showed that IGF-1 delivered by retrograde adeno-associated virus (AAV) prolonged survival and delayed disease progression of SOD1<sup>G93A</sup> mice when treated both before and at disease onset. The effect of IGF-1 was due to its presence in motor neurons themselves given that delivery of muscle-targeted IGF-1 viral vectors (which could not be retrogradely transported) only increased survival very modestly (Kaspar et al. 2003). However, muscle-specific expression of IGF-1 (by a transgenic mouse approach) in SOD1<sup>G93A</sup> mice reduced spinal cord inflammation and increased their survival (Dobrowolny et al. 2005), but this was not confirmed by a similar approach where IGF-1 was expressed exclusively in the skeletal muscle or in the CNS (Messi et al. 2007). Beneficial effects of IGF-1 were also obtained by intraparenchymal delivery of AAV in the lumbar spinal cord of presymptomatic SOD1<sup>G93A</sup> mice, leading to increased motor performance with delayed

disease onset but a modest increased survival selectively in males (Lepore et al. 2007). IGF-1 expression was found in neuronal cell bodies (including ventral horn motor neurons) but not in astrocytes, therefore in accordance with an absence of effect on the disease duration, which as previously discussed, is mostly dependent on glial cells. Injection of AAV-IGF-1 vectors this time, into the deep cerebellar nuclei of SOD1<sup>G93A</sup> mice at disease onset, to deliver IGF-1 in the brain stem and spinal cord through axonal transport led to increased motor neuron survival, reduction in microglial and astroglial activation, improved motor function and a significant extension of life span (Dodge et al. 2008). A different method for continuous delivery used infusion of IGF-1 into the intrathecal space (CSF) of the lumbar spinal cord in presymptomatic SOD1<sup>G93A-low</sup> mice and showed a positive outcome with improved performance of motor functions, delayed onset and extended survival (Nagano et al. 2005a).

A similar technique was used in a small number of ALS patients where intrathecal delivery of IGF-1 showed a modest, but significant benefit (Nagano et al. 2005b). Other clinical trials with recombinant human IGF-I used subcutaneous delivery that resulted in modest effects in one trial (Lai et al. 1997), or did not show significant differences in two other studies (Borasio et al. 1998; Sorenson et al. 2008).

IGF-1 levels have been shown to be altered in the blood and CSF of ALS patients, with circulating concentrations either decreased (Torres-Aleman et al. 1998), increased (Hosback et al. 2007; Pellecchia et al. 2009) but also unchanged (Braunstein and Reviczky 1987; Bilic et al. 2006; Corbo et al. 2009) as compared to controls. In addition, alterations in the levels of IGF-1 binding proteins, which regulate IGF-1 bioavailability have been reported (Torres-Aleman et al. 1998; Hosback et al. 2007). These alterations that could influence the effect of IGF-1 treatments point out the need to evaluate the best administration route for assuring a correct availability of IGF-1 to the CNS and whether it needs to be delivered to motor neurons and/or other non-neuronal cell types.

### **Increased expression of microglial mitogenic and chemoattractant factors**

Colony stimulating factors (CSF) are implicated in myeloid cell production with macrophage-CSF (M-CSF or CSF-1) being the archetypal mitogenic factor for macrophages/microglial cells. Although secreted by microglial cells, a main source of CSF-1 is coming from astrocytes. Granulocyte macrophage CSF (GM-CSF) is also a mitogenic factor for macrophages while granulocyte-CSF (G-CSF) mostly affects neutrophil production. Lack of M-CSF in osteopetrotic (op/op) mice or lack of its receptor (fms) leads to severe deficiency in monocytes and tissue macrophages including microglia (Wiktor-Jedrzejczak et al. 1982; Wegiel et al. 1998; Dai et al. 2002). In op/op mice, macrophage production seems to increase with age but post-lesion proliferation of microglia remains impaired (Begg et al. 1993; Raivich et al. 1994; Kalla et al. 2001). M-CSF mRNA levels were found to be increased from onset of symptoms onward as disease progressed in the CNS of SOD1<sup>G93A-low</sup> mice, correlating with the increase in microglial reactivity (Elliott 2001) while levels of GM-CSF were measured in SOD1<sup>G93A</sup> mice and showed no alteration (Hensley et al. 2003). Intra-peritoneal administration of M-CSF to SOD1<sup>G37R</sup> mice induced an amoeboid morphology and increased proliferation of microglia, but not sufficiently to

translate to a higher number of microglial cells in the spinal cord, or peripheral macrophages in the sciatic nerve. However, M-CSF treatment exacerbated the disease of ALS mice (Gowing et al. 2009). CSFs also have neuroprotective capabilities apparently due to direct effects on the neurons expressing their receptors (Schneider et al. 2005; Schabitz et al. 2008). This is the case for G-CSF, which improved motor neuron survival, delayed onset of symptoms and increased lifespan (though modestly) when administered subcutaneously after onset of denervation or when overexpressed transgenically in SOD1<sup>G93A</sup> mice (Pitzer et al. 2008). However, in a pilot clinical study G-CSF, which was well tolerated, showed no benefit in ALS patients (Nefussy et al. 2009).

Increased inflammatory processes also include chemoattraction of macrophages/microglial cells to the lesion site. Although as previously described, macrophages from the periphery do not seem to contribute to the increase in microglial cells number in the spinal cord, increased chemokine levels could still reflect extended microglial reactivity and attraction of local microglia to specific regions of the CNS. Monocyte chemoattractant protein-1 (MCP-1), a chemotactic factor for macrophages/microglia, was found to be increased in the CSF and serum of ALS patients while immunohistochemical studies localized its expression principally in glial cells in human ALS spinal cords (Wilms et al. 2003; Henkel et al. 2004; Simpson et al. 2004; Baron et al. 2005; Nagata et al. 2007; Kuhle et al. 2009). Interestingly, a trend to higher MCP-1 levels in the CSF was found in ALS patients with shorter lifespan which, if confirmed in a bigger cohort, could be of prognostic potential for ALS (Kuhle et al. 2009). MCP-1 was also found to be elevated in animal models of ALS (Nguyen et al. 2001; Henkel et al. 2006). Surprisingly, MCP-1 mRNA levels were already elevated at the age of 15 days in SOD1<sup>G93A</sup> mouse spinal cords and therefore before microglial activation revealing a very early intrinsic modification of MCP-1 synthesis in SOD1<sup>G93A</sup> mice and consistent with the increased production of MCP-1 by activated SOD1<sup>G93A</sup> cultured microglia from neonates when compared to control cells (Henkel et al. 2006; Sargsyan et al. 2009). MCP-1 immunoreactivity was found in neuronal and glial cells and overtly increased at end-stage of disease in ALS mice (Henkel et al. 2006). Although MCP-1 has been the most studied chemokine in ALS, other chemokines such as IL-8 in CSF, eotaxin in serum and RANTES in CSF and serum have also been found at higher concentrations in ALS cases (Rentzos et al. 2007; Kuhle et al. 2009). Accordingly, protein levels of RANTES/CCL5 but also GRO/KC (the mouse analog of IL-8) were also increased in the SOD1<sup>G93A</sup> mouse spinal cords (Hensley et al. 2003).

### **Motor neuron signals to microglial cells**

Microglial cells through release of trophic or toxic factors could have an impact on motor neuron survival and disease progression. Initiation of the inflammatory process could in theory come from several sources. (1) Factors coming from the periphery through disrupted blood spinal cord barrier (Garbuzova-Davis et al. 2008; Zhong et al. 2008), however, the origin of this rupture is not yet clear and could likely be linked to the motor neuron degenerative processes. (2) Intrinsic pathways in microglial cells expressing mutations leading to ALS could be the second hypothesis since as described previously microglial cells expressing mutant SOD1 can produce more toxic and less trophic factors in

culture (Beers et al. 2006; Xiao et al. 2007) and downregulating mutant SOD1 in microglia/macrophages has a beneficial effect on disease progression in mice (Beers et al. 2006; Boillee et al. 2006b; Wang et al. 2009b). However, replacing wild-type microglia/macrophages by mutant expressing cells is not enough to induce death of motor neurons not expressing mutant SOD1 (Beers et al. 2006). (3) A likely pathway would, therefore, be signals coming from motor neurons when degenerating (and even when not yet showing signs of death that could explain early microglial activation in ALS mouse spinal cord) and an exacerbation of the inflammatory process by factors coming from microglia (described above) or even astrocytes and most likely also due to the intrinsic expression of ALS linked mutated proteins (Fig. 1). Factors coming from neurons and directly activating microglial cells are still not well defined in the context of ALS.

Communication between neurons and microglia is an important feature for normal function of the CNS. For example, fractalkine and its receptor CX3CR1, as well as CD200-CD200R and SIRP $\alpha$ -CD47 actively maintain microglial cells in a resting state (Hoek et al. 2000; Cardona et al. 2006; Bessis et al. 2007). Microglia can sense a wide range of stimuli including the ones released from damaged cells in pathological conditions (Fig. 1), which is first believed to be protective to reconstitute the damaged area. However, its chronic persistence is most likely becoming deleterious and participates in the degenerative process of many neurodegenerative diseases including ALS (Wyss-Coray and Mucke 2002; Hanisch and Kettenmann 2007).

Among the factors released by damaged neurons, ATP can act on microglia through cell surface purinergic P2 receptors either the ionotropic (P2X) or the metabotropic (P2Y) subtypes (Fig. 1), which can activate inflammatory responses (Inoue 2006). Recently, upregulation of P2X4, P2X7 and P2Y6 receptors as well as down-regulation of ATP-hydrolyzing activities have been found in mutant SOD1 microglia. This activation translated first, into increased expression of TNF- $\alpha$  and COX-2 by mutant SOD1 microglia activated by ATP and second, into toxicity towards neuronal cell lines expressing human SOD1<sup>G93A</sup> and exposed to conditioned media of 2'-3'-O-(benzoyl-benzoyl) ATP-activated microglia (D'Ambrosi et al. 2009). P2X receptors have also been related to ALS pathology in humans, since a greater density of P2X7-immunoreactive microglial cells/macrophages was found in affected regions of ALS spinal cords (Yiangou et al. 2006).

One obvious candidate to participate in motor neuron-microglia interactions in ALS mouse models is mutant SOD1 itself, but this evidence only became clear when chromogranin A (CgA) was found as an interacting partner to SOD1<sup>G93A</sup> in a yeast two hybrid approach (Urushitani et al. 2006). Indeed, CgA which was expressed by motor neurons (but not microglial cells) seemed to act as a chaperone-like factor promoting secretion of mutant SOD1 in a CgA-SOD1 complex (Fig. 1). CgA and CgB co-localized with mutant SOD1, but not wild-type SOD1, in ALS mice *in vivo*. Of interest, a recent genetic screen showed implication of a mutation in the CgB gene as a risk factor and a modifier of onset in ALS patients (Gros-Louis et al. 2009). In mixed mouse spinal cord cell culture experiments adding mutant SOD1 was toxic for motor neurons in contrast to wild-type SOD1 and this toxicity was dependent on the presence of microglial cells (Urushitani et al. 2006). These observations, therefore, proposed a scheme where mutant SOD1 secreted by motor neurons would activate microglial cells that would in turn secrete neurotoxic factors and

affect motor neurons. In fact, mutant SOD1 added to microglial cell cultures changed their morphology towards a round activated shape and turned microglia to a pro-inflammatory type with increased production of TNF- $\alpha$ , IL-1 $\beta$  and superoxides (Zhao et al. 2010). Mutant SOD1 seemed to activate microglial cells through binding to CD14. CD14 is a LPS receptor that mediates signal transduction in concert with TLR2 and TLR4. These data are also consistent with studies on BV2 cells expressing mutant SOD1 and producing high amounts of TNF- $\alpha$  especially when the TLR2 pathway is activated (Liu et al. 2009). However, deletion of CD14 (CD14<sup>-/-</sup> mice) in SOD1<sup>G93A</sup> mice had no effect on disease progression and survival (Zhao et al. 2010).

Because mutant SOD1 release by motor neurons was implicated in the disease mechanism, vaccination approaches have been tested in different ALS lines. Using a bacterially purified recombinant mutant SOD1<sup>G93A</sup> protein as the immunogen, in the slowly progressing SOD1<sup>G37R</sup> line, the vaccination extended their lifespan by 4 weeks. This strategy did not impact the survival of the fast-progressing SOD1<sup>G93A</sup> mice (most probably due to too high expression levels of the transgenic protein). However, direct intraventricular infusion of antibodies raised against the SOD1<sup>G93A</sup> protein achieved to prolong lifespan of the fast-progressing SOD1<sup>G93A</sup> mice (Urushitani et al. 2007). Therefore, immunization could be considered for the treatment of ALS patients, but this strategy would only be pertinent for familial ALS linked to SOD1 mutations unless one could show that human SOD1 in sporadic ALS patients behaves like mutant SOD1. Indeed, studies in mice have shown that adding wild-type human SOD1 in mice expressing mutant human SOD1 could speed up the disease (Deng et al. 2006; Wang et al. 2009a) most likely through a stabilization of the mutant protein by the wild-type one, although wild-type SOD1 could also acquire a toxic deleterious conformation through oxidation (Rakhit et al. 2002; Ezzi et al. 2007; Kabashi et al. 2007). In any case, since microglial cells are activated in familial and sporadic ALS cases, finding the motor neuron pathways implicated in microglial activation could help in slowing motor neuron degeneration.

### **The role of the adaptive immune system in ALS mice**

Besides microglia and astrocytes other cells participate in the inflammatory process occurring in ALS (Fig. 1). Evidence of T lymphocytes infiltration was described in ALS tissues (Lampson et al. 1990; Troost et al. 1990; Kawamata et al. 1992) and in mutant SOD1 mice (Alexianu et al. 2001; Beers et al. 2008; Chiu et al. 2008). In ALS patients, lymphocytic infiltration of both T-helper (CD4+) and cytotoxic (CD8+) classes (but predominantly CD8+ T cells) was described in the corticospinal tracts and anterior horns of the spinal cord (Troost et al. 1989; Lampson et al. 1990; Troost et al. 1990; Kawamata et al. 1992), leading to a proposed autoimmune reaction or viral infection as a cause of ALS disease (McGeer and McGeer 2002). However, these hypotheses were weakened by the lack of further evidence. The absence of specific antibodies or T cells directly attacking motor neurons or the failure of therapeutic immunization in mouse models and ALS patients (detailed below) are not in favor of an autoimmune process (Appel et al. 1993); moreover, the absence of viral DNA or RNA in tissues of ALS patients do not support a viral hypothesis (Swanson et al. 1995;

Rosener et al. 1996; Andrews et al. 1997, 2000; Walker et al. 2001). But still, the presence of T cells in the affected tissues of ALS patients and ALS mice raised the question of the possible role of these infiltrated cells and different mouse mating strategies were used to show the involvement of T cells in ALS mice. First, to block recruitment of peripheral cells in the CNS, PU.1<sup>-/-</sup> / SOD1<sup>G93A</sup> mice were grafted with bone marrow cells lacking the receptor for MCP-1 (CCR2) which recruits CCR2 expressing cells meaning, activated T cells and monocytes. Because CCR2<sup>-/-</sup> bone marrow transplanted ALS mice had shortened lifespan as compared to the mice that received wild-type bone marrow, cells coming from the periphery into the CNS had an influence on the disease. However, this positive effect could have also come from CCR2<sup>-/-</sup> microglial cells in the CNS (since all the microglial cells in the PU.1<sup>-/-</sup> / SOD1<sup>G93A</sup> got replaced by CCR2<sup>-/-</sup> cells). Therefore, SOD1<sup>G93A</sup> mice were crossed with RAG2<sup>-/-</sup> mice that lack functional T and B cells which led to a shorter lifespan. When these mice received SOD1<sup>G93A</sup> or WT bone marrow, they survived longer with a slower disease progression showing that lymphocytes had a beneficial effect on the disease (Beers et al. 2008). Of note, mutant SOD1 expressed in lymphocytes did not seem to affect the disease. Crossing SOD1<sup>G93A</sup> mice with TCRβ<sup>-/-</sup> mice that lack T cells, which led to decreased survival of ALS mice, showed the trophic capabilities of T cells in ALS mice (Chiu et al. 2008). To determine the role of subpopulations of T cells, SOD1<sup>G93A</sup> mice were bred with CD4<sup>-/-</sup> mice. These mice showed the same disease acceleration than SOD1<sup>G93A</sup> / RAG2<sup>-/-</sup> mice meaning that CD4<sup>+</sup> T cells were responsible for the neuroprotection measured, probably by enhancing the expression of neurotrophic factors for motor neurons, such as IGF-1 and also by increasing the expression of glutamate transporters (GLT-1 and GLAST) as measured in the spinal cord of these mice (Beers et al. 2008). Furthermore, T cells also modulated the reactivity of microglial cells since SOD1<sup>G93A</sup> / CD4<sup>-/-</sup> mice showed attenuated staining with microglial activation markers. This observation also means that acceleration of disease did not correlate with the level of microglial activation or that microglial activated morphology did not predict their toxic function (Beers et al. 2008).

Concerning the kinetics of infiltration of T cells that could be influenced by alterations in the BBB permeability described in ALS mice (Zhong et al. 2008; Zhong et al. 2009), CD4<sup>+</sup> T cells were present at onset of disease and accumulated with disease progression while CD8<sup>+</sup> T cells were only present at the end-stage of disease in SOD1<sup>G93A</sup> mice consistent with observations in postmortem tissues of ALS patients, where the majority of the lymphocytes were CD8<sup>+</sup> (Troost et al. 1990). No B cells were observed in the spinal cord of these ALS mice (Chiu et al. 2008) or in human ALS spinal cords (Troost et al. 1990; Engelhardt et al. 1993). The lack of contribution of B cells in SOD1<sup>G93A</sup> mice was shown by mating them with Ig-μ<sup>-/-</sup> mice (where B cell development is blocked at pro-B stage) resulting in no difference in survival. In addition, no evidence of altered phenotype of B cells expressing mutant SOD1 was found (Naor et al. 2009).

Analyzing the T cell immune response and lymphocyte proliferation in SOD1<sup>G93A</sup> mice showed that ALS mice had impaired T cell function and increased number of apoptotic T cells (Banerjee et al. 2008). In addition, spleen size and weight as well as spleen cells counts were reduced at the end-stage of the disease in SOD1<sup>G93A</sup> mice (Banerjee et al. 2008). This T cell malfunction was hypothesized to occur in parallel to the motor neuron dysfunction. Indeed, a significant reduction in thymic progenitor-cell content and abnormal

thymic histology was observed in ALS animals (Seksenyán et al. 2009). Importantly, ALS patients also showed reduction in blood levels of T cell receptor rearrangement excision circles (sjTRECs) and a restricted T cell repertoire accompanied by an increase in proapoptotic markers (Seksenyán et al. 2009).

A reconstitution strategy (adoptive transfer with polyclonal wild-type T regulator (Treg CD4<sup>+</sup> CD25<sup>+</sup>) or T effector (Teff CD4<sup>+</sup> CD25<sup>-</sup>)) cells beginning at the presymptomatic stage in ALS mice, added another evidence to the T cell impairment since it delayed motor neuron loss and extended survival with Treg rather delaying symptom onset and Teff increasing latency between disease onset and entry into late stage, suggesting that these two subsets act on independent pathways (Banerjee et al. 2008). This impairment in T cell response could explain the fact that copolymer-1 (cop-1), a glatiramer acetate derivate (GA), treatment was not efficient in ALS mice (or modestly in females only (Banerjee et al. 2008). Cop-1 is an FDA-approved treatment for MS that has also shown some benefit in mouse models of Alzheimer's and Parkinson's disease (Benner et al. 2004; Frenkel et al. 2005; Butovsky et al. 2006; Laurie et al. 2007). Although the action of cop-1 is not clearly established, it seems to act as an immunomodulatory agent by enhancing the CD4<sup>+</sup> Th2-like response, resulting in anti-inflammatory effects. A previous study using different doses of TV-5010 (another GA derivate) on three different mutant SOD1 mouse lines had shown the same outcome of no delay in disease onset or survival of ALS mice (Haenggeli et al. 2007); conversely, another study reported that GA immunization in low copy (SOD1<sup>G93A-low</sup>), but not high copy, SOD1<sup>G93A</sup> mice delayed disease progression (Angelov et al. 2003). A phase II clinical trial for GA was conducted on ALS patients and had no impact on disease progression, but since it was well tolerated GA could still be used as a supplement treatment for future trials targeting the immune system (Meininger et al. 2009).

## **Conclusion**

Neuroinflammation is part of ALS physiopathology and immune cells participate in the disease progression. Although microglial cells are activated prior to the appearance of the symptoms, motor neuron dysfunction seems to be the primary affection in ALS. Factors coming from sick motor neurons would be at the origin of microglial activation leading to increased neurotoxicity and diminished neuroprotective action of the glial cells. This glial cell reactivity becoming self-sustained would amplify the neuroinflammatory process, hitting back at the suffering motor neurons and increasing their vulnerability to the primary source of damage (Fig. 1). Degenerating motor neurons or reactive glial factors would also influence the blood spinal cord barrier tightness allowing leakage of potential neurotoxic factors from the periphery and increasing the attraction of lymphoid cells from the periphery. CD4<sup>+</sup> T lymphocytes entering the spinal cord can release trophic factors that could act directly on motor neurons or modify microglial reactivity to lead to a more trophic environment. However, mutant proteins that lead to ALS (in first instance mutant SOD1) act in different cell types and modify their response to the injury. The different studies highlighted in this review converge to the conclusion that the immune system participates to motor neuron degeneration in ALS. To this point, the main question is, therefore, why anti-inflammatory drugs have not yet proven their benefice to ALS patients? Several explanations could be

emphasized including (1) a potential interaction with Riluzol in the clinical trials, (2) results in animal pre-clinical trials that are often based on the beneficial effect of presymptomatic administration of the drug. Indeed, all of the potential therapeutic compounds used in mice starting after the onset has shown less benefit than when used at presymptomatic stages. (3) A prescreening of the factors on mutant SOD1 linked ALS models that might not reflect the multifactorial causes of ALS and which explains the current keen interest on building new ALS models expressing new mutated genes responsible for ALS. (4) The adaptive immune response in ALS is defective which could cause imbalance of the immune response to the neurodegeneration and could influence the response to therapies targeting the immune system. (5) The immune reaction is finely tuned, microglial cells can exert both neurotoxic or neurotrophic functions on motor neurons, depending on their activation state and specific microenvironment therefore anti-inflammatory drugs could have beneficial effects at specific stages of the disease and for a defined period of time highlighting the need to better understand the mechanisms of cell interactions, to clarify the different actors of neuroinflammation and the time course of expression/regulation of the inflammatory pathways potentially implicated in motor neuron death.

Targeting the symptomatic phase of the disease being the only way to slow down motor neuron degeneration in a disease mainly of sporadic etiology like ALS, neuroinflammation is still one of the most promising candidate pathway for therapeutic intervention.

## References

- Ajami B, Bennett JL, Krieger C, Tetzlaff W, Rossi FM (2007) Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. *Nat Neurosci* 10: 1538-1543
- Alexianu ME, Kozovska M, Appel SH (2001) Immune reactivity in a mouse model of familial ALS correlates with disease progression. *Neurology* 57: 1282-1289
- Almer G, Vukosavic S, Romero N, Przedborski S (1999) Inducible nitric oxide synthase up-regulation in a transgenic mouse model of familial amyotrophic lateral sclerosis. *J Neurochem* 72: 2415-2425
- Almer G, Guegan C, Teismann P, Naini A, Rosoklija G, Hays AP, Chen C, Przedborski S (2001) Increased expression of the pro-inflammatory enzyme cyclooxygenase-2 in amyotrophic lateral sclerosis. *Ann Neurol* 49: 176-185
- Andersson PB, Perry VH, Gordon S (1991) The kinetics and morphological characteristics of the macrophage-microglial response to kainic acid-induced neuronal degeneration. *Neuroscience* 42: 201-214
- Andrews WD, Al-Chalabi A, Garson JA (1997) Lack of evidence for HTLV tax-rex DNA in motor neurone disease. *J Neurol Sci* 153: 86-90
- Andrews WD, Tuke PW, Al-Chalabi A, Gaudin P, Ijaz S, Parton MJ, Garson JA (2000) Detection of reverse transcriptase activity in the serum of patients with motor neurone disease. *J Med Virol* 61: 527-532
- Angelov DN, Waibel S, Guntinas-Lichius O, Lenzen M, Neiss WF, Tomov TL, Yoles E, Kipnis J, Schori H, Reuter A, Ludolph A, Schwartz M (2003) Therapeutic vaccine for acute and chronic motor neuron diseases: implications for amyotrophic lateral sclerosis. *Proc Natl Acad Sci U S A* 100: 4790-4795
- Appel SH, Engelhardt JI, Henkel JS, Siklos L, Beers DR, Yen AA, Simpson EP, Luo Y, Carrum G, Heslop HE, Brenner MK, Popat U (2008) Hematopoietic stem cell transplantation in patients with sporadic amyotrophic lateral sclerosis. *Neurology* 71: 1326-1334
- Appel SH, Smith RG, Engelhardt JI, Stefani E (1993) Evidence for autoimmunity in amyotrophic lateral sclerosis. *J Neurol Sci* 118: 169-174
- Banerjee R, Mosley RL, Reynolds AD, Dhar A, Jackson-Lewis V, Gordon PH, Przedborski S, Gendelman HE (2008) Adaptive immune neuroprotection in G93A-SOD1 amyotrophic lateral sclerosis mice. *PLoS One* PMID: 18648532
- Barbeito LH, Pehar M, Cassina P, Vargas MR, Peluffo H, Viera L, Estevez AG, Beckman JS (2004) A role for astrocytes in motor neuron loss in amyotrophic lateral sclerosis. *Brain Res Brain Res Rev* 47: 263-274
- Barber SC, Mead RJ, Shaw PJ (2006) Oxidative stress in ALS: a mechanism of neurodegeneration and a therapeutic target. *Biochim Biophys Acta* 1762: 1051-1067
- Baron P, Bussini S, Cardin V, Corbo M, Conti G, Galimberti D, Scarpini E, Bresolin N, Wharton SB, Shaw PJ, Silani V (2005) Production of monocyte chemoattractant protein-1 in amyotrophic lateral sclerosis. *Muscle Nerve* 32: 541-544
- Bedard K, Krause KH (2007) The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. *Physiol Rev* 87: 245-313
- Beers DR, Henkel JS, Xiao Q, Zhao W, Wang J, Yen AA, Siklos L, McKercher SR, Appel SH (2006) Wild-type microglia extend survival in PU.1 knockout mice with familial amyotrophic lateral sclerosis. *Proc Natl Acad Sci U S A* 103: 16021-16026
- Beers DR, Henkel JS, Zhao W, Wang J, Appel SH (2008) 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* 105: 15558-15563
- Begg SK, Radley JM, Pollard JW, Chisholm OT, Stanley ER, Bertoncello I (1993) Delayed hematopoietic development in osteopetrotic (op/op) mice. *J Exp Med* 177: 237-242
- Benner EJ, Mosley RL, Destache CJ, Lewis TB, Jackson-Lewis V, Gorantla S, Nemachek C, Green SR, Przedborski S, Gendelman HE (2004) Therapeutic immunization protects dopaminergic neurons in a mouse model of Parkinson's disease. *Proc Natl Acad Sci U S A* 101: 9435-9440

- Bessis A, Bechade C, Bernard D, Roumier A (2007) Microglial control of neuronal death and synaptic properties. *Glia* 55: 233-238
- Bilic E, Rudan I, Kusec V, Zurak N, Delimar D, Zagar M (2006) Comparison of the growth hormone, IGF-1 and insulin in cerebrospinal fluid and serum between patients with motor neuron disease and healthy controls. *Eur J Neurol* 13: 1340-1345
- Boillee S, Vande Velde C, Cleveland DW (2006a) ALS: a disease of motor neurons and their nonneuronal neighbors. *Neuron* 52: 39-59
- Boillee S, Yamanaka K, Lobsiger CS, Copeland NG, Jenkins NA, Kassiotis G, Kollias G, Cleveland DW (2006b) Onset and progression in inherited ALS determined by motor neurons and microglia. *Science* 312: 1389-1392
- Borasio GD, Robberecht W, Leigh PN, Emile J, Guiloff RJ, Jerusalem F, Silani V, Vos PE, Wokke JH, Dobbins T (1998) A placebo-controlled trial of insulin-like growth factor-I in amyotrophic lateral sclerosis. European ALS/IGF-I Study Group. *Neurology* 51: 583-586
- Braunstein GD, Reviczky AL (1987) Serum insulin-like growth factor-I levels in amyotrophic lateral sclerosis. *J Neurol Neurosurg Psychiatry* 50: 792-794
- Breidert T, Callebert J, Heneka MT, Landreth G, Launay JM, Hirsch EC (2002) Protective action of the peroxisome proliferator-activated receptor-gamma agonist pioglitazone in a mouse model of Parkinson's disease. *J Neurochem* 82: 615-624
- Brujin LI, Becher MW, Lee MK, Anderson KL, Jenkins NA, Copeland NG, Sisodia SS, Rothstein JD, Borchelt DR, Price DL, Cleveland DW (1997) ALS-linked SOD1 mutant G85R mediates damage to astrocytes and promotes rapidly progressive disease with SOD1-containing inclusions. *Neuron* 18: 327-338
- Butovsky O, Koronyo-Hamaoui M, Kunis G, Ophir E, Landa G, Cohen H, Schwartz M (2006) Glatiramer acetate fights against Alzheimer's disease by inducing dendritic-like microglia expressing insulin-like growth factor 1. *Proc Natl Acad Sci U S A* 103: 11784-11789
- Cardona AE, Pioro EP, Sasse ME, Kostenko V, Cardona SM, Dijkstra IM, Huang D, Kidd G, Dombrowski S, Dutta R, Lee JC, Cook DN, Jung S, Lira SA, Littman DR, Ransohoff RM (2006) Control of microglial neurotoxicity by the fractalkine receptor. *Nat Neurosci* 9: 917-924
- Carri MT (2008) Minocycline for patients with ALS. *Lancet Neurol* 7: 118-119; author reply 120-111
- Cereda C, Baiocchi C, Bongioanni P, Cova E, Guareschi S, Metelli MR, Rossi B, Sbalsi I, Cuccia MC, Ceroni M (2008) TNF and sTNFR1/2 plasma levels in ALS patients. *J Neuroimmunol* 194: 123-131
- Chen K, Northington FJ, Martin LJ (2009) Inducible nitric oxide synthase is present in motor neuron mitochondria and Schwann cells and contributes to disease mechanisms in ALS mice. *Brain Struct Funct*
- Cheret C, Gervais A, Lelli A, Colin C, Amar L, Ravassard P, Mallet J, Cumano A, Krause KH, Mallat M (2008) Neurotoxic activation of microglia is promoted by a nox1-dependent NADPH oxidase. *J Neurosci* 28: 12039-12051
- Chiu IM, Chen A, Zheng Y, Kosaras B, Tsiftoglou SA, Vartanian TK, Brown RH, Jr., Carroll MC (2008) T lymphocytes potentiate endogenous neuroprotective inflammation in a mouse model of ALS. *Proc Natl Acad Sci U S A* 105: 17913-17918
- Chiu IM, Phatnani H, Kuligowski M, Tapia JC, Carrasco MA, Zhang M, Maniatis T, Carroll MC (2009) Activation of innate and humoral immunity in the peripheral nervous system of ALS transgenic mice. *Proc Natl Acad Sci U S A* 106: 20960-20965
- Chow CY, Landers JE, Bergren SK, Sapp PC, Grant AE, Jones JM, Everett L, Lenk GM, McKenna-Yasek DM, Weisman LS, Figlewicz D, Brown RH, Meisler MH (2009) Deleterious variants of FIG4, a phosphoinositide phosphatase, in patients with ALS. *Am J Hum Genet* 84: 85-88
- 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 (2003) Wild-type nonneuronal cells extend survival of SOD1 mutant motor neurons in ALS mice. *Science* 302: 113-117
- Colville-Nash PR, Qureshi SS, Willis D, Willoughby DA (1998) Inhibition of inducible nitric oxide synthase by peroxisome proliferator-activated receptor agonists: correlation with induction of heme oxygenase 1. *J Immunol* 161: 978-984

- Consilvio C, Vincent AM, Feldman EL (2004) Neuroinflammation, COX-2, and ALS--a dual role? *Exp Neurol* 187: 1-10
- Corbo M, Lunetta C, Magni P, Dozio E, Ruscica M, Adobbati L, Silani V (2009) Free insulin-like growth factor (IGF)-1 and IGF-binding proteins-2 and -3 in serum and cerebrospinal fluid of amyotrophic lateral sclerosis patients. *Eur J Neurol* 17:398-404
- Corti S, Locatelli F, Donadoni C, Guglieri M, Papadimitriou D, Strazzer S, Del Bo R, Comi GP (2004) Wild-type bone marrow cells ameliorate the phenotype of SOD1-G93A ALS mice and contribute to CNS, heart and skeletal muscle tissues. *Brain* 127: 2518-2532
- Cuadros MA, Navascues J (1998) The origin and differentiation of microglial cells during development. *Prog Neurobiol* 56: 173-189
- Cudkovic ME, Shefner JM, Schoenfeld DA, Zhang H, Andreasson KI, Rothstein JD, Drachman DB (2006) Trial of celecoxib in amyotrophic lateral sclerosis. *Ann Neurol* 60: 22-31
- D'Ambrosi N, Finocchi P, Apolloni S, Cozzolino M, Ferri A, Padovano V, Pietrini G, Carri MT, Volonte C (2009) The proinflammatory action of microglial P2 receptors is enhanced in SOD1 models for amyotrophic lateral sclerosis. *J Immunol* 183: 4648-4656
- Dai XM, Ryan GR, Hapel AJ, Dominguez MG, Russell RG, Kapp S, Sylvestre V, Stanley ER (2002) Targeted disruption of the mouse colony-stimulating factor 1 receptor gene results in osteopetrosis, mononuclear phagocyte deficiency, increased primitive progenitor cell frequencies, and reproductive defects. *Blood* 99: 111-120
- Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, Littman DR, Dustin ML, Gan WB (2005) ATP mediates rapid microglial response to local brain injury in vivo. *Nat Neurosci* 8: 752-758
- Dehmer T, Heneka MT, Sastre M, Dichgans J, Schulz JB (2004) Protection by pioglitazone in the MPTP model of Parkinson's disease correlates with I kappa B alpha induction and block of NF kappa B and iNOS activation. *J Neurochem* 88: 494-501
- Deng HX, Shi Y, Furukawa Y, Zhai H, Fu R, Liu E, Gorrie GH, Khan MS, Hung WY, Bigio EH, Lukas T, Dal Canto MC, O'Halloran TV, Siddique T (2006) Conversion to the amyotrophic lateral sclerosis phenotype is associated with intermolecular linked insoluble aggregates of SOD1 in mitochondria. *Proc Natl Acad Sci U S A* 103: 7142-7147
- Di Giorgio FP, Carrasco MA, Siao MC, Maniatis T, Eggan K (2007) Non-cell autonomous effect of glia on motor neurons in an embryonic stem cell-based ALS model. *Nat Neurosci* 10: 608-614
- Dobrowolny G, Giacinti C, Pelosi L, Nicoletti C, Winn N, Barberi L, Molinaro M, Rosenthal N, Musaro A (2005) Muscle expression of a local IGF-1 isoform protects motor neurons in an ALS mouse model. *J Cell Biol* 168: 193-199
- Dodge JC, Haidet AM, Yang W, Passini MA, Hester M, Clarke J, Roskelley EM, Treleaven CM, Rizo L, Martin H, Kim SH, Kaspar R, Taksir TV, Griffiths DA, Cheng SH, Shihabuddin LS, Kaspar BK (2008) Delivery of AAV-IGF-1 to the CNS extends survival in ALS mice through modification of aberrant glial cell activity. *Mol Ther* 16: 1056-1064
- Drachman DB, Frank K, Dykes-Hoberg M, Teismann P, Almer G, Przedborski S, Rothstein JD (2002) Cyclooxygenase 2 inhibition protects motor neurons and prolongs survival in a transgenic mouse model of ALS. *Ann Neurol* 52: 771-778
- Duplan L, Bernard N, Casseron W, Dudley K, Thouvenot E, Honnorat J, Rogemond V, De Bovis B, Aebischer P, Marin P, Raoul C, Henderson CE, Pettmann B (2010) Collapsin response mediator protein 4a (CRMP4a) is upregulated in motoneurons of mutant SOD1 mice and can trigger motoneuron axonal degeneration and cell death. *J Neurosci* 30: 785-796
- Elliott JL (2001) Cytokine upregulation in a murine model of familial amyotrophic lateral sclerosis. *Brain Res Mol Brain Res* 95: 172-178
- Ende N, Weinstein F, Chen R, Ende M (2000) Human umbilical cord blood effect on sod mice (amyotrophic lateral sclerosis). *Life Sci* 67: 53-59
- Engelhardt JL, Tajti J, Appel SH (1993) Lymphocytic infiltrates in the spinal cord in amyotrophic lateral sclerosis. *Arch Neurol* 50: 30-36
- Ezzi SA, Urushitani M, Julien JP (2007) Wild-type superoxide dismutase acquires binding and toxic properties of ALS-linked mutant forms through oxidation. *J Neurochem* 102: 170-178

- Facchinetti F, Sasaki M, Cutting FB, Zhai P, MacDonald JE, Reif D, Beal MF, Huang PL, Dawson TM, Gurney ME, Dawson VL (1999) Lack of involvement of neuronal nitric oxide synthase in the pathogenesis of a transgenic mouse model of familial amyotrophic lateral sclerosis. *Neuroscience* 90: 1483-1492
- Fendrick SE, Xue QS, Streit WJ (2007) Formation of multinucleated giant cells and microglial degeneration in rats expressing a mutant Cu/Zn superoxide dismutase gene. *J Neuroinflammation* 4: 9
- Flugel A, Bradl M, Kreutzberg GW, Graeber MB (2001) Transformation of donor-derived bone marrow precursors into host microglia during autoimmune CNS inflammation and during the retrograde response to axotomy. *J Neurosci Res* 66: 74-82
- Frenkel D, Maron R, Burt DS, Weiner HL (2005) Nasal vaccination with a proteasome-based adjuvant and glatiramer acetate clears beta-amyloid in a mouse model of Alzheimer disease. *J Clin Invest* 115: 2423-2433
- Garbuzova-Davis S, Saporta S, Sanberg PR (2008) Implications of blood-brain barrier disruption in ALS. *Amyotroph Lateral Scler* 9: 375-376
- Garbuzova-Davis S, Willing AE, Zigova T, Saporta S, Justen EB, Lane JC, Hudson JE, Chen N, Davis CD, Sanberg PR (2003) Intravenous administration of human umbilical cord blood cells in a mouse model of amyotrophic lateral sclerosis: distribution, migration, and differentiation. *J Hematother Stem Cell Res* 12: 255-270
- Gitcho MA, Baloh RH, Chakraverty S, Mayo K, Norton JB, Levitch D, Hatanpaa KJ, White CL, 3rd, Bigio EH, Caselli R, Baker M, Al-Lozi MT, Morris JC, Pestronk A, Rademakers R, Goate AM, Cairns NJ (2008) TDP-43 A315T mutation in familial motor neuron disease. *Ann Neurol* 63: 535-538
- Gong YH, Parsadanian AS, Andreeva A, Snider WD, Elliott JL (2000) Restricted expression of G86R Cu/Zn superoxide dismutase in astrocytes results in astrocytosis but does not cause motoneuron degeneration. *J Neurosci* 20: 660-665
- Gordon PH, Moore DH, Miller RG, Florence JM, Verheijde JL, Doorish C, Hilton JF, Spitalny GM, MacArthur RB, Mitsumoto H, Neville HE, Boylan K, Mozaffar T, Belsh JM, Ravits J, Bedlack RS, Graves MC, McCluskey LF, Barohn RJ, Tandan R (2007) Efficacy of minocycline in patients with amyotrophic lateral sclerosis: a phase III randomised trial. *Lancet Neurol* 6: 1045-1053
- Gowing G, Dequen F, Soucy G, Julien JP (2006) Absence of tumor necrosis factor-alpha does not affect motor neuron disease caused by superoxide dismutase 1 mutations. *J Neurosci* 26: 11397-11402
- Gowing G, Lalancette-Hebert M, Audet JN, Dequen F, Julien JP (2009) Macrophage colony stimulating factor (M-CSF) exacerbates ALS disease in a mouse model through altered responses of microglia expressing mutant superoxide dismutase. *Exp Neurol* 220: 267-275
- Gowing G, Philips T, Van Wijmeersch B, Audet JN, Dewil M, Van Den Bosch L, Billiau AD, Robberecht W, Julien JP (2008) Ablation of proliferating microglia does not affect motor neuron degeneration in amyotrophic lateral sclerosis caused by mutant superoxide dismutase. *J Neurosci* 28: 10234-10244
- Graber DJ, Hickey WF, Harris BT (2010) Progressive changes in microglia and macrophages in spinal cord and peripheral nerve in the transgenic rat model of amyotrophic lateral sclerosis. *J Neuroinflammation* 7: 8
- Griffin JW, George R, Ho T (1993) Macrophage systems in peripheral nerves. A review. *J Neuropathol Exp Neurol* 52: 553-560
- Gros-Louis F, Andersen PM, Dupre N, Urushitani M, Dion P, Souchon F, D'Amour M, Camu W, Meininger V, Bouchard JP, Rouleau GA, Julien JP (2009) Chromogranin B P413L variant as risk factor and modifier of disease onset for amyotrophic lateral sclerosis. *Proc Natl Acad Sci U S A* 106: 21777-21782
- Gurney ME, Pu H, Chiu AY, Dal Canto MC, Polchow CY, Alexander DD, Caliendo J, Hentati A, Kwon YW, Deng HX, et al. (1994) Motor neuron degeneration in mice that express a human Cu,Zn superoxide dismutase mutation. *Science* 264: 1772-1775

- Haenggeli C, Julien JP, Mosley RL, Perez N, Dhar A, Gendelman HE, Rothstein JD (2007) Therapeutic immunization with a glatiramer acetate derivative does not alter survival in G93A and G37R SOD1 mouse models of familial ALS. *Neurobiol Dis* 26: 146-152
- Hall ED, Oostveen JA, Gurney ME (1998) Relationship of microglial and astrocytic activation to disease onset and progression in a transgenic model of familial ALS. *Glia* 23: 249-256
- Hanisch UK, Kettenmann H (2007) Microglia: active sensor and versatile effector cells in the normal and pathologic brain. *Nat Neurosci* 10: 1387-1394
- Harratz MM, Marden JJ, Zhou W, Zhang Y, Williams A, Sharov VS, Nelson K, Luo M, Paulson H, Schoneich C, Engelhardt JF (2008) SOD1 mutations disrupt redox-sensitive Rac regulation of NADPH oxidase in a familial ALS model. *J Clin Invest* 118: 659-670
- Henkel JS, Beers DR, Siklos L, Appel SH (2006) The chemokine MCP-1 and the dendritic and myeloid cells it attracts are increased in the mSOD1 mouse model of ALS. *Mol Cell Neurosci* 31: 427-437
- Henkel JS, Engelhardt JI, Siklos L, Simpson EP, Kim SH, Pan T, Goodman JC, Siddique T, Beers DR, Appel SH (2004) Presence of dendritic cells, MCP-1, and activated microglia/macrophages in amyotrophic lateral sclerosis spinal cord tissue. *Ann Neurol* 55: 221-235
- 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 (2003) 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* 14: 74-80
- Hoek RM, Ruuls SR, Murphy CA, Wright GJ, Goddard R, Zurawski SM, Blom B, Homola ME, Streit WJ, Brown MH, Barclay AN, Sedgwick JD (2000) Down-regulation of the macrophage lineage through interaction with OX2 (CD200). *Science* 290: 1768-1771
- Hosback S, Hardiman O, Nolan CM, Doyle MA, Gorman G, Lynch C, O'Toole O, Jakeman P (2007) Circulating insulin-like growth factors and related binding proteins are selectively altered in amyotrophic lateral sclerosis and multiple sclerosis. *Growth Horm IGF Res* 17: 472-479
- 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 (2002) 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* 99: 1604-1609
- Ilieva H, Polymenidou M, Cleveland DW (2009) Non-cell autonomous toxicity in neurodegenerative disorders: ALS and beyond. *J Cell Biol* 187: 761-772
- Inoue K (2006) The function of microglia through purinergic receptors: neuropathic pain and cytokine release. *Pharmacol Ther* 109: 210-226
- Jaarsma D, Teuling E, Haasdijk ED, De Zeeuw CI, Hoogenraad CC (2008) Neuron-specific expression of mutant superoxide dismutase is sufficient to induce amyotrophic lateral sclerosis in transgenic mice. *J Neurosci* 28: 2075-2088
- Kabashi E, Valdmanis PN, Dion P, Rouleau GA (2007) Oxidized/misfolded superoxide dismutase-1: the cause of all amyotrophic lateral sclerosis? *Ann Neurol* 62: 553-559
- Kabashi E, Valdmanis PN, Dion P, Spiegelman D, McConkey BJ, Vande Velde C, Bouchard JP, Lacomblez L, Pochigaeva K, Salachas F, Pradat PF, Camu W, Meininger V, Dupre N, Rouleau GA (2008) TARDBP mutations in individuals with sporadic and familial amyotrophic lateral sclerosis. *Nat Genet* 40: 572-574
- Kalla R, Liu Z, Xu S, Koppius A, Imai Y, Kloss CU, Kohsaka S, Gschwendtner A, Moller JC, Werner A, Raivich G (2001) Microglia and the early phase of immune surveillance in the axotomized facial motor nucleus: impaired microglial activation and lymphocyte recruitment but no effect on neuronal survival or axonal regeneration in macrophage-colony stimulating factor-deficient mice. *J Comp Neurol* 436: 182-201
- Kaspar BK, Llado J, Sherkat N, Rothstein JD, Gage FH (2003) Retrograde viral delivery of IGF-1 prolongs survival in a mouse ALS model. *Science* 301: 839-842
- Kawamata T, Akiyama H, Yamada T, McGeer PL (1992) Immunologic reactions in amyotrophic lateral sclerosis brain and spinal cord tissue. *Am J Pathol* 140: 691-707

- Kiaei M, Kipiani K, Chen J, Calingasan NY, Beal MF (2005) Peroxisome proliferator-activated receptor-gamma agonist extends survival in transgenic mouse model of amyotrophic lateral sclerosis. *Exp Neurol* 191: 331-336
- Kiaei M, Petri S, Kipiani K, Gardian G, Choi DK, Chen J, Calingasan NY, Schafer P, Muller GW, Stewart C, Hensley K, Beal MF (2006) Thalidomide and lenalidomide extend survival in a transgenic mouse model of amyotrophic lateral sclerosis. *J Neurosci* 26: 2467-2473
- Kreutzberg GW (1996) Microglia: a sensor for pathological events in the CNS. *Trends Neurosci* 19: 312-318
- Kriz J, Nguyen MD, Julien JP (2002) Minocycline slows disease progression in a mouse model of amyotrophic lateral sclerosis. *Neurobiol Dis* 10: 268-278
- Kuhle J, Lindberg RL, Regeniter A, Mehling M, Steck AJ, Kappos L, Czaplinski A (2009) Increased levels of inflammatory chemokines in amyotrophic lateral sclerosis. *Eur J Neurol* 16: 771-774
- Kwiatkowski TJ, Jr., Bosco DA, Leclerc AL, Tamrazian E, Vanderburg CR, Russ C, Davis A, Gilchrist J, Kasarskis EJ, Munsat T, Valdmanis P, Rouleau GA, Hosler BA, Cortelli P, de Jong PJ, Yoshinaga Y, Haines JL, Pericak-Vance MA, Yan J, Ticozzi N, Siddique T, McKenna-Yasek D, Sapp PC, Horvitz HR, Landers JE, Brown RH, Jr. (2009) Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. *Science* 323: 1205-1208
- Lai EC, Felice KJ, Festoff BW, Gawel MJ, Gelinis DF, Kratz R, Murphy MF, Natter HM, Norris FH, Rudnicki SA (1997) Effect of recombinant human insulin-like growth factor-I on progression of ALS. A placebo-controlled study. The North America ALS/IGF-I Study Group. *Neurology* 49: 1621-1630
- Lampson LA, Kushner PD, Sobel RA (1990) Major histocompatibility complex antigen expression in the affected tissues in amyotrophic lateral sclerosis. *Ann Neurol* 28: 365-372
- Laurie C, Reynolds A, Coskun O, Bowman E, Gendelman HE, Mosley RL (2007) CD4+ T cells from Copolymer-1 immunized mice protect dopaminergic neurons in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of Parkinson's disease. *J Neuroimmunol* 183: 60-68
- Lawson LJ, Perry VH, Gordon S (1992) Turnover of resident microglia in the normal adult mouse brain. *Neuroscience* 48: 405-415
- Leigh PN, Meininger V, Bensimon G, Cudkowicz M, Robberecht W (2008) Minocycline for patients with ALS. *Lancet Neurol* 7: 119-120; author reply 120-111
- Leong SK, Ling EA (1992) Amoeboid and ramified microglia: their interrelationship and response to brain injury. *Glia* 6: 39-47
- Lepore AC, Haenggeli C, Gasmi M, Bishop KM, Bartus RT, Maragakis NJ, Rothstein JD (2007) Intraparenchymal spinal cord delivery of adeno-associated virus IGF-1 is protective in the SOD1G93A model of ALS. *Brain Res* 1185: 256-265
- Lepore AC, Rauck B, Dejea C, Pardo AC, Rao MS, Rothstein JD, Maragakis NJ (2008) Focal transplantation-based astrocyte replacement is neuroprotective in a model of motor neuron disease. *Nat Neurosci* 11: 1294-1301
- Lewis CA, Solomon JN, Rossi FM, Krieger C (2009) Bone marrow-derived cells in the central nervous system of a mouse model of amyotrophic lateral sclerosis are associated with blood vessels and express CX(3)CR1. *Glia* 57: 1410-1419
- Liang X, Wang Q, Shi J, Lokteva L, Breyer RM, Montine TJ, Andreasson K (2008) The prostaglandin E2 EP2 receptor accelerates disease progression and inflammation in a model of amyotrophic lateral sclerosis. *Ann Neurol* 64: 304-314
- Ling EA, Penney D, Leblond CP (1980) Use of carbon labeling to demonstrate the role of blood monocytes as precursors of the 'ameboid cells' present in the corpus callosum of postnatal rats. *J Comp Neurol* 193: 631-657
- Lino MM, Schneider C, Caroni P (2002) Accumulation of SOD1 mutants in postnatal motoneurons does not cause motoneuron pathology or motoneuron disease. *J Neurosci* 22: 4825-4832
- Liu Y, Hao W, Dawson A, Liu S, Fassbender K (2009) Expression of amyotrophic lateral sclerosis-linked SOD1 mutant increases the neurotoxic potential of microglia via TLR2. *J Biol Chem* 284: 3691-3699

- Lobsiger CS, Boillee S, Cleveland DW (2007) Toxicity from different SOD1 mutants dysregulates the complement system and the neuronal regenerative response in ALS motor neurons. *Proc Natl Acad Sci U S A* 104: 7319-7326
- Lobsiger CS, Boillee S, McAlonis-Downes M, Khan AM, Feltri ML, Yamanaka K, Cleveland DW (2009) Schwann cells expressing dismutase active mutant SOD1 unexpectedly slow disease progression in ALS mice. *Proc Natl Acad Sci U S A* 106: 4465-4470
- Ludolph AC, Bendotti C, Blaugrund E, Chio A, Greensmith L, Loeffler JP, Mead R, Niessen HG, Petri S, Pradat PF, Robberecht W, Ruegg M, Schwalenstocker B, Stiller D, van den Berg L, Vieira F, von Horsten S (2010) Guidelines for preclinical animal research in ALS/MND: A consensus meeting. *Amyotroph Lateral Scler* 11: 38-45
- Maihofner C, Probst-Cousin S, Bergmann M, Neuhuber W, Neundorfer B, Heuss D (2003) Expression and localization of cyclooxygenase-1 and -2 in human sporadic amyotrophic lateral sclerosis. *Eur J Neurosci* 18: 1527-1534
- Marden JJ, Harraz MM, Williams AJ, Nelson K, Luo M, Paulson H, Engelhardt JF (2007) Redox modifier genes in amyotrophic lateral sclerosis in mice. *J Clin Invest* 117: 2913-2919
- Marin-Teva JL, Dusart I, Colin C, Gervais A, van Rooijen N, Mallat M (2004) Microglia promote the death of developing Purkinje cells. *Neuron* 41: 535-547
- Martin LJ, Liu Z, Chen K, Price AC, Pan Y, Swaby JA, Golden WC (2007) Motor neuron degeneration in amyotrophic lateral sclerosis mutant superoxide dismutase-1 transgenic mice: mechanisms of mitochondriopathy and cell death. *J Comp Neurol* 500: 20-46
- Marty S, Dusart I, Peschanski M (1991) Glial changes following an excitotoxic lesion in the CNS--I. Microglia/macrophages. *Neuroscience* 45: 529-539
- McGeer PL, Kawamata T, Walker DG, Akiyama H, Tooyama I, McGeer EG (1993) Microglia in degenerative neurological disease. *Glia* 7: 84-92
- McGeer PL, McGeer EG (1995) The inflammatory response system of brain: implications for therapy of Alzheimer and other neurodegenerative diseases. *Brain Res Brain Res Rev* 21: 195-218
- McGeer PL, McGeer EG (2002) Inflammatory processes in amyotrophic lateral sclerosis. *Muscle Nerve* 26: 459-470
- McKercher SR, Torbett BE, Anderson KL, Henkel GW, Vestal DJ, Baribault H, Klemsz M, Feeney AJ, Wu GE, Paige CJ, Maki RA (1996) Targeted disruption of the PU.1 gene results in multiple hematopoietic abnormalities. *EMBO J* 15: 5647-5658
- Meininger V, Drory VE, Leigh PN, Ludolph A, Robberecht W, Silani V (2009) Glatiramer acetate has no impact on disease progression in ALS at 40 mg/day: a double-blind, randomized, multicentre, placebo-controlled trial. *Amyotroph Lateral Scler* 10: 378-383
- Messi ML, Clark HM, Prevette DM, Oppenheim RW, Delbono O (2007) The lack of effect of specific overexpression of IGF-1 in the central nervous system or skeletal muscle on pathophysiology in the G93A SOD-1 mouse model of ALS. *Exp Neurol* 207: 52-63
- Mildner A, Schmidt H, Nitsche M, Merkler D, Hanisch UK, Mack M, Heikenwalder M, Bruck W, Priller J, Prinz M (2007) Microglia in the adult brain arise from Ly-6ChiCCR2+ monocytes only under defined host conditions. *Nat Neurosci* 10: 1544-1553
- Miller TM, Kim SH, Yamanaka K, Hester M, Umapathi P, Arnson H, Rizo L, Mendell JR, Gage FH, Cleveland DW, Kaspar BK (2006) 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* 103: 19546-19551
- Nagai M, Re DB, Nagata T, Chalazonitis A, Jessell TM, Wichterle H, Przedborski S (2007) Astrocytes expressing ALS-linked mutated SOD1 release factors selectively toxic to motor neurons. *Nat Neurosci* 10: 615-622
- Nagano I, Ilieva H, Shiote M, Murakami T, Yokoyama M, Shoji M, Abe K (2005a) Therapeutic benefit of intrathecal injection of insulin-like growth factor-1 in a mouse model of Amyotrophic Lateral Sclerosis. *J Neurol Sci* 235: 61-68
- Nagano I, Shiote M, Murakami T, Kamada H, Hamakawa Y, Matsubara E, Yokoyama M, Moritaz K, Shoji M, Abe K (2005b) Beneficial effects of intrathecal IGF-1 administration in patients with amyotrophic lateral sclerosis. *Neurol Res* 27: 768-772

- Nagata T, Nagano I, Shiote M, Narai H, Murakami T, Hayashi T, Shoji M, Abe K (2007) Elevation of MCP-1 and MCP-1/VEGF ratio in cerebrospinal fluid of amyotrophic lateral sclerosis patients. *Neurol Res* 29: 772-776
- Naor S, Keren Z, Bronshtein T, Goren E, Machluf M, Melamed D (2009) Development of ALS-like disease in SOD-1 mice deficient of B lymphocytes. *J Neurol* 256: 1228-1235
- Nefussy B, Artamonov I, Deutsch V, Naparstek E, Nagler A, Drory VE (2009) Recombinant human granulocyte-colony stimulating factor administration for treating amyotrophic lateral sclerosis: A pilot study. *Amyotroph Lateral Scler*: 1-7
- Neymotin A, Petri S, Calingasan NY, Wille E, Schafer P, Stewart C, Hensley K, Beal MF, Kiaei M (2009) Lenalidomide (Revlimid) administration at symptom onset is neuroprotective in a mouse model of amyotrophic lateral sclerosis. *Exp Neurol* 220: 191-197
- Nguyen MD, D'Aigle T, Gowing G, Julien JP, Rivest S (2004) Exacerbation of motor neuron disease by chronic stimulation of innate immunity in a mouse model of amyotrophic lateral sclerosis. *J Neurosci* 24: 1340-1349
- Nguyen MD, Julien JP, Rivest S (2001) Induction of proinflammatory molecules in mice with amyotrophic lateral sclerosis: no requirement for proapoptotic interleukin-1beta in neurodegeneration. *Ann Neurol* 50: 630-639
- Nimmerjahn A, Kirchhoff F, Helmchen F (2005) Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. *Science* 308: 1314-1318
- Ohnishi S, Ito H, Suzuki Y, Adachi Y, Wate R, Zhang J, Nakano S, Kusaka H, Ikehara S (2009) Intra-bone marrow-bone marrow transplantation slows disease progression and prolongs survival in G93A mutant SOD1 transgenic mice, an animal model mouse for amyotrophic lateral sclerosis. *Brain Res* 1296: 216-224
- Olsen MK, Roberds SL, Ellerbrock BR, Fleck TJ, McKinley DK, Gurney ME (2001) Disease mechanisms revealed by transcription profiling in SOD1-G93A transgenic mouse spinal cord. *Ann Neurol* 50: 730-740
- Pardo AC, Wong V, Benson LM, Dykes M, Tanaka K, Rothstein JD, Maragakis NJ (2006) Loss of the astrocyte glutamate transporter GLT1 modifies disease in SOD1(G93A) mice. *Exp Neurol* 201: 120-130
- Pellecchia MT, Pivonello R, Monsurro MR, Trojsi F, Longo K, Piccirillo G, Pivonello C, Rocco M, Di Somma C, Colao A, Tedeschi G, Barone P (2009) The GH-IGF system in amyotrophic lateral sclerosis: correlations between pituitary GH secretion capacity, insulin-like growth factors and clinical features. *Eur J Neurol* PMID: 20002733
- Perry VH, Hume DA, Gordon S (1985) Immunohistochemical localization of macrophages and microglia in the adult and developing mouse brain. *Neuroscience* 15: 313-326
- Pitzer C, Kruger C, Plaas C, Kirsch F, Dittgen T, Muller R, Laage R, Kastner S, Suess S, Spoelgen R, Henriques A, Ehrenreich H, Schabitz WR, Bach A, Schneider A (2008) Granulocyte-colony stimulating factor improves outcome in a mouse model of amyotrophic lateral sclerosis. *Brain* 131: 3335-3347
- Poloni M, Facchetti D, Mai R, Micheli A, Agnoletti L, Francolini G, Mora G, Camana C, Mazzini L, Bachetti T (2000) Circulating levels of tumour necrosis factor-alpha and its soluble receptors are increased in the blood of patients with amyotrophic lateral sclerosis. *Neurosci Lett* 287: 211-214
- Pompl PN, Ho L, Bianchi M, McManus T, Qin W, Pasinetti GM (2003) A therapeutic role for cyclooxygenase-2 inhibitors in a transgenic mouse model of amyotrophic lateral sclerosis. *FASEB J* 17: 725-727
- Pramatarova A, Laganiere J, Roussel J, Brisebois K, Rouleau GA (2001) Neuron-specific expression of mutant superoxide dismutase 1 in transgenic mice does not lead to motor impairment. *J Neurosci* 21: 3369-3374
- Priller J, Flugel A, Wehner T, Boentert M, Haas CA, Prinz M, Fernandez-Klett F, Prass K, Bechmann I, de Boer BA, Frotscher M, Kreutzberg GW, Persons DA, Dirnagl U (2001) Targeting gene-modified hematopoietic cells to the central nervous system: use of green fluorescent protein uncovers microglial engraftment. *Nat Med* 7: 1356-1361

- Raivich G, Moreno-Flores MT, Moller JC, Kreutzberg GW (1994) Inhibition of posttraumatic microglial proliferation in a genetic model of macrophage colony-stimulating factor deficiency in the mouse. *Eur J Neurosci* 6: 1615-1618
- Rakhit R, Cunningham P, Furtos-Matei A, Dahan S, Qi XF, Crow JP, Cashman NR, Kondejewski LH, Chakrabarty A (2002) Oxidation-induced misfolding and aggregation of superoxide dismutase and its implications for amyotrophic lateral sclerosis. *J Biol Chem* 277: 47551-47556
- Raoul C, Buhler E, Sadeghi C, Jacquier A, Aebischer P, Pettmann B, Henderson CE, Haase G (2006) Chronic activation in presymptomatic amyotrophic lateral sclerosis (ALS) mice of a feedback loop involving Fas, Daxx, and FasL. *Proc Natl Acad Sci U S A* 103: 6007-6012
- Raoul C, Estevez AG, Nishimune H, Cleveland DW, deLapeyriere O, Henderson CE, Haase G, Pettmann B (2002) Motoneuron death triggered by a specific pathway downstream of Fas. potentiation by ALS-linked SOD1 mutations. *Neuron* 35: 1067-1083
- Reaume AG, Elliott JL, Hoffman EK, Kowall NW, Ferrante RJ, Siwek DF, Wilcox HM, Flood DG, Beal MF, Brown RH, Jr., Scott RW, Snider WD (1996) Motor neurons in Cu/Zn superoxide dismutase-deficient mice develop normally but exhibit enhanced cell death after axonal injury. *Nat Genet* 13: 43-47
- Rentzos M, Nikolaou C, Rombos A, Boufidou F, Zoga M, Dimitrakopoulos A, Tsoutsou A, Vassilopoulos D (2007) RANTES levels are elevated in serum and cerebrospinal fluid in patients with amyotrophic lateral sclerosis. *Amyotroph Lateral Scler* 8: 283-287
- Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK (1998) The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. *Nature* 391: 79-82
- Ripps ME, Huntley GW, Hof PR, Morrison JH, Gordon JW (1995) Transgenic mice expressing an altered murine superoxide dismutase gene provide an animal model of amyotrophic lateral sclerosis. *Proc Natl Acad Sci U S A* 92: 689-693
- Rosen DR, Siddique T, Patterson D, Figlewicz DA, Sapp P, Hentati A, Donaldson D, Goto J, O'Regan JP, Deng HX, et al. (1993) Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* 362:59-62.
- Rosen DR (1993) Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* 364: 362
- Rosener M, Hahn H, Kranz M, Heeney J, Rethwilm A (1996) Absence of serological evidence for foamy virus infection in patients with amyotrophic lateral sclerosis. *J Med Virol* 48: 222-226
- Rothstein JD, Van Kammen M, Levey AI, Martin LJ, Kuncl RW (1995) Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. *Ann Neurol* 38: 73-84
- Rutherford NJ, Zhang YJ, Baker M, Gass JM, Finch NA, Xu YF, Stewart H, Kelley BJ, Kuntz K, Crook RJ, Sreedharan J, Vance C, Sorenson E, Lippa C, Bigio EH, Geschwind DH, Knopman DS, Mitumoto H, Petersen RC, Cashman NR, Hutton M, Shaw CE, Boylan KB, Boeve B, Graff-Radford NR, Wszolek ZK, Caselli RJ, Dickson DW, Mackenzie IR, Petrucelli L, Rademakers R (2008) Novel mutations in TARDBP (TDP-43) in patients with familial amyotrophic lateral sclerosis. *PLoS Genet* PMID: 18802454
- Sargsyan SA, Blackburn DJ, Barber SC, Monk PN, Shaw PJ (2009) Mutant SOD1 G93A microglia have an inflammatory phenotype and elevated production of MCP-1. *Neuroreport* 20: 1450-1455
- Sarlette A, Krampfl K, Grothe C, Neuhoff N, Dengler R, Petri S (2008) Nuclear erythroid 2-related factor 2-antioxidative response element signaling pathway in motor cortex and spinal cord in amyotrophic lateral sclerosis. *J Neuropathol Exp Neurol* 67: 1055-1062
- Schabitz WR, Kruger C, Pitzer C, Weber D, Laage R, Gassler N, Aronowski J, Mier W, Kirsch F, Dittgen T, Bach A, Sommer C, Schneider A (2008) A neuroprotective function for the hematopoietic protein granulocyte-macrophage colony stimulating factor (GM-CSF). *J Cereb Blood Flow Metab* 28: 29-43
- Schnabel J (2008) Neuroscience: Standard model. *Nature* 454: 682-685
- Schneider A, Kruger C, Steigleder T, Weber D, Pitzer C, Laage R, Aronowski J, Maurer MH, Gassler N, Mier W, Hasselblatt M, Kollmar R, Schwab S, Sommer C, Bach A, Kuhn HG, Schabitz WR (2005) The hematopoietic factor G-CSF is a neuronal ligand that counteracts programmed cell death and drives neurogenesis. *J Clin Invest* 115: 2083-2098

- Schutz B, Reimann J, Dumitrescu-Ozimek L, Kappes-Horn K, Landreth GE, Schurmann B, Zimmer A, Heneka MT (2005) The oral antidiabetic pioglitazone protects from neurodegeneration and amyotrophic lateral sclerosis-like symptoms in superoxide dismutase-G93A transgenic mice. *J Neurosci* 25: 7805-7812
- Scott S, Kranz JE, Cole J, Lincecum JM, Thompson K, Kelly N, Bostrom A, Theodoss J, Al-Nakhala BM, Vieira FG, Ramasubbu J, Heywood JA (2008) Design, power, and interpretation of studies in the standard murine model of ALS. *Amyotroph Lateral Scler* 9: 4-15
- Seksenyan A, Ron-Harel N, Azoulay D, Cahalon L, Cardon M, Rogeri P, Ko MK, Weil M, Bulvik S, Rechavi G, Amariglio N, Konen E, Koronyo-Hamaoui M, Somech R, Schwartz M (2009) Thymic Involution in Amyotrophic Lateral Sclerosis. *J Cell Mol Med* PMID: 19650830
- Shibata N, Nagai R, Uchida K, Horiuchi S, Yamada S, Hirano A, Kawaguchi M, Yamamoto T, Sasaki S, Kobayashi M (2001) Morphological evidence for lipid peroxidation and protein glycooxidation in spinal cords from sporadic amyotrophic lateral sclerosis patients. *Brain Res* 917: 97-104
- Simard AR, Rivest S (2004) Bone marrow stem cells have the ability to populate the entire central nervous system into fully differentiated parenchymal microglia. *FASEB J* 18: 998-1000
- Simpson EP, Henry YK, Henkel JS, Smith RG, Appel SH (2004) Increased lipid peroxidation in sera of ALS patients: a potential biomarker of disease burden. *Neurology* 62: 1758-1765
- Smith RG, Henry YK, Mattson MP, Appel SH (1998) Presence of 4-hydroxynonenal in cerebrospinal fluid of patients with sporadic amyotrophic lateral sclerosis. *Ann Neurol* 44: 696-699
- Solomon JN, Lewis CA, Ajami B, Corbel SY, Rossi FM, Krieger C (2006) Origin and distribution of bone marrow-derived cells in the central nervous system in a mouse model of amyotrophic lateral sclerosis. *Glia* 53: 744-753
- Son M, Fathallah-Shaykh HM, Elliott JL (2001) Survival in a transgenic model of FALS is independent of iNOS expression. *Ann Neurol* 50: 273
- Sorenson EJ, Windbank AJ, Mandrekar JN, Bamlet WR, Appel SH, Armon C, Barkhaus PE, Bosch P, Boylan K, David WS, Feldman E, Glass J, Gutmann L, Katz J, King W, Luciano CA, McCluskey LF, Nash S, Newman DS, Pascuzzi RM, Pioro E, Sams LJ, Scelsa S, Simpson EP, Subramony SH, Tiryaki E, Thornton CA (2008) Subcutaneous IGF-1 is not beneficial in 2-year ALS trial. *Neurology* 71: 1770-1775
- Sreedharan J, Blair IP, Tripathi VB, Hu X, Vance C, Rogelj B, Ackerley S, Durnall JC, Williams KL, Buratti E, Baralle F, de Bellerocche J, Mitchell JD, Leigh PN, Al-Chalabi A, Miller CC, Nicholson G, Shaw CE (2008) TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. *Science* 319: 1668-1672
- Stommel EW, Cohen JA, Fadul CE, Cogbill CH, Graber DJ, Kingman L, Mackenzie T, Channon Smith JY, Harris BT (2009) Efficacy of thalidomide for the treatment of amyotrophic lateral sclerosis: a phase II open label clinical trial. *Amyotroph Lateral Scler* 10: 393-404
- Streit WJ (2002) Microglia as neuroprotective, immunocompetent cells of the CNS. *Glia* 40: 133-139
- Swanson NR, Fox SA, Mastaglia FL (1995) Search for persistent infection with poliovirus or other enteroviruses in amyotrophic lateral sclerosis-motor neurone disease. *Neuromuscul Disord* 5: 457-465
- Torres-Aleman I, Barrios V, Berciano J (1998) The peripheral insulin-like growth factor system in amyotrophic lateral sclerosis and in multiple sclerosis. *Neurology* 50: 772-776
- Troost D, van den Oord JJ, de Jong JM, Swaab DF (1989) Lymphocytic infiltration in the spinal cord of patients with amyotrophic lateral sclerosis. *Clin Neuropathol* 8: 289-294
- Troost D, Van den Oord JJ, Vianney de Jong JM (1990) Immunohistochemical characterization of the inflammatory infiltrate in amyotrophic lateral sclerosis. *Neuropathol Appl Neurobiol* 16: 401-410
- Turner BJ, Ackerley S, Davies KE, Talbot K (2009) Dismutase-competent SOD1 mutant accumulation in myelinating Schwann cells is not detrimental to normal or transgenic ALS model mice. *Hum Mol Genet*
- Turner MR, Cagnin A, Turkheimer FE, Miller CC, Shaw CE, Brooks DJ, Leigh PN, Banati RB (2004) Evidence of widespread cerebral microglial activation in amyotrophic lateral sclerosis: an [<sup>11</sup>C](R)-PK11195 positron emission tomography study. *Neurobiol Dis* 15: 601-609

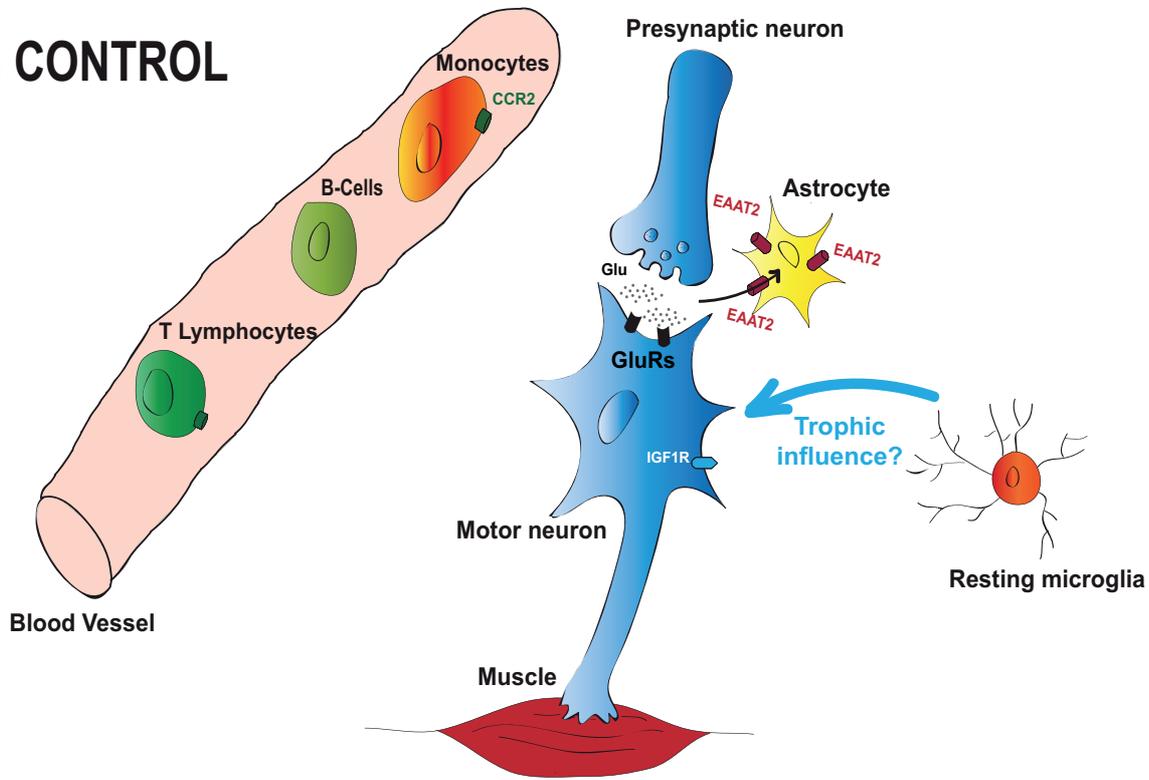
- Urushitani M, Ezzi SA, Julien JP (2007) Therapeutic effects of immunization with mutant superoxide dismutase in mice models of amyotrophic lateral sclerosis. *Proc Natl Acad Sci U S A* 104: 2495-2500
- Urushitani M, Sik A, Sakurai T, Nukina N, Takahashi R, Julien JP (2006) Chromogranin-mediated secretion of mutant superoxide dismutase proteins linked to amyotrophic lateral sclerosis. *Nat Neurosci* 9: 108-118
- Van Den Bosch L, Robberecht W (2008) Crosstalk between astrocytes and motor neurons: what is the message? *Exp Neurol* 211: 1-6
- Van Den Bosch L, Tilkin P, Lemmens G, Robberecht W (2002) Minocycline delays disease onset and mortality in a transgenic model of ALS. *Neuroreport* 13: 1067-1070
- Van Den Bosch L, Van Damme P, Bogaert E, Robberecht W (2006) The role of excitotoxicity in the pathogenesis of amyotrophic lateral sclerosis. *Biochim Biophys Acta* 1762: 1068-1082
- Vance C, Rogelj B, Hortobagyi T, De Vos KJ, Nishimura AL, Sreedharan J, Hu X, Smith B, Ruddy D, Wright P, Ganesalingam J, Williams KL, Tripathi V, Al-Saraj S, Al-Chalabi A, Leigh PN, Blair IP, Nicholson G, de Belleruche J, Gallo JM, Miller CC, Shaw CE (2009) Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. *Science* 323: 1208-1211
- Vargas MR, Johnson DA, Sirkis DW, Messing A, Johnson JA (2008) Nrf2 activation in astrocytes protects against neurodegeneration in mouse models of familial amyotrophic lateral sclerosis. *J Neurosci* 28: 13574-13581
- Vargas MR, Pehar M, Cassina P, Beckman JS, Barbeito L (2006) Increased glutathione biosynthesis by Nrf2 activation in astrocytes prevents p75NTR-dependent motor neuron apoptosis. *J Neurochem* 97: 687-696
- Walker MP, Schlager R, Hays AP, Bowser R, Lipkin WI (2001) Absence of echovirus sequences in brain and spinal cord of amyotrophic lateral sclerosis patients. *Ann Neurol* 49: 249-253
- Wang J, Xu G, Gonzales V, Coonfield M, Fromholt D, Copeland NG, Jenkins NA, Borchelt DR (2002) Fibrillar inclusions and motor neuron degeneration in transgenic mice expressing superoxide dismutase 1 with a disrupted copper-binding site. *Neurobiol Dis* 10: 128-138
- Wang L, Deng HX, Grisotti G, Zhai H, Siddique T, Roos RP (2009a) Wild-type SOD1 overexpression accelerates disease onset of a G85R SOD1 mouse. *Hum Mol Genet* 18: 1642-1651
- Wang L, Sharma K, Grisotti G, Roos RP (2009b) The effect of mutant SOD1 dismutase activity on non-cell autonomous degeneration in familial amyotrophic lateral sclerosis. *Neurobiol Dis* 35: 234-240
- Wegiel J, Wisniewski HM, Dziewiatkowski J, Tarnawski M, Kozielski R, Trenkner E, Wiktor-Jedrzejczak W (1998) Reduced number and altered morphology of microglial cells in colony stimulating factor-1-deficient osteopetrotic op/op mice. *Brain Res* 804: 135-139
- Wegorzewska I, Bell S, Cairns NJ, Miller TM, Baloh RH (2009) TDP-43 mutant transgenic mice develop features of ALS and frontotemporal lobar degeneration. *Proc Natl Acad Sci U S A* 106: 18809-18814
- Weydt P, Yuen EC, Ransom BR, Moller T (2004) Increased cytotoxic potential of microglia from ALS-transgenic mice. *Glia* 48: 179-182
- Wiktor-Jedrzejczak WW, Ahmed A, Szczylik C, Skelly RR (1982) Hematological characterization of congenital osteopetrosis in op/op mouse. Possible mechanism for abnormal macrophage differentiation. *J Exp Med* 156: 1516-1527
- Wilms H, Sievers J, Dengler R, Bufler J, Deuschl G, Lucius R (2003) Intrathecal synthesis of monocyte chemoattractant protein-1 (MCP-1) in amyotrophic lateral sclerosis: further evidence for microglial activation in neurodegeneration. *J Neuroimmunol* 144: 139-142
- Wils H, Kleinberger G, Janssens J, Pereson S, Joris G, Cuijt I, Smits V, Ceuterick-de Groote C, Van Broeckhoven C, Kumar-Singh S (2010) TDP-43 transgenic mice develop spastic paralysis and neuronal inclusions characteristic of ALS and frontotemporal lobar degeneration. *Proc Natl Acad Sci U S A* 107: 3858-3863
- Wong PC, Pardo CA, Borchelt DR, Lee MK, Copeland NG, Jenkins NA, Sisodia SS, Cleveland DW, Price DL (1995) An adverse property of a familial ALS-linked SOD1 mutation causes motor

- neuron disease characterized by vacuolar degeneration of mitochondria. *Neuron* 14: 1105-1116
- Wu DC, Re DB, Nagai M, Ischiropoulos H, Przedborski S (2006) The inflammatory NADPH oxidase enzyme modulates motor neuron degeneration in amyotrophic lateral sclerosis mice. *Proc Natl Acad Sci U S A* 103: 12132-12137
- Wyss-Coray T, Mucke L (2002) Inflammation in neurodegenerative disease--a double-edged sword. *Neuron* 35: 419-432
- Xiao Q, Zhao W, Beers DR, Yen AA, Xie W, Henkel JS, Appel SH (2007) Mutant SOD1(G93A) microglia are more neurotoxic relative to wild-type microglia. *J Neurochem* 102: 2008-2019
- Yamanaka K, Boillee S, Roberts EA, Garcia ML, McAlonis-Downes M, Mikse OR, Cleveland DW, Goldstein LS (2008a) Mutant SOD1 in cell types other than motor neurons and oligodendrocytes accelerates onset of disease in ALS mice. *Proc Natl Acad Sci U S A* 105: 7594-7599
- Yamanaka K, Chun SJ, Boillee S, Fujimori-Tonou N, Yamashita H, Gutmann DH, Takahashi R, Misawa H, Cleveland DW (2008b) Astrocytes as determinants of disease progression in inherited amyotrophic lateral sclerosis. *Nat Neurosci* 11: 251-253
- Yasojima K, Tourtellotte WW, McGeer EG, McGeer PL (2001) Marked increase in cyclooxygenase-2 in ALS spinal cord: implications for therapy. *Neurology* 57: 952-956
- Yiangou Y, Facer P, Durrenberger P, Chessell IP, Naylor A, Bountra C, Banati RR, Anand P (2006) COX-2, CB2 and P2X7-immunoreactivities are increased in activated microglial cells/macrophages of multiple sclerosis and amyotrophic lateral sclerosis spinal cord. *BMC Neurol* 6: 12
- Yoshihara T, Ishigaki S, Yamamoto M, Liang Y, Niwa J, Takeuchi H, Doyu M, Sobue G (2002) Differential expression of inflammation- and apoptosis-related genes in spinal cords of a mutant SOD1 transgenic mouse model of familial amyotrophic lateral sclerosis. *J Neurochem* 80: 158-167
- Yrjanheikki J, Tikka T, Keinanen R, Goldsteins G, Chan PH, Koistinaho J (1999) A tetracycline derivative, minocycline, reduces inflammation and protects against focal cerebral ischemia with a wide therapeutic window. *Proc Natl Acad Sci U S A* 96: 13496-13500
- Zhao W, Beers DR, Henkel JS, Zhang W, Urushitani M, Julien JP, Appel SH (2010) Extracellular mutant SOD1 induces microglial-mediated motoneuron injury. *Glia* 58: 231-243
- Zhao W, Xie W, Xiao Q, Beers DR, Appel SH (2006a) Protective effects of an anti-inflammatory cytokine, interleukin-4, on motoneuron toxicity induced by activated microglia. *J Neurochem* 99: 1176-1187
- Zhao X, Ou Z, Grotta JC, Waxham N, Aronowski J (2006b) Peroxisome-proliferator-activated receptor-gamma (PPARgamma) activation protects neurons from NMDA excitotoxicity. *Brain Res* 1073-1074: 460-469
- Zhong Z, Deane R, Ali Z, Parisi M, Shapovalov Y, O'Banion MK, Stojanovic K, Sagare A, Boillee S, Cleveland DW, Zlokovic BV (2008) ALS-causing SOD1 mutants generate vascular changes prior to motor neuron degeneration. *Nat Neurosci* 11: 420-422
- 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 (2009) Activated protein C therapy slows ALS-like disease in mice by transcriptionally inhibiting SOD1 in motor neurons and microglia cells. *J Clin Invest* 119: 3437-3449
- Zhou H, Huang C, Chen H, Wang D, Landel CP, Xia PY, Bowser R, Liu YJ, Xia XG (2010) Transgenic rat model of neurodegeneration caused by mutation in the TDP gene. *PLoS Genet* PMID: 20361056
- Zhu S, Stavrovskaya IG, Drozda M, Kim BY, Ona V, Li M, Sarang S, Liu AS, Hartley DM, Wu DC, Gullans S, Ferrante RJ, Przedborski S, Kristal BS, Friedlander RM (2002) Minocycline inhibits cytochrome c release and delays progression of amyotrophic lateral sclerosis in mice. *Nature* 417: 74-78

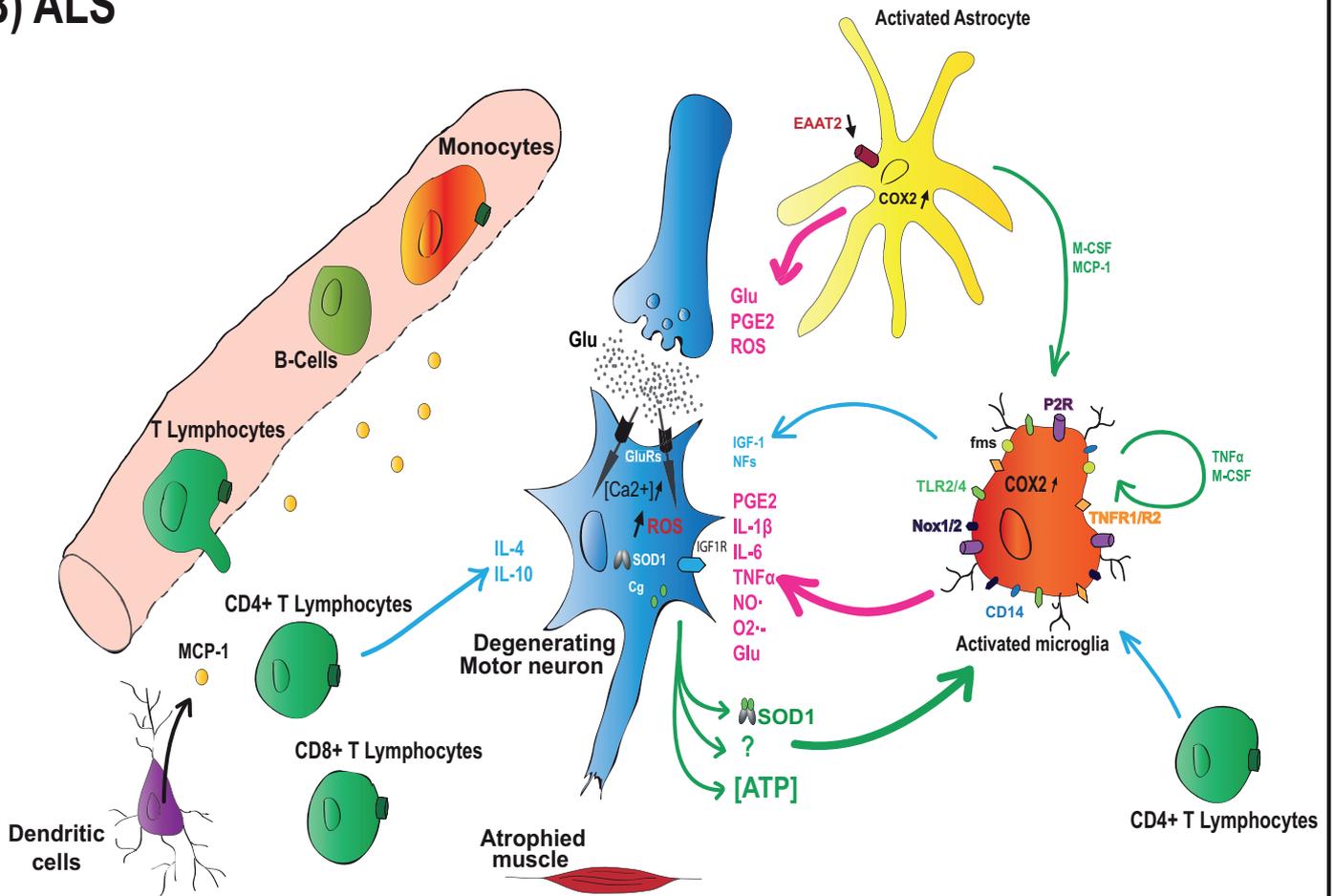
## Figure Legend

**Fig. 1** Immune cells of the CNS including microglia and T lymphocytes affect motor neuron survival. **a** Control situation: the presynaptic neuron releases glutamate (Glu), which binds to glutamate receptors (GluRs) on the postsynaptic motor neuron resulting in excitation through calcium influx. Extracellular glutamate is quickly cleared from the synaptic cleft by astrocytes through EAAT2 transporters. In a non-inflamed environment, microglial cells remain in a resting or “surveying” state, most likely releasing factors with neurotrophic influence (*blue arrows*) **b** ALS situation: decreased expression of astrocytic glutamate transporters EAAT2 could lead to prolonged glutamate excitation of motor neurons and participate in their degeneration (excitotoxic hypothesis). Activated microglial cells and astrocytes produce toxic factors (*pink arrows*). Among the factors released by astrocytes, macrophage- colony stimulating Factor (M-CSF) and monocyte chemoattractant protein-1 (MCP-1) are capable of activating microglial cells, increasing their proliferation (M-CSF) or migration (MCP-1) (*green arrows*). Microglial cells are also prone to self-activation by releasing Tumor Necrosis Factor- $\alpha$  (TNF $\alpha$ ) for which they express the receptors 1 and 2 (TNFR1/2) and M-CSF acting on the receptor fms. Activated microglial cells will produce more reactive oxygen species (ROS) like nitric oxide (NO) through the inducible NO synthase and superoxide (O<sub>2</sub><sup>-</sup>) through activation of NADPH oxidases (Nox1/2), but also proinflammatory cytokines like interleukines 1 $\beta$  and 6 (IL-1 $\beta$ , IL-6) and prostaglandins (PGE2) through activation of the cyclooxygenase 2 (COX2). Extracellular ATP, likely coming from damaged motor neurons, binds to microglial purinergic P2 receptors, therefore, contributing to microglial activation. Motor neurons can also participate to glial cell activation by releasing mutant SOD1 co-secreted with chromogranine (Cg) that can bind to CD14 acting in concert with the Toll-like receptors (TLR2/4). The adaptive immune system is also part of the degenerating motor neuron response. T lymphocytes (CD4<sup>+</sup> and CD8<sup>+</sup>) coming from the periphery enter the spinal cord during the inflammatory process in ALS. CD4<sup>+</sup> lymphocytes seem to have a neuroprotective effect by directly releasing anti-inflammatory factors like interleukines 4 and 10 (IL-4, IL-10) or by acting on microglial cells to increase their neurotrophic function (production of insulin-like growth factor-1 (IGF-1)). The role of infiltrating CD8<sup>+</sup> T cells remains unclear and B cells are not present in ALS spinal cords. Dendritic (antigen-presenting) cells secrete MCP-1, which probably participates in the infiltration of peripheral immune cells. All together, the inflammatory environment and increased oxidative stress take part in the degeneration of the motor neurons that leads to muscle atrophy in ALS.

# A) CONTROL



# B) ALS



Barbeito et al. Figure 1