Published in final edited form as:
Neurobiol Dis. 2010 March ; 37(3): 493–502. doi:10.1016/j.nbd.2009.10.005.

Inflammation in ALS and SMA: sorting out the good from the evil

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Abstract

Indices of neuroinflammation are found in a variety of diseases of the CNS including amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA). Over the years, neuroinflammation, in degenerative disorders of the CNS, has evolved from being regarded as an innocent bystander accomplishing its housekeeping function secondary to neurodegeneration to being considered as a *bona fide* contributor to the disease process and, in some situations, as a putative initiator of the disease. Herein, we will review not only neuroinflammation in both ALS and SMA from the angle of neuropathology, but also from the angle of its potential role in the pathogenesis and treatment of these two dreadful paralytic disorders.

Keywords

Neuroinflammation; Gliosis; Astrocytosis; Astrocytes; Microgliosis; Microglia; Amyotrophic lateral sclerosis (ALS); superoxide dismutase 1 (SOD1); Spinal muscular atrophy (SMA)

Introduction

Neuroinflammation is a term coined to describe cellular and molecular processes which encompasses activation of microglia and astrocytes and infiltration of peripheral immune cells. It occurs in the CNS in a variety of pathological conditions including neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA). Traditionally, it was thought that neuroinflammation is a secondary, complex cellular event, arising in response to a loss of neurons and aimed, first, at limiting cell damage and at promoting CNS regeneration. Yet, mounting evidence supports the possibility that neuroinflammation may, in fact, impair neuronal survival thus stimulating the progression and propagation of the degenerative process. More provocative, it is now even believed that among neuroinflammatory cells, astrocytes and microglia can, under certain circumstances, initiate

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neurodegeneration. It is around these two proposed deleterious roles of neuroinflammation, specifically in ALS and SMA, that this review will be articulated.

ALS and SMA, two prominent motor neuron diseases

Prior to embarking on our discussion of neuroinflammation, briefly it is worth introducing the two paralytic disorders at the center of this review, namely ALS and SMA. First, it should be remembered that ALS is a relentless, adult-onset, fatal paralytic disorder confined to the voluntary motor system (Rowland, 1995). It is characterized, mainly, by the loss of upper motor neurons (MN) or lower MNs or both, and a robust activation of astrocytes and microglia (Almer et al., 1999; Hall et al., 1998; Hirano, 1996; Schiffer et al., 1996). ALS arises essentially as a sporadic condition, but ~10% of the disease is inherited, and these rare familial occurrences are clinically and pathologically almost indistinguishable from the sporadic cases of ALS (Pasinelli and Brown, 2006). Approximately 20% of the familial cases of ALS are linked to mutations in the gene encoding for the ubiquitous cytosolic enzyme, superoxide dismutase-1 (SOD1) (Pasinelli and Brown, 2006). Transgenic (Tg) rodents expressing either catalytically active (Gurney et al., 1994; Wong et al., 1995) or inactive SOD1 mutants (Bruijn et al., 1997; Wang et al., 2003) recapitulate the clinical and neuropathological hallmarks of ALS, including selective spinal MN degeneration and glial activation (Almer et al., 1999). Tg mice expressing high levels of wild-type human *SOD1* are healthy (Gurney et al., 1994; Wong et al., 1995). Altogether, these results argue for mutant SOD1 causing the ALS-like phenotype, not via a loss-of-, but rather via a gain-of-function effect. However, despite intense research efforts, the nature of this mutant SOD1 acquired adverse property remains elusive. To date, it has been proposed that mutant SOD1 cytotoxicity may involve oxidative stress, aberrant protein aggregation/interaction, mitochondrial dysfunction, apoptosis, and neuroinflammation, none of which are mutually exclusive (Bruijn et al., 2004; Strong et al., 2005; Turner and Talbot, 2008). Aside from the mechanism of toxicity, mutant SOD1 being ubiquitous, its cellular site of action is also a source of discussion. Studies support the notion that mutant SOD1, present in both MNs and non-neuronal cells, such as microglia and astrocytes, contributes to the disease process *in vivo* (Beers et al., 2006; Boillee et al., 2006; Clement et al., 2003).

As for SMA, it is the second most common autosomal-recessive disorder of infancy, often leading to death (Pearn, 1978). It is characterized by a selective loss of MNs within the anterior horns and, in some cases, the motor nuclei of the brain stem. Another pathological hallmark of SMA is glial bundle formation, predominantly in the anterior roots and in the lumbar region where MN demise and glial activation occur (Araki et al., 2003; Chou and Nonaka, 1978; Garcia-Cabezas et al., 2004). SMA is caused by homozygous deletions or rare missense mutations in the *survival MN 1* (*SMN1*) gene at chromosome 5q13 (Frugier et al., 2002; Lefebvre et al., 1995). Humans also possess a *SMN2* gene, which is almost identical to the *SMN1* gene, but produces predominantly mRNA (~90%) that lacks exon 7 leading to a truncated and unstable SMN protein (Lorson et al., 1999; Monani et al., 2000c). Therefore, SMA is caused by a loss of function of the *SMN1* gene and insufficient levels of SMN protein. There is an inverse correlation between the number of *SMN2* copies and the disease's severity (Feldkotter et al., 2002; Lefebvre et al., 1997; Parsons et al., 1998). On the basis of age of onset and clinical course, SMA is classified into three types: type I (infant most severe form), type II (toddler intermediate form) and the type III (mild juvenile form). Unlike humans, rodents have a single survival MN gene (*Smn*). Inactivation of this gene leads to early embryonic death (Schrank et al., 1997). Mice, that lack the endogenous *Smn* gene, but have two copies of a Tg human *SMN2* gene, develop a type I SMA phenotype, with a median survival of approximately six days (Monani et al., 2000a). SMA mice, that also possess an additional transgene expressing high levels of the SMN transcript lacking exon 7, have, for unclear reasons, a median survival of approximately two weeks, indicating that the truncated SMN protein can be partially

functional (Le et al., 2005). Importantly, mice, that express 8–16 copies of the *SMN2* gene, are completely rescued from the disease phenotype, thus recapitulating the relationship between *SMN2* copy number and disease severity observed in humans (Monani et al., 2000b). Although the biological roles of the SMN protein are still unclear, it is known that SMN mediates the assembly of small nuclear ribonucleoproteins essential for pre-mRNA splicing machinery (Yong et al., 2004).

Gliosis a consistent feature of neuroinflammation in motor neuron diseases

Indices of neuroinflammation are recognized pathological features of nearly all acute and chronic disorders of the CNS. In perusing the literature on neuroinflammation, readers will often encounter the term “gliosis” also sometime called “reactive astrocytosis”. Thus, before summarizing the changes in astrocytes and microglial cells in ALS and SMA, it may be worth discussing briefly the meaning of gliosis. Typically gliosis refers to scarring produced by astrocytes, which is often loosely used to define no more than increased immunoreactivity for the intermediate filament, glial fibrillary acid protein (GFAP). As stressed elsewhere (Przedborski and Goldman, 2004), the pervasive use of this term, especially in old landmark studies, makes the interpretation of non-neuronal pathological changes murky, as the limited range of techniques employed does not always allow a precise analysis of the data. For instance, on many occasions it is unclear whether gliosis, evidenced by increased GFAP immunostaining, meant simply increased stainability of the tissue, increased numbers of astrocytes, increased size of astrocytes, or a combination of all of the above. Nor is it always possible to comment on the status of other glial cells such as oligodendrocytes and microglia. In all forms of human ALS, increased GFAP immunostaining and, at least in some reports, evidence of increased labeling for microglial makers, such as ionized calcium-binding adaptor molecule 1 and macrophage antigen 1 also called CD11b, are observed in areas of MN degeneration, including the motor cortex, motor nuclei of the brainstem, the corticospinal tract and the ventral horn of the spinal cord (Ince et al., 1996; Kawamata et al., 1992). Likewise, an augmentation of astrocytic and microglial immunostaining has been reported in symptomatic mutant *SOD1* Tg mice (Almer et al., 1999; Bruijn et al., 1997; Hall et al., 1998). However, in these animals, since the brunt of the pathology is at the level of the spinal cord, little, if no information is available about glial cell alterations in other regions of the CNS. Despite this caveat of modeling, these Tg mice have provided invaluable insights into the time course of the glial response by showing that both the intensity of immunostaining and the number of immunostained spinal astrocytes and microglia seem to parallel the degree of spinal MN degeneration (Fischer et al., 2004; Hall et al., 1998). Gliosis was also reported to be associated with areas of MN degeneration in the spinal cord and brain stem in all three types of human SMA (Araki et al., 2003; Garcia-Cabezas et al., 2004; Kuru et al., 2009). In addition, in this pediatric paralytic disorder, a singular pathological feature, called glial bundles, can be observed at the level of spinal roots (Chou and Fakadej, 1971; Ghatak, 1983; Kumagai and Hashizume, 1982; Kuru et al., 2009); these glial bundles are thought to correspond to a protrusion of reactive astrocytes into the neurilemmal tubes containing degenerating myelinated axons (Ghatak, 1983). Unlike in the mutant *SOD1* model of ALS, gliosis has not been reported in any of the current experimental models of SMA, despite the facts that all, developed a paralytic phenotype and, when symptomatic, exhibit a reduced number of spinal MNs (Kariya et al., 2008; Tsai et al., 2006). Could it be that the degree of MN loss in these SMA rodent models is insufficient to trigger a noticeable glial response? This is unlikely, since investigations in mutant *SOD1* Tg animals reveal that reactive astrocytes (Fischer et al., 2004) and microglia (Henkel et al., 2006) can be detected in the anterior horn of the spinal cord quite early in the disease process, even before overt loss of MNs can be documented. Thus, subtle changes in spinal microenvironment, elicited by compromised but not yet dead neurons, may suffice to activate neighboring glial cells. Relevant to this view is the demonstration that MNs, stressed by trophic factor deprivation only (Estévez et al., 1998) or by FAS ligation

(Raoul et al., 2002), respond by producing nitric oxide (NO) and superoxide. These two can react with each other to form peroxynitrite (Beckman and Koppenol, 1996), which can trigger reactive morphological changes and GFAP and inducible nitric oxide synthase (iNOS) expression in neighboring astrocytes (Cassina et al., 2002). Similarly, microglial cells can be activated *in vitro* by the supernatant from cultured MN-like clonal cell lines deprived of trophic support (He et al., 2002). Consequently, it can be assumed that the SMA models cited-above should have shown at least some signs of a glial response within the CNS regions where neurodegeneration occurs unless, the loss of *smn* impairs the responsiveness of glial cells to pathological signals.

Astrocyte and microglia interactions

In ALS, the areas of neuroinflammation are composed of primarily astrocytes and microglia, and to a much lesser extent, T-lymphocytes. Although some have argued that alterations in the blood-brain barrier and microvasculature may occur in ALS (Apostolski et al., 1991; Garbuzova-Davis et al., 2007; Zhong et al., 2008), inflamed regions of the brain typically lack blood-borne polynuclear cells, which one would expect if the blood-brain barrier or the vascular walls were disrupted. A similar characterization of the cellular composition of the inflamed CNS regions in SMA is, to our knowledge, not available. However, it is likely the same as in ALS since, by analogy to other neurodegenerative diseases such as Parkinson's disease (Przedborski, 2007), it seems that the aforementioned cellular composition of inflamed areas is rather generic and not disease-specific. It is also remarkable to note that in the diseases of the CNS for which detailed cellular descriptions are available, the abundance and the activation of both astrocytes and microglia are consistently stressed. This suggests that astrocytes and microglia may react in concert to similar pathological signals emanating from, for example, compromised neighboring neurons. Alternatively, both microglia and astrocytes are thought to possibly influence each other; thus, the coincidence of astrocytosis and microgliosis may not be the fruit of a simultaneous, but rather a sequential activation of these two inflammatory cell populations. For instance, microglial priming can be triggered by cytokines and chemokines originating from astrocytes (Moisse and Strong, 2006). Conversely, microglia are a source of proinflammatory mediators such as cytokines and NO which can modulate astrocyte activation (von Bernhardi and Eugenin, 2004). Yet, a compelling demonstration of such cell-cell interactions is still lacking as conflicting data have been generated by using engineered animals models. A targeted ablation of proliferating microglia using CD11b-TK^{mut-30};*SOD1G93A* doubly Tg mice with the administration of the nucleoside analogue ganciclovir, has demonstrated that microgliosis reduction also reduces astrocytosis (Gowing et al., 2008). However, in mutant *SOD1* Tg mice, numerous studies using pharmacological or genetic intervention aimed at protecting MNs without directly targeting inflammation, showed that microgliosis can be reduced without any detectable effect on astrocytosis (Tanaka et al., 2008; Yamanaka et al., 2008b). And, in experiments using GFAP-TK;*SOD1G93A* mice, the administration of ganciclovir attenuated neither astrocytosis nor microgliosis (Lepore et al., 2008); these results must be taken with caution as the use of the GFAP promoter might not have been the most appropriate strategy for this work since, as indicated below, proliferating astrocytes in this ALS model originate, almost exclusively, from glial precursors cells called "NG2" for their specific expression of the NG2 chondroitin sulphate proteoglycan and not from mature GFAP-positive astrocytes. In aggregate, these data show how much remains to be elucidated about the signal triggering glial activation and the sequence in which the different inflammatory cells and mediators are recruited into play.

Gliosis, innate immunity and active role in motor neuron diseases

If gliosis is now an accepted neuropathological feature of many diseases of the brain, it should be remembered that, for many years, it was thought to be an innocent bystander. It is more

recently that the role played by gliosis in pathological situations has been recognized as not restricted to its housekeeping functions but also to include modulation of the death of neurons, especially in neurodegenerative disorders like ALS. Yet, several lines of evidence indicate that gliosis may actually exert very different effects in the diseased brain as, depending upon the situation, it may mediate either beneficial or harmful events. This novel view of gliosis is, at least in part, linked to our increased understanding of innate immunity in healthy or diseased CNS. Indeed, if innate immunity is a non-specific (antigen-independent) immune response (Moisse and Strong, 2006), it must be emphasized that microglia are the professional resident immune cells of the CNS, providing surveillance and acting as the first line of immunological defense by scavenging foreign microorganisms and compromised cells and acting as immunoeffectors, i.e. able to produce a host of inflammatory mediators. As for astrocytes, while they are the most abundant glial cells in the CNS, they are not professional immune cells but, upon activation, they can still contribute, to some extent, to the local innate immune response triggered by a variety of insults (Farina et al., 2007).

In the normal adult brain, glial cells such as microglia and astrocytes are present in a resting state. However, when the brain becomes affected by an injury or a disease, these cells quickly become activated in response to specific molecular signals. For microglia, this transition operates as a dramatic morphological transformation from a resting ramified phenotype to an active amoeboid phagocytic phenotype, through an intermediate, early activated or “primed” state (Kreutzberg, 1996). Microglial priming can be triggered by cytokines and chemokines, such as interferon γ (IFN-γ), tumor necrosis factor α (TNFα), macrophage colony stimulating factor, and granulocyte macrophage colony stimulating factor, originating from neurons and astrocytes (Moisse and Strong, 2006). Primed microglia are in an intensified local surveillance state. They exhibit thicker cell bodies and proximal processes and they express major histocompatibility class II molecules to exert their antigen-presentation role. It is only following a secondary stimulation by TNF-α or interleukins (IL) such as IL-1 and IL-6 that microglia will evolve into their maximal activation state and secrete a battery of inflammatory mediators. Aside from parenchyma microglia, CNS macrophages can supposedly also originate from infiltrating bone marrow (BM)-derived microglial precursors and blood-borne macrophages as well as from resident perivascular macrophages (Flugel et al., 2001). However, by transplanting green fluorescent protein-labeled BM cells into myelo-ablated ALS Tg mice (Lewis et al., 2009; Solomon et al., 2006) or by a systematic analysis of cellular markers expressed by reactive microglia (Gowing et al., 2008), it was found that, in mutant *SOD1* Tg mice, the principal source of spinal reactive microglia originated from parenchyma myeloid precursors and less than 10% from BM-derived precursors. Although very likely, whether a similar situation applies to SMA is unknown.

In contrast to resting microglia, resting astrocytes exert numerous roles essential for neuronal survival, function, and homeostasis (Pehar et al., 2005). For instance, quiescent astrocytes provide neurons with highly specialized and localized structural, metabolic and trophic support and participate actively in neuronal synaptic transmission. In the injured brain, astrocytes become reactive and respond to pro-inflammatory cytokines with an increase in proliferation and a dramatic morphological change. In this glial reaction, astrocytes harbor a typically reactive morphology with hypertrophic nuclei and with cell bodies that develop numerous processes highly immunopositive for GFAP. They also exhibit robust vimentin, glutamine synthetase and calcium binding protein, S-100β immunopositivity and express a host of inflammatory markers such as iNOS (Almer et al., 1999) and cyclooxygenase 2 (COX-2) (Maihofner et al., 2003). In mutant *SOD1* Tg mice, the major source of reactive, proliferating astrocytes do not derive from mature GFAP-positive astrocytes, but from NG2 glial precursor cells (Gowing et al., 2008; Lepore et al., 2008). Such dependence on precursors to provide a pool of proliferating glial cells seems to be stronger for astrocytes than for microglia, as for

the latter evidence of dividing, mature microglia has been documented (Gowing et al., 2008; Lepore et al., 2008).

The Janus face of neuroinflammation and its contribution to ALS and SMA

As mentioned above, neuroinflammation may mediate both beneficial and harmful effects within diseased area of the CNS (Wyss-Coray and Mucke, 2002). The purpose of the present review, is to highlight the neuroprotective and deleterious effects of reactive or diseased glial cells in particular in ALS and SMA. These main dual effects of microglia, astrocytes and glia of undetermined type are summarized in Table 1, Table 2, and Table 3, respectively.

Among beneficial phenomena, it can be mentioned that reactive microglia may enhance neuronal repair with controlled-phagocytosis via the removal of pre-synaptic axon terminals from damaged neurons, a process called synaptic stripping (Moisse and Strong, 2006). In the inflamed brain, reactive astrocytes lead to the formation of a glial scar which functions as a physical barrier isolating the injured area to limit the spread of damage, even if it was shown to impede axonal re-growth (Dong and Benveniste, 2001). However, other data show that reactive astrocytes can serve as a permissive substratum for axonal regeneration. If resting astrocytes produce low levels of nerve growth factor (NGF), reactive astrocytes have been reported to produce significantly larger amounts (Strauss et al., 1994; Wu et al., 1998) and NGF was shown to induce axonal sprouting *in vivo* (Chalmers et al., 1996). Furthermore, regenerating axons expressing the adhesion molecule L1 can be supported by reactive astrocytic processes expressing NCAM. Astrocytes also synthesize molecules involved in axonal elongation such as proteoglycans, including heparan-, chondroitin- and keratan-sulfate (Mckeon et al., 1995). These beneficial effects of reactive glial cells may be relevant to damaged CNS areas in ALS as post-lesion sprouting of corticospinal axons within the adult spinal cord was observed in the presence of gliosis (Li and Raisman, 1995). From a neuroprotection perspective, both activated microglia and astrocytes can preserve neuronal survival and CNS tissue through the release of anti-inflammatory cytokines, neurotrophins and growth factors (Dong and Benveniste, 2001; Moisse and Strong, 2006). For instance, microglial cells can produce brain-derived neurotrophic factor (Nakajima et al., 2002) and can stimulate astrocytes to eliminate extracellular excitotoxic glutamate during an inflammatory response (Zhao et al., 2004). Although insulin growth factor (IGF)-I is only detected in neurons in the intact CNS, it is induced in reactive astrocytes, suggesting a role for IGF-1 in the astroglial response to injury, possibly to protect neurons from delayed post-lesion death (Garcia-Estrada et al., 1992). In human ALS tissue, rod-like activated microglia were evidenced to express IGF-II and the pro-neuronal survival leukemia inhibitory factor in the vicinity of MNs expressing their receptors (Kihira et al., 2007), suggesting a protective effect of reactive microglia.

Conversely, in many pathological situations, the glial response has also been implicated in harmful events (Gonzalez-Scarano and Baltuch, 1999; Ridet et al., 1997). Supporting a detrimental role for activated glial cells in ALS are the studies showing that the chronic administration of lipopolysaccharide or the deletion of the receptor for the chemokine, fractalkine, is associated with a robust astrocytosis and microgliosis and an exacerbated ALS-like phenotype in mutant *SOD1* Tg mice (Cardona et al., 2006; Nguyen et al., 2004). Likewise, *in vitro*, it has been shown (Cassina et al., 2002) that, once activated, astrocytes become capable of killing even previously healthy neighboring MNs. After activation, glial cells can embark on the production of a host of toxic molecules (Kreutzberg, 1996; Wyss-Coray and Mucke, 2002) which are the mediators of the glial noxious action on neighboring neurons. Among these, one key mechanism by which activated-glial cells can cause tissue damage is through oxidative injury to macromolecules caused by a variety of reactive oxygen species (ROS) and NO (Klebanoff, 1992; MacMicking et al., 1997). Relevant to this are the demonstrations that

many markers of oxidative damage are enhanced in ALS spinal cord (Bogdanov et al., 1998; Ferrante et al., 1997; Pedersen et al., 1998) and, although none of these studies demonstrate that the observed oxidative damage in ALS originates from activated glial cells, they do provide major impetus for this hypothesis. The pathological consequences of glial-derived oxidants can be multiple. For instance, by damaging plasma membrane lipids and proteins, ROS can compromise the integrity of neighboring cells; they can oxidize proteins of particular importance to MN survival, such as the IGF-1 receptor (Wu et al., 2006); they can alter the redox status of intraneuronal signaling proteins such as peroxiredoxines; and finally, they can promote excitotoxicity to neighboring neurons by damaging the glutamate uptake site EAAT2/GLT-1 present on astrocytes (Trotti et al., 1998). In SMA, several markers of oxidative injury were also reported to be associated with the inflammatory and neurodegenerative processes. In SMA type II, it was shown that gliosis in the spinal cord and in the brainstem is associated with a severe reduction of MN number and that oxidative stress-related products are deposited into the remaining atrophic MNs (Araki et al., 2003). In contrast to ALS, the expression of glial glutamate transporters in SMA cases was not altered or oxidatively damaged (Hayashi et al., 2002).

Factors, such as the pro-inflammatory cytokines, IL-6 and IL-1β, may also mediate some of the glial-related deleterious effects via activation of the apoptotic machinery (Pettmann and Henderson, 1998). We (Kostic et al., 1997; Li et al., 2000; Vukosavic et al., 1999) and others (Pasinelli et al., 1998) have found evidence to this effect. It should be mentioned that IL-6 and IL-1β are elevated in the cerebrospinal fluid (Sekizawa et al., 1998) and spinal cord (Kostic et al., 1997) of ALS patients. Increased levels of IL-1β, TNFα and several other pro-inflammatory factors have also been described in the spinal cords of Tg mutant *SOD1* mice, even before MN loss (Elliott, 2001; Hensley et al., 2002; Yoshihara et al., 2002). Further, the inhibition of either IL-1β activation or TNFα binding in Tg mutant *SOD1* mice (Friedlander et al., 1997; West et al., 2004) is associated with some clinical benefit. Previously, in Tg *SOD1G93A* mice, our group demonstrated that, at the early symptomatic stages, microgliosis development was associated with an increase in pro-inflammatory cyclooxygenase-2 (Cox-2) mRNA and protein, and an augmentation in prostaglandin E2 (PGE2) content in the region of MN pathology (Almer et al., 2001), confirming further a role for microglial activation in MN pathology. The Cox-2 alterations were not restricted to the mutant *SOD1* mouse model of ALS as similar changes were found in spinal cord tissues from sporadic ALS patients (Almer et al., 2001). Along this same line, a genetic deletion of the PGE2 receptor, which is induced significantly in astrocytes and microglia in the *SOD1G93A* ALS mouse, has been shown to decrease pro-inflammatory effectors such as Cox-1, Cox-2 and iNOS, to improve motor strength and to extend animal survival (Liang et al., 2008). This latter study reinforces the view that PGE2 signaling represents one of the pathways through which gliosis is detrimental in the ALS pathogenic process. Interestingly, it has also been shown that contents of D-serine, which can play the role of a NMDA receptor co-agonist, and its producing enzyme are increased within activated astrocytes from spinal cord of mutant *SOD1* Tg mice and ALS patients (Sasabe et al., 2007). More importantly, the authors demonstrate that D-serine can potentiate, in a dose dependent manner, NMDA toxicity to cultured MNs (Sasabe et al., 2007). Unexpectedly, it was also found that trophic factors such as NGF, while known to support neuronal survival and to be secreted by activated astrocytes, can, in the presence of low NO concentrations, induce apoptosis in MNs by a $p75^{NTR}$-dependent mechanism (Pehar et al., 2004).

To date, the involvement of inflammatory pathways in SMA remains ambiguous. Cardiotrophin-1 (CT-1), a cytokine belonging to the IL-6 family and shown to delay motor axonal degeneration in ALS Tg mice (Bordet et al., 2001) was tested in a mouse model of SMA in which the *smn* gene was selectively deleted in neuronal cells (Lesbordes et al., 2003). Intramuscular administration of adenoviral vectors expressing CT-1 (AdCT-1) to SMA mutant mice increased survival and improved motor performance and was associated with a marked

reduction in abnormal neuromuscular junctions (Bordet et al., 1999). This attenuation of the SMA phenotype by CT-1 indicates that some cytokines are able to delay the neurodegenerative process in SMA. On the other hand, the neuronal apoptosis inhibitory protein (NAIP) gene is located in a region adjacent to the *smn1* gene, and is deleted along with *smn1* in 50% of type I SMA and 10–20% of type II and III patients (Roy et al., 1995). There is such a strong correlation between *NAIP* deletion and disease severity that the *NAIP* gene is often referred to as a SMA-modifying gene. NAIP is a member of the inhibitor of apoptosis (IAP) protein family, of which several members interact with the TNF-α receptor binding proteins (e.g. TRAF-1 and TRAF-2) to block cell death (Rothe et al., 1996). In neuronally-differentiated PC12 cells, over-expression of NAIP prevented apoptotic death induced by NGF withdrawal or TNF-α receptor activation (Gotz et al., 2000). It is known that the TNF-α pathway contributes to neuroinflammation through a number of different mechanisms, as well as being a key regulator of cell death. In SMA, there appears to be an inverse relationship between disease severity and activation of the TNF-α pathway. For example, in SMA type I (most severe) compared to normal controls (Millino et al., 2009), p38 kinase, as well as the MAP kinase interacting serine/threonine kinase (MNK2) are down-regulated in muscle. The pro-inflammatory cytokine, IL-32, which stimulates TNF-α and p38, is also down-regulated in these patients compared to controls and a negative upstream regulator of a p38-kinase, MEKK5, is increased in SMA type I samples. Conversely, in the mildest form of the disease, type III, transcript levels of these genes show up-regulation compared to control samples; e.g. p38 expression is increased (Millino et al., 2009). In contrast to an enhancement of this pathway in milder cases, these findings point to a decrease in inflammatory signaling in the most severe forms of the disease. Systemic overexpression of IL-3 results in a MN disease (Chavany et al., 1998) in which neuronal vacuolizations and astrocytosis were found in the brainstem and spinal cord. The observed degeneration involved primarily the lower MNs of the lumbar spinal cord, which resembles SMA rather than ALS. Thus, the *in vivo* overproduction of IL-3 may be a contributing factor to the generation of MN disease.

Glia-mediated non-cell autonomous component to the neurodegenerative process

All of the aforementioned data do provide insights into how secondary inflammatory-related events can positively or negatively modulate the progression and propagation of MN degeneration. However, they do not inform us about whether astrocytes and microglia can initiate the disease *per se*. Although evidence for such a non-cell autonomous mechanism for MN degeneration in SMA is lacking, mounting evidence support the notion that, at least in the mutant *SOD1* form of ALS, the expression of mutant SOD1 in non-neuronal cells, and especially in glial cells, may trigger the disease phenotype in neighboring MNs (Beers et al., 2006; Boillee et al., 2006; Clement et al., 2003). A study done in chimeric mice, involving cells expressing either wild-type or mutant SOD1 (Clement et al., 2003), revealed that wild-type MNs surrounded by mutant non-neuronal cells acquire ubiquitin-positive protein aggregates, a sign of neuronal damage in this ALS model. Results from studies in other kinds of chimeras made of both mutant SOD1 MNs/oligodendrocytes and a mixture of wild-type and mutant SOD1 non-MN cells (Yamanaka et al., 2008a) strengthen further the notion that mutant SOD1 in cell types other than MNs and oligodendrocytes may contribute to the initiation of the MN degenerative process in this ALS model.

Contribution of microglia expressing mutant SOD1 to the disease process

Regarding the specific involvement of microglia in this non-cell autonomous paradigm, it has been shown that the selective lowering of mutant SOD1, in either MNs or in microglia with a Cre-Lox system, prolongs survival of Tg *SOD1G37R* mice compared to their germline littermates, with a more pronounced effect for microglia (99 days vs. 64 days) (Boillee et al.,

2006). Similarly, Beers et al. (2006) have found that mutant mice, originally lacking in microglia due to a defect in the transcription factor *PU.1* and carrying the *SOD1G93A* mutation, then reconstituted by the transplantation of wild-type bone marrow cells, have a longer lifespan than those transplanted with *SOD1G93A* bone marrow cells. In contrast to the study of Boillee et al., here only microglia devoted of mutant SOD1 are present in the CNS and MN protection is associated with a reduction in microgliosis as attested to by a decrease in CD68/CD11b immunostaining. Further, they showed that *PU.1*-deficient mice, carrying the normal SOD1 allele and transplanted with bone marrow from mutant *SOD1G93A* mice, did not develop MN disease. Even though the CNS here is populated with mutant SOD1 microglia, this experiment demonstrates that the expression of mutant SOD1 in microglia is not sufficient to induce the disease *in vivo*, assuming that the correct density of mutant SOD1 expressing microglia in the spinal cord was achieved. Nonetheless, altogether these results support the importance of the toxic role played by microglial cells expressing mutant SOD1 in the ALS neurodegenerative process and show that both reactive and resting wild-type microglia may contribute to the expression of the disease's phenotype.

Several mechanisms have been proposed for mutant SOD1 expression-mediated microglial toxicity. Cultured primary or clonal microglia expressing mutant SOD1 (Beers et al., 2006; Liu et al., 2009; Weydt et al., 2004) were shown to have increased cytotoxic potential due to an enhanced secretion of TNF-α, NO and superoxides, and a decreased production of neuroprotective IL-6. Although Beers et al. (2006) report a slight increase in NO and superoxide release under basal conditions in SOD1G93A versus wild-type microglial cultures, both Lui et al. (2009) and Weydt et al. (2004) have found that this toxic potential is only revealed following LPS exposure or toll-like receptor 2 ligation. In agreement with the later studies, we did not observe any significant increase in NO in SOD1G93A microglial cultures (unpublished data) and medium conditioned for 7 days with these microglia did not cause any overt degeneration of primary or embryonic stem (ES) cell-derived MNs (Nagai et al., 2007). On the other hand, intracellular mutant SOD1 presumably disrupts the redox sensitive Rac regulation of NOX, thereby, causing excessive NOX activation in Tg mouse tissues and in cell lines expressing mutant SOD1 (Harraz et al., 2008). Relevant to this observation, we have reported that NOX is activated in spinal cords from sporadic ALS patients and from *SOD1G93A* mice and that its abrogation extends the survival of mutant *SOD1* Tg mice (Wu et al., 2006).

Extracellular mutant SOD1 was also found to be toxic through its chromagraninmediated secretion (Urushitani et al., 2006). Once in the extracellular milieu in mixed embryonic primary cultures, mutant SOD1 can trigger the activation of microglia directly, leading to the death of MNs. Of note, an intracerebral injection of mutant SOD1 recombinant in adult mice did trigger an inflammatory response, but not neurotoxicity (Kang and Rivest, 2007). In the latter study, it was also reported that extracellular mutant SOD1 protein did activate microglia through a myeloid differentiation factor 88(Myd88)-dependent signaling pathway. Moreover, these authors demonstrated that *SOD1G37R* Tg mice, chimeric for or transplanted with Myd88-deficient BM cells, exhibit a more pronounced gliosis and a reduced lifespan. This study further exemplifies how complex the regulation of the microglial phenotype is in pathological situations like ALS.

Contribution of astrocyte expressing mutant SOD1 to the disease process

Tg mice expressing mutant *SOD1*, driven by the astrocytic promoter GFAP, exhibit a subtle pathological phenotype characterized by gliosis, but no overt MN degeneration (Gong et al., 2000). This experiment speaks against the ability of astrocytes expressing mutant SOD1 to trigger the MN degenerative process. However, without knowing the percentage of astrocytes present expressing mutant SOD1 or the level of expression of this toxic protein per astrocyte,

this elegant study does not allow one to conclude whether or not mutant astrocytes can provoke MN degeneration. In contrast, Wang et al. (2005) reported that mice Tg for *SOD1G37R* cDNA, driven by a mouse prion promoter, had the highest CNS expression of the transgene in both neurons and astrocytes, and developed MN disease. Yamanaka et al. (2008b) on other hand elected to mate Tg Lox*SOD1G37R* mice with Tg GFAP-Cre-LacZ mice and found that, upon reducing mutant SOD1 in GFAP-positive cells, which included not only reactive astrocytes, but also Schwann cells and oligodendrocytes (Imura et al., 2003), the survival of these animals was extended. The reduction of mutant SOD1 in GFAP-positive cells did not modify the degree of astrocytosis but did reduce microgliosis and iNOS activation in microglia. This suggests that astrocyte expression of mutant SOD1 influences, in some way, the microglial response.

We and others (Di Giorgio et al., 2007; Nagai et al., 2007; Vargas et al., 2006) found that primary or ES-derived MNs die when co-cultured with astrocytes expressing mutant SOD1. These studies argue for mutant astrocytes being able to transmit the disease phenotype to wild-type MNs. There are two schools of thought on how mutant SOD1 astrocytes can achieve this. The first one, which includes Van Damme and collaborators, posits that mutant astrocytes are defective in mediating a beneficial effect, e.g. proper regulation of GluR2 AMPA subunit expression (Van Damme et al., 2007). The second one, which includes the authors of the following studies (Di Giorgio et al., 2007; Marchetto et al., 2008; Vargas et al., 2006) and ourselves, think that mutant SOD1 astrocytes exert toxic effects. Based on our ongoing, unpublished investigations, it appears that the effects of mutant astrocytes on wild-type MNs are mediated by a released, soluble toxic factor. Our cultured mutant astrocytes do not differ morphologically from their wild-type counterparts. Yet, mutant SOD1 astrocytes were reported by others to have elevations in both basal and TNF-α-stimulated levels of the proinflammatory PGE2 as well as leukotriene B4, iNOS and NO, and protein oxidation products (Hensley et al., 2006). Specific cytokine- and TNF-α death-receptor-associated components are also upregulated in these cultured mutant astrocytes. In a co-culture model composed of human ES-derived MNs exposed to rodent astrocytes, a gene array screen revealed that PGD2 receptor expression was increased in mutant SOD1 astrocytes and an antagonist to this receptor was protective (Di Giorgio et al., 2008). In a similar model using both ES-derived MNs with primary astrocytes of human origin, others (Marchetto et al., 2008) reported an inflammatory response in mutant SOD1 astrocytes that was characterized by an increase in NOX-2 expression. Here, the authors showed that inhibiting NOX with 300 μM apocynin was protective to the MNs. Three different forms of NGF potentially produced by reactive astrocytes were reported to induce MN degeneration by a $p75^{NTR}$-dependent mechanism: pro-NGF (Domeniconi et al., 2007), β-NGF (in cooperation with NO) (Pehar et al., 2004), and NGF oxidized by peroxynitrite (Pehar et al., 2006). Collectively, the above findings suggest that mutant SOD1-expressing astrocytes, at baseline, may be intrinsically not quiescent and more prone to enter an activated neuroinflammatory state compared to their wild-type counterparts. We already know from our previous study (Nagai et al., 2007) that glutamate is an unlikely candidate, as its levels were not increased in our Tg co-culture model and the use of the AMPA/kainate receptor antagonist CNQX, failed to protect MNs. Other excluded suspects include IL-1β, IL-6, INF-γ and TNF-α, as levels of these were either non-detectable or similar between mutant and wild-type astrocyte culture medium.

Conclusions and potential therapeutic strategies targeting inflammation in ALS and SMA

To date, only a few approved treatments, such as mechanical ventilation and riluzole, do prolong survival, to some extent, in ALS patients. From the above, it has emerged that several glial-derived targets may be considered for therapy. In keeping with this view, over the past several years, diverse strategies aimed at targeting a single neuroinflammatory event or

mediator have been tested in ALS Tg mice and have yielded some positive outcomes in these animals (Beers et al., 2006; Beers et al., 2008; Drachman et al., 2002; Kang and Rivest, 2007; Kiaei et al., 2005; Klivenyi et al., 2004; Kriz et al., 2002; Wu et al., 2006; Zhu et al., 2002). However, so far, the few that have been tested clinically have not shown significant benefit in patients. While many explanations can be formulated, it should be remembered that, as illustrated above, neuroinflammation is a multifaceted phenomena. Thus, the likelihood of providing benefit in patients by targeting a single factor is low. Even more important is the observation that studies targeting the ablation of proliferating microglia (Gowing et al., 2008) have generated confusing results, perhaps because preventing microgliosis may abrogate both the beneficial and the detrimental effects exerted by these immune cells upon their activation. Thus, the recognition that glial cells may have a dual nature and can adopt a variety of different phenotypes with opposite effects suggests that glial cell ablation may not be the correct avenue to take for the treatment of neurodegenerative disorders. Over the years, insights into the molecular signaling pathways involved in glial phenotype have been revealed. It is thus tempting IPT to suggest that perhaps the most promising therapeutic avenue focused on neuroinflammation would be to devise strategies able to harmonize and shift the glial phenotype from pro-inflammatory cytotoxic to anti-inflammatory cytoprotective. However, prior to embarking on this path, it is essential to ascertain the immune system of ALS patients to assure that immunomodulation is applicable. This last cautionary remark is prompted by the demonstration of a severe defect in the immune system of *SOD1G93A* mice (Banerjee et al., 2008) making this model, unless shown otherwise, improper for such an approach and whether the same issue applies to humans remains to be demonstrated.

Regarding SMA, currently, there is no treatment, although clinical trials exploring the effectiveness of inhibitors of histone deacetylases (HDAC) have shown some promise. Relevant to this review is the fact that HDAC inhibitors such as valproic acid, sodium butyrate, or trichostatin A were reported to reduce the proliferation and activation of microglia in different *in vivo* brain injury models (Kim et al., 2007; Zhang et al., 2008). *In vitro*, the same inhibitors prevented microglia-derived pro-inflammatory toxicity to dopamine neurons induced by LPS (Chen et al., 2007; Peng et al., 2005). Given these findings, it is tempting to speculate that one possible mechanism for the beneficial effects conferred by HDAC inhibition in SMA models is through an anti-inflammatory action. However, in light of the presence of limited markers of inflammation in SMA human tissue, and none in animal models of the disease, this possibility may be far-fetched.

Acknowledgments

The authors wish to thank Dr. Jackson-Lewis for her comments, advice, and assistance in preparing this manuscript. This study is supported by Muscular Dystrophy Association/Wings-over-Wall Street, US National Institutes of Health NS042269, NS062180, NS064191, NS38370, NS11766, AG 21617 and NS062055, US Department of Defense Grant DAMD 17-03-1, W81XWH-08-1-0522, and W81XWH-08-1-0465, the Hartman Foundation and the Parkinson's Disease Foundation. A.J. is the recipient of a post-doctoral fellowship from the Association Francaise contre les Myopathies (AFM).

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Table 1

Summary of neuroprotective and deleterious effects of reactive or diseased microglia highlighted in this review.

MICROGLIA	
Neuroprotective effects	**Deleterious effects**
Enhancement of neuronal repair (i.e. synaptic stripping) by reactive microglia (Moisse and Strong, 2006) Release of anti-inflammatory cytokines, neurotrophins (Dong and Benveniste, 2001; Moisse and Strong, 2006), and growth factors (such as BDNF) (Nakajima, 2002) Specific to ALS: Expression of IGFII and pro-neuronal survival LIF P12 (Kihira, 2007)	Specific to ALS: Production of toxic molecules (Kreutzber, 1996; Wyss-Coray and Mucke, 2002), and induction of oxidative stress (Klebanoff, 1992; MacMicking, 1997) Increase of pro-inflammatory Cox-2 mRNA and protein, and augmentation of PGE2 in the vicinity of MN pathology (Almer, 2001) Enhanced secretion of TNF-α, NO and superoxides, and a decreased production of neuroprotective IL-6 (Beers, 2006; Liu, 2009; Weydt, 2004) Indution of PGE2 signaling associated with Cox-1, Cox-2 and iNOS (Liang, 2008) Specific to SMA: Induction of oxidative stress (Araki, 2003)

Table 2

Summary of neuroprotective and deleterious effects of reactive or diseased astrocytes highlighted in this review.

ASTROCYTES	
Neuroprotective effects	**Deleterious effects**
Formation of a glial scar isolating physically the injured area. (Dong and Benveniste, 2001) Permissive substrate for axonal regeneration (by expression of NCAM, proteoglycans) (Mckeon, 1995) Release of NGF that induces axonal sprouting (in vitro: Strauss, 1994; Wu, 1998/ in vivo: Chalmers, 1996) Release of anti-inflammatory cytokines, neurotrophins, growth factors (IGF1) (Dong and Benveniste, 2001; Moisse and Strong, 2006) Release of IGF (Garcia-Estrada, 1992)	Impediment of axonal regeneration by glial scar (Dong and Benveniste, 2001) Specific to ALS: Production of toxic molecules (Kreutzber, 1996; Wyss-Coray and Mucke, 2002), and induction of oxidative stress (Klebanoff, 1992; MacMicking, 1997) Release by mutant SOD1 astrocytes of an unknown toxic factor(s) that selectively kills motor neurons (Di Giorgio, 2007; Nagai, 2007; Vargas, 2006) Elevations in both basal and TNF-α stimulated levels of proinflammatory PGE2, leukotriene B4, iNOS and NO, and protein oxidation products in mutant SOD1 astrocytes (Hensley, 2006), upregulated cytokine- and TNF-alpha death-receptor-associated components Induction of motor neuron degeneration by activated astrocyte producing pro-NGF (Domeniconi, 2007), Beta-NGF in cooperation with NO (Pehar, 2004) or NGF oxidized by peroxynitrite (Pehar, 2006) in a p75-NTR – dependent mechanism Increase in the astrocyte production of D-serine, an NMDA receptor co-agonist. (Sasabe, 2007) Indution of PGE2 signaling associated with Cox-1, Cox-2 and iNOS (Liang, 2008) Specific to SMA: Induction of oxidative stress (Araki, 2003)

Table 3

Summary of neuroprotective and deleterious effects of reactive or diseased glia of undetermined type highlighted in this review.

UNDETERMINED GLIAL CELLS	
Neuroprotective effects	**Deleterious effects**
Specific to SMA: Release of cytokines delaying neurodegerative process (CT1) (Bordet, 2001)	Release of pro-inflammatory cytokines (IL6, IL1β, TNFα) activating apoptotic machinery (Pettmann and Henderson, 1998; Kostic, 1997; Li, 2000; Vukosavic, 1999; Pasinelli, 1998)