Unraveling the Mechanisms Involved in Motor Neuron Degeneration in ALS

Lucie I. Bruijn,[1] Timothy M. Miller,[2] and Don W. Cleveland[2]

[1]*The ALS Association, Guilford, Connecticut 06437; email: lbruijn@snet.net*
[2]*Departments of Medicine and Neurosciences and the Ludwig Institute for Cancer Research, University of California at San Diego, La Jolla, California 92093; email: dcleveland@ucsd.edu; timiller@ucsd.edu*

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■ **Abstract**   Although Charcot described amyotrophic lateral sclerosis (ALS) more than 130 years ago, the mechanism underlying the characteristic selective degeneration and death of motor neurons in this common adult motor neuron disease has remained a mystery. There is no effective remedy for this progressive, fatal disorder. Modern genetics has now identified mutations in one gene [Cu/Zn superoxide dismutase (SOD1)] as a primary cause and implicated others [encoding neurofilaments, cytoplasmic dynein and its processivity factor dynactin, and vascular endothelial growth factor (VEGF)] as contributors to, or causes of, motor neuron diseases. These insights have enabled development of model systems to test hypotheses of disease mechanism and potential therapies. Along with errors in the handling of synaptic glutamate and the potential excitotoxic response this provokes, these model systems highlight the involvement of nonneuronal cells in disease progression and provide new therapeutic strategies.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS), commonly known in the United States as Lou Gehrig's disease, is the most common adult motor neuron disease. Described in 1869 by the great French neurobiologist and physician Jean-Martin Charcot, the disease's primary hallmark is the selective dysfunction and death of the neurons in the motor pathways. This leads to spasticity, hyperreflexia (upper motor neurons), generalized weakness, muscle atrophy, and paralysis (lower motor neurons) (Mulder et al. 1986). Failure of the respiratory muscles is generally the fatal event, occurring within one to five years of onset. Selectivity of killing occurs even among motor neurons: The neurons that control the bladder (i.e., Onuf's nucleus in the sacral cord) and eye movements are relatively spared.

Although approximately 5–10% of ALS cases are inherited (familial), the majority of cases have no genetic component (sporadic). The typical age of onset

for both forms is between 50 and 60 years. The lifetime risk is approximately 1 in 2000. This corresponds to ~30,000 affected individuals in the United States and ~5000 in the United Kingdom. Motor neuron loss is accompanied by reactive gliosis (Leigh & Swash 1991), intracytoplasmic neurofilament abnormalities, and axonal spheroids (Carpenter 1968, Gonatas et al. 1992, Hirano et al. 1984, Leigh et al. 1991). In end-stage disease, there is significant loss of large myelinated fibers in the corticospinal tracts and ventral roots as well as evidence of Wallerian degeneration and atrophy of the myelinated fibers (Delisle & Carpenter 1984).

The causes for most cases of ALS are unknown and the clinical course is highly variable, suggesting that multiple factors underly the disease mechanism. This review focuses on the proposed mechanisms involved in ALS, why motor neurons are a key target in the disease, and how other cell types may contribute to disease.

The Genetics of Motor Neuron Disease

ALS is a member of a group of heterogeneous disorders that affect the survival and function of upper or lower motor neurons. For some of these disorders, a genetic link has been determined (Table 1). Approximately 10% of classical ALS is familial. Among the familial cases, approximately 20% are caused by dominantly inherited mutations in the protein Cu/Zn superoxide dismutase (SOD1) (Rosen et al. 1993). The striking pathological and clinical similarity between familial and sporadic disease has sparked enthusiasm that the animal models based on mutant SOD1 might provide insight into mechanisms of both sporadic and familial disease. However, to date, there is no direct evidence validating this assumption.

Using modern gene mapping methods, researchers have identified a new gene linked to a rare, recessively inherited juvenile or infantile onset form that progresses slowly (Hadano et al. 2001, Yang et al. 2001). The gene, localized to chromosome 2, encodes a 184-kD protein (named ALS2 or alsin) with three putative guanine-nucleotide-exchange factor (GEF) domains. Small GTP-binding proteins of the Ras superfamily act as molecular switches in signal transduction, affecting cytoskeletal dynamics, intracellular trafficking, and other important biological processes. GEFs catalyze the dissociation of the tightly bound GDP from the small G protein in response to upstream signals. Although widely expressed, the ALS2 protein is enriched in nervous tissue, where it is peripherally bound to the cytoplasmic face of endosomal membranes, an association that requires the amino-terminal RCC1-like GEF domain (Yamanaka et al. 2003). The G protein(s) upon which the ALS2 GEFs act has not been identified, although an initial report has shown that ALS2 can act in vitro as an exchange factor for Rab5a (Otomo et al. 2003), which functions in endosomal trafficking. All of the disease-causing mutants are highly unstable (Yamanaka et al. 2003): This has led to the conclusion that early-onset motor neuron disease is caused by loss of activity of one or more of the GEF domains of this endosomal GEF.

TABLE 1 Genetics of ALS-related diseases

Disease	Inheritance	Linkage	Gene/protein	Onset	Features
Sporadic ALS	None	None	Largely unknown, ?NF-H, ?EAAT2	Adult	>90% of ALS
Familial ALS					
ALS	Dominant	21q22.1	SOD1	Adult	20% of inherited cases; more than 90 mutants known, all but one of which are dominant (Rosen et al. 1993)
ALS	Dominant	16	?	Adult	Typical adult onset, familial ALS without SOD1 mutations (Abalkhail et al. 2003, Ruddy et al. 2003, Sapp et al. 2003)
ALS	Dominant	18	?	Adult	Typical adult onset, familial ALS without SOD1 mutations (Hand et al. 2002, Sapp et al. 2003)
ALS	Dominant	20	?	Adult	Typical adult onset, familial ALS without SOD1 mutations (Sapp et al. 2003)
ALS with frontotemporal dementia	Dominant	9q21-22	?	Adult	Dementia (Hosler et al. 2000)
ALS with dementia, Parkinsonism	Dominant	17q21	Tau	Adult	Dementia > Parkinsonism, amotrophy (Wilhermsen 1997)
ALS	X-linked	Xp11-Xq12	?	Adult	Slowly progressing (Hentati et al. 1998)
Juvenile-type 1	Recessive	15q15-22	?	Adolescence	
Juvenile-type 3	Recessive	2q33	ALS2	Adolescence	Slowly progressing; mutant gene product appears to be a guanine exchange factor (GEF) (Hadano et al. 2001, Yang et al. 2001)
Juvenile	Dominant	9q34 (ref 84)	?	Before 25	Slowly progressing (Blair et al. 2000)

Efforts to find new genes linked to the remainder of familial ALS cases continue. The identification of three separate families with linkage to chromosome 16 (Abalkhail et al. 2003, Ruddy et al. 2003, Sapp et al. 2003) has significantly narrowed the region of interest, and rapid identification of the gene involved is anticipated. In addition, efforts to identify disease genes for chromosomes 18 and 20 are under way (Table 1). Researchers have just begun to identify other genetic contributors, including one dominant locus on mouse chromosome 13 that can sharply slow initiation of SOD1 mutant mediated disease (Kunst et al. 2000).

Mechanisms of Selective Motor Neuron Death

There are many animal models of motor neuron degeneration, ranging from chronic aluminum toxicity in rodents to spinal muscular atrophy in cows. Because each of these models may provide new, important information about the motor neuron, they may be relevant to ALS. However, given space constraints, the disease mechanism part of this review focuses almost exclusively on rodent models of mutations in SOD1. This bias is based on the phenotypic and pathologic correlation between sporadic and familial human SOD1 ALS cases and the close resemblance of the mouse model to human disease (discussed below).

A TOXIC PROPERTY FROM CU/ZN SOD1 MUTATIONS

The best-known function of SOD1, a homodimer of an ubiquitously expressed 153–amino acid polypeptide, is to convert superoxide, a toxic by-product of mitochondrial oxidative phosphorylation, to water or hydrogen peroxide. Catalysis by SOD1 is mediated in two asymmetric steps by an essential copper atom, which is alternately reduced and oxidized by superoxide. The disease-causing mutations are scattered throughout the primary and three-dimensional structure of the protein. More than 100 mutations are known (Andersen 2000, Andersen et al. 2001, Gaudette et al. 2000), and all but one mutation, SOD1^{D90A} (aspartate substituted to alanine at reside 90), cause dominantly inherited disease. An updated list of the mutations can be found at the online database for ALS genetics, http://www.alsod.org.

How mutant SOD1 leads to motor neuron degeneration remains unclear. However, it is well established that SOD1-mediated toxicity in ALS is not due to loss of function but instead to a gain of one or more toxic properties that are independent of the levels of SOD1 activity. The main arguments against the importance of loss of dismutase function are that (*a*) SOD1 null mice do not develop motor neuron disease (Reaume et al. 1996) and (*b*) removal of the normal SOD1 genes in mice that develop motor neuron disease from expressing a dismutase inactive mutant (SOD1^{G85R}) does not affect onset or survival (Bruijn et al. 1998). In addition,

levels of SOD1 activity do not correlate with disease in mice or humans; in fact, some mutant enzymes retain full dismutase activity (Borchelt et al. 1994, Bowling et al. 1995). Finally, chronic increase in the levels of wild-type SOD1 (and dismutase activity) either has no effect on disease (Bruijn et al. 1998) or accelerates it (Jaarsma et al. 2000).

Of the more than 100 mutations in humans, 3 ($SOD1^{G85R}$, $SOD1^{G37R}$, and $SOD1^{G93A}$) have been extensively characterized in transgenic mouse models of ALS (Bruijn & Cleveland 1996, Gurney 1994, Ripps et al. 1995, Wong et al. 1995). In these mice, the mutant human protein is ubiquitously expressed (under control of the human or mouse SOD1 gene promoter) at levels equal to or several fold higher than the level of endogenous SOD1. Unlike the variable pattern of weakness in humans, weakness typically starts in the hind limbs in mice between 3 and 12 months of age, depending on both the mutation and the level to which it is expressed. Hind limb weakness coincides with increased astrogliosis, activation of microglia, and loss of spinal cord motor neurons. Pathology in these mice closely mimics many aspects of the human disease.

A feature common to all examples of SOD1 mutant-mediated disease in mice is prominent ubiquitin-positive, intracellular aggregates of SOD1 in motor neurons and astroctyes (Figure 1). Vacuolar pathology that at least partially represents damaged mitochondria is seen in motor neurons of mice or rats expressing high levels of $SOD1^{G93A}$ (Dal Canto & Gurney 1994, Jaarsma et al. 2001, Howland et al. 2002) and $SOD1^{G37R}$ (Wong et al. 1995), but it is not seen in disease from other mutants (Bruijn et al. 1997b, Nagai et al. 2001). This may be due to the higher (by as much as 20-fold) accumulated levels of the catalytically active $SOD1^{G93A}$ and $SOD1^{G37R}$ mutants, as compared with the inactive $SOD1^{G85R}$, which are required to provoke disease in mice. This difference is even more provocative for the frameshift mutation $SOD1^{G127X}$ truncated in the last 26 residues of SOD1. Despite accumulation to only ~1% the level of endogenous wild-type SOD1 in mice (i.e., ~1/400 the level required to cause disease from the dismutase-active mutants in mice), this highly toxic mutant provokes disease accompanied by prominent aggregates (Jonsson et al. 2004).

SOD1-MEDIATED TOXICITY IS NONCELL AUTONOMOUS: ARE MOTOR NEURONS DIRECT TARGETS OF DISEASE?

The obvious loss of motor neurons in the spinal cord initially focused attention on how mutant SOD1 may act within motor neurons to provoke neuronal degeneration and death. However, as in almost all prominent examples of inherited human neurodegenerative diseases, the mutant gene products are expressed widely. In the case of SOD1, expression is ubiquitous, raising the possibility that the toxic cascade may be achieved wholly or in part by mutant SOD1 action in the

adjacent nonneuronal cells. In the first set of experiments to address this question, mutant SOD1 was expressed selectively in astrocytes (Gong et al. 2000) or in neurons (Pramatarova et al. 2001, Lino et al. 2002). Although there was astrocytosis in the mice with astrocyte-specific expression, none of the mice in these three sets developed motor neuron disease. Because high mutant SOD1 levels were sustained in the mice accumulating mutant SOD1 only in the astrocytes, mutant action solely within astroctyes appears insufficient to cause disease. In the case of neuron-specific expression [using either neurofilament (Pramatarova et al. 2001) or neural-specific enolase promoters (Lino et al. 2002)], no clear outcome emerged. However, the levels of mutant SOD1 may have been too low to yield disease. Further efforts, however, made it clear that toxicity to motor neurons from SOD1 mutants is noncell autonomous, that is, it requires mutant damage not just within motor neurons but also to nonneuronal cells. Clement et al. (2003) demonstrated this with three sets of chimeric mice including both normal and SOD1 mutant–expressing cells. Mutant motor neurons that chronically expressed $SOD1^{G37R}$ or $SOD1^{G93A}$ mutants at levels that cause early-onset disease when expressed ubiquitously could escape degeneration and death if surrounded by a sufficient number of normal nonneuronal cells (Figure 2). In some cases, a relatively small minority (5–20%) of normal cells eliminated disease, as well as degeneration and death of mutant motor neurons. Mice with these cells survived to ages at least twice those of the longest lived parental mice that expressed either mutant ubiquitously. Moreover, even within the same animal, the proportions of wild-type cells frequently differed on the two sides of the spinal cord. In two chimeras, all spinal motor neurons were mutant expressing. Although both animals developed disease, in each case twice as many of these mutant-expressing motor neurons survived on the side of the spinal cord with the higher number of wild-type, nonneuronal cells. Perhaps just as important, normal motor neurons surrounded by mutant-expressing nonneuronal cells acquired intraneuronal ubiquitinated deposits, indicating that the mutant-expressing cells had transfered damage to them. These data clearly demonstrate that the cellular neighborhood does matter: The death of the motor neurons depends, at least in part, on a contribution from surrounding glia and possibly other cell types.

OXIDATIVE DAMAGE AND METAL MISHANDLING AS SUSPECTS IN MUTANT SOD1–MEDIATED DISEASE

Hypotheses for Toxicity from Active-Site Copper-Mediated Aberrant Chemistry

The discovery of mutations in SOD1 immediately prompted hypotheses that toxicity is caused by aberrant chemistry of the active copper and zinc sites of the misfolded enzyme (Beckman et al. 1993). This aberrant chemistry could involve

greater access of abnormal substrates to the active site or clumsy handling of the copper and/or zinc ions. Even wild-type SOD1 can exhibit additional enzymatic activities including superoxide reductase or oxidase activities (Liochev & Fridovich 2000) in which either the oxidized or reduced forms use substrates other than superoxide, respectively. Two specific candidate chemistries involve use of inappropriate enzymatic substrates, which produces peroxynitrite and/or hydroxyl radical. Peroxynitrite can nitrate tyrosine residues (Beckman et al. 1994), thus damaging proteins in affected cells. It has been proposed that peroxynitrite can be produced when the enzyme runs catalysis backwards, converting oxygen to superoxide, which then combines with nitric oxide (NO) within the active site (Estevez et al. 1999). In vitro, such chemistry can be done by mutants that bind the catalytic copper, and this backwards catalysis is strongly exacerbated by depletion of zinc from SOD1 (Estevez et al. 1999). Acting as chaperones during the synthesis of metalloenzymes, metallothioneins are important in the regulation of zinc bioavailability within cells (Jacob et al. 1998, Palmiter 1998). Three isoforms exist: MT-I and -II are largely glial; MT-III is neuronal. Metallothionein expression is markedly upregulated in the spinal cord of ALS patients and transgenic mice (Blaauwgeers et al. 1996, Gong & Elliott 2000, Nagano et al. 2001). Consistent with zinc depletion as a factor in toxicity, SOD1^{G93A} mice deficient in either MT-I and MT-II (both expressed in glia) or MT-III (accmulated in neurons) exhibit significant accelerations in disease onset (Nagano et al. 2001), although it is not clear that acceleration in either case is due to altered zinc binding to SOD1.

The other proposed substrate is hydrogen peroxide, the normal end product of the oxidized form of the enzyme (SOD1-Cu^{2+}). Use of peroxide by the reduced form of SOD1 (SOD-Cu^{1+}) may produce the highly reactive hydroxyl radical. Wiedau-Pazos et al. (1996) reported a two- to fourfold increase in the use of hydrogen peroxide by two dismutase-active mutants relative to wild-type SOD1 in vitro. Despite their initial attractiveness, neither hypothesis is likely to represent an underlying toxicity common to the ALS-causing mutants. Evidence against these hypotheses includes the following:

1. Although both of the proposed oxidative mechanisms require active-site copper, a mutant missing all four copper-coordinating histidines still causes progressive motor neuron disease (Wang et al. 2003).

2. Although increased levels of free 3-nitrotyrosine have been reported in the spinal cords of human patients (Beal et al. 1997) and in mouse models of ALS (Bruijn et al. 1997a), there is no evidence of increased levels of nitrotyrosine bound to proteins in ALS patients or in most mutant SOD1 mice (Bruijn et al. 1997a). Furthermore, using mass spectrometry, Williamson et al. (2000) detected no increased nitration in neurofilaments isolated from transgenic mice expressing dismutase-active or -inactive mutants.

3. Although increases in hydroxyl radicals, measured by high-performance liquid chromatography, have been reported (Andrus et al. 1998, Hall et al.

1998) in tissue samples from transgenic mice expressing the catalytically active mutant SOD1^{G93A}, these increases have not been detected in other mouse models (Bruijn et al. 1997a).

4. Because NO is critical for the formation of peroxynitrate, alteration in NO synthesis would be expected to substantially alter the disease course if peroxynitrite were an important contributor to disease. However, reduction of neuronal nitric oxide synthase (nNOS) either pharmacologically or by genetic manipulation did not result in a change in disease progression (Dawson 2000). Similarly, in mice deletion of an inducible NOS (iNOS), localized to astrocytes and microglia, did not change the survival of mutant SOD1 mice (Son et al. 2001).

Toxicity Despite Reduced or Absent Catalytic Copper

The discovery that copper acquisition by SOD1 in yeast requires a specific copper chaperone for SOD1 (CCS) (Culotta et al. 1997) provided the basis for a test of whether the catalytic copper loading is important in disease. Both human wild-type and mutant SOD1 subunits load copper in vivo through the action of a mammalian CCS (Corson et al. 1998, Wong et al. 2000). Although eliminating CCS in transgenic mice expressing three different mutations, SOD1^{G37R}, SOD1^{G85R}, and SOD1^{G93A}, significantly lowered copper loading onto mutant SOD1 in transgenic mice [measured either with in vivo labeling or by in vitro catalytic activity of the active mutants (Subramaniam et al. 2002)], onset and progression of motor neuron disease was not affected. Furthermore, mutations in SOD1 that disrupt some or all the copper-coordinating residues do not eliminate toxicity (Wang et al. 2002, Wang et al. 2003). A variant of the oxidative proposal is that the mutants may handle copper more clumsily, thereby releasing the metal that can catalyze aberrant chemistry. Such mishandling, however, is unlikely to be an important contributor to disease. Even though the CCS is the major delivery source of copper to mutant SOD1, toxicity is the same in the presence or absence of the CCS (Subramaniam et al. 2002).

Hypothesis of Aberrant Catalysis from Surface-Bound Copper

A final proposal suggests that copper contributes to toxicity via aberrant chemistry of copper bound not to the active site of SOD1 but rather to one or more residues, including Cys111, on the surface of the subunit (Bush 2002). This idea arose from the observation that SOD1 in vitro can bind copper at its surface (Liu et al. 2000) and that a CCS-independent, ~30% elevation in tissue copper occurred in some SOD1 mouse models of disease (Subramaniam et al. 2002). Although this hypothesis cannot be formally excluded, no in vivo evidence supports such surface-copper binding, and the source of such copper is perplexing given evidence that the average pool of free copper is only a small fraction of a molecule per cell (Rae et al. 1999).

A COMMON FEATURE OF SOD1-MEDIATED TOXICITY IS PROTEIN AGGREGATION: BUT WHY SHOULD AGGREGATES BE TOXIC?

The presence of abnormal protein aggregates or inclusions has been described in many neurodegenerative diseases [amyloid and tau in Alzheimer's disease (Selkoe 2001), α-synuclein in Parkinson's disease (Selkoe 2001), and huntingtin in Huntington's disease (Steffan et al. 2001)]. For ALS, prominent, intracellular, cytoplasmic inclusions in motor neurons and in some cases within the astrocytes surrounding them are found in each of the prominent mouse models of SOD1-mediated disease and in all reported instances of human ALS (Bruijn et al. 1998). In the mouse models, these accumulations are highly immunoreactive for SOD1 (Figure 1). At least some misfolded SOD1 aggregates cannot be readily dissociated and are resistant to strong ionic detergents; some also contain covalent adducts to other components that can be detected biochemically in spinal cord extracts of transgenic SOD1 mice long before (Johnston et al. 2000, Wang et al. 2002), or contemporaneous with (Bruijn et al. 1997b), onset of disease.

An unsolved puzzle is whether these aggregates damage motor neurons (or other cells in the spinal cord), and if so through what mechanism(s). Several possible toxicities of the protein aggregates have been proposed (Figure 3), including aberrant chemistry; loss of protein function through coaggregation with the aggregates; depletion of protein folding chaperones; dysfunction of the proteasome overwhelmed with undigestable, misfolded protein; and inhibition of specific organelle function, including mictochondria and peroxisomes, through mutant aggregation onto or within such organelles. Consistent with involvement of the ubiquitin-proteasome system, the aggregates are intensely immunoreactive with antibodies to ubiquitin, a feature common to all SOD1 ALS mouse models (Bruijn et al. 1998, Jonsson et al. 2004, Wang et al. 2003) and human SOD1-mediated familial ALS (Bruijn et al. 1998, Kato et al. 2000).

Partial inhibition of the proteasome is sufficient to provoke large aggregates in cultured nonneuronal cells that express SOD1 mutants. This suggests that proteasome activity is limiting so that decrease in it either prevents the appropriate removal of the mutant protein or compromises the removal of even more important components (Johnston et al. 2000). Mutant SOD1s are selectively degraded by action of the RING finger-type E3 ubiquitin ligase, dorfin, followed by subsequent proteosomal degradation. Like its substrate, dorphin is predominantly localized in inclusion bodies in familial and sporadic ALS (Niwa et al. 2002). The finding that ubiquitin-containing aggregates are a frequent feature of sporadic disease (Mather et al. 1993. Leigh & Swash 1991) could link the mechanisms of familial and sporadic ALS. However, the complete picture must involve other proteins besides SOD1 as the source of the aggregates because SOD1-containing aggregates are not a characteristic feature of sporadic disease (Shibata et al. 1996).

In addition to their effect on the ubiquitin-proteasome system, aggregates affect the functional aspects of protein-folding chaperones, including those normally

present and those induced by heat or other stresses. The coimmunoprecipitation of HSP70, HSP40, and $\alpha\beta$-crystallin with mutant SOD1 (Shinder et al. 2001) confirms a likely involvement of such components. Perhaps more important is the finding that cultured motor neurons die when exposed to acute expression of mutant SOD1, but this is ameliorated by contemporaneous expression of high levels of HSP70 (Bruening et al. 1999). Although the conspicuous nature of large aggregates in affected tissue makes them attractive candidates for causing disease, whether these aggregates are integrally involved in the disease process or are beneficial in sequestering toxic by-products is unknown.

A CASCADE OF CASPASES MEDIATES MOTOR NEURON DEATH

Although the primary toxicities of the familial ALS–linked mutations of SOD1 remain unresolved, the final event in the death cascade has been partially clarified. Activation of caspase-1, one of the early events in the mechanism of toxicity of SOD1 mutants, occurs months prior to neuronal death and phenotypic disease onset (Pasinelli et al. 2000, Vukosavic et al. 2000). A central feature in cell death mediated by mutant SOD1 is the activation of caspase-3, one of the major cysteine-aspartate proteases responsible for degradation of many key cellular constituents in apoptotic cell death. Caspase-3 activation occurs in motor neurons (Li et al. 2000b, Pasinelli et al. 2000, Vukosavic et al. 2000) and astrocytes (Pasinelli et al. 2000) contemporaneous with the first stages of motor neuron death in all three of the best-studied mouse models. For SOD1[G93A], release of cytochrome c from mitochondria is followed by activation of caspase-9 (Guegan et al. 2001), which may be the effector for the subsequent activation of caspases-3 and -7. In vitro, this temporal cascade of caspase activation occurs within the same neuronal cell (Pasinelli et al. 2000), although this has not been firmly established in mice.

A common step toward toxicity of mutant SOD1 is a sequential activation of at least two caspases, which act more slowly here than they do during the apoptotic death processes of development. Moreover, apparent inhibition of one or more caspases in this cascade is beneficial: Despite a short half-life once in aqueous solution, long-term intrathecal administration of the pan-caspase inhibitor (N-benzylocarbonyl-Val-Ala-Asp-fluoromethylketone or zVAD-fmk) prolongs the life of SOD1[G93A] mice by approximately 27 days (Li et al. 2000). Further evidence that proteins of the cell death pathways are important for SOD1-mediated neuron death includes the demonstration that increasing expression of the anti-apoptotic factor Bcl2 slows disease onset and survival of SOD1[G93A] mice by three to four weeks (Kostic et al. 1997).

The best-case scenario for modulation of the final apoptotic cell death pathway is to find key mediators of programmed cell death that affect motor, but not other, neurons, thereby providing the basis for specificity. The demonstration that cultured embryonic motor neurons, but not other neuronal populations, are sensitive to killing by Fas ligand may provide such an example (Raoul et al. 2002). Activation of Fas receptors is an important pathway in the immune system,

where it eliminates virus-infected cells, cancerous cells, or mature T cells at the end of an immune response. Whether Fas activation is important for the survival of adult motor neurons threatened with abnormal SOD1 proteins remains to be determined.

ARE MITOCHONDRIA TARGETS FOR MUTANT SOD1–MEDIATED DAMAGE?

The presence of what appear to be vacuolated mitochondrial remnants within spinal motor neurons very early in the lines of mice accumulating the highest levels of SOD1^{G93A} and SOD1^{G37R} provided an initial suggestion that mitchondria may be primary targets for SOD1 mutant–mediated damage (Dal Canto & Gurney 1994, Kong & Xu 1998, Wong et al. 1995). For SOD1^{G93A}, Mattiazzi et al. (2002) later reported an ∼25% decrement in some enzymatic activities of the respiratory chain in isolated mitochondria, although this was neither seen before disease onset nor selective to mitochondria from spinal cord. Decreased activity of mitochondrial cytochrome oxidase has been reported for spinal cord extracts from end-stage sporadic human disease. Such enzymatic losses could arise from action of the mutant SOD1 within mitochondria: Using electron microscopy (Higgins et al. 2002, Jaarsma et al. 2001) and its apparent resistance to protease digestion with intact mitochondria isolated from brain (Mattiazzi et al. 2002), mutant SOD1^{G93A} has been reported to be present in spinal cord mitochondria, presumably within the intermembrane space. Partial deficiency of manganese superoxide dismutase 2 (a mitochondrial enzyme localized to the mitochondrial matrix) exacerbates disease in transgenic SOD1 mice (Andreassen et al. 2000), providing further evidence of mitochondria's role in ALS. Moreover, mis-targeting mutant SOD1 to the mitocondrial matrix of cultured cells is toxic (Takeuchi et al. 2002), although there is no evidence that mutant SOD1 ever enters the matrix without addition of a specific targeting sequence. Administration of creatine (Klivenyi et al. 1999), which may enhance energy storage capacity and inhibit the opening of the mitochondrial transition pore, and minocycline (Van Den Bosch et al. 2002, Zhu et al. 2002), a tetracycline derivative believed to inhibit microglial activation and block release of cytochrome c from the mitochondria (Kriz et al. 2002), slows disease by at least two to four weeks in mice expressing dismutase-active mutants SOD1^{G93A} and SOD1^{G37R}.

Despite these findings, the evidence that mitochondria are important targets for damage common to SOD1 mutants with different biochemical characters remains contradictory. Mitochondrial pathology has not been found in other rodent models that develop motor neuron disease from expression of lower levels of the same mutants or in any of the models that develop motor neuron disease from expression of mutants without dismutase activity (Ripps et al. 1995, Bruijn et al. 1997a, Wang et al. 2002, Jonsson et al. 2004, Wang et al. 2003). Moreover, although import of mutant SOD1 into mitochondria requires the CCS in yeast (Field et al. 2003), toxicity from SOD1 mutants is unaffected by the absence of the CCS (Subramanium et al. 2002).

NEUROFILAMENTS: A COMPONENT OF SELECTIVITY OF MOTOR NEURON TOXICITY IN ALS

Neurofilaments, the most abundant structural proteins in many types of mature motor neurons, have long been thought to confer some selective vulnerability to the neurons at risk in disease. Neurofilament assembly in axons is essential for establishing proper axonal diameters (Lee & Cleveland 1996, Garcia et al. 2003). Only the largest caliber, neurofilament-rich, lower motor neurons are at risk either in sporadic ALS (Kawamura et al. 1981) or SOD1-mediated disease in mice (Bruijn et al. 1997b). The discovery that accumulation and abnormal assembly of neurofilaments are common pathological hallmarks in several neurodegenerative diseases, including sporadic (Carpenter 1968, Chou & Fakadej 1971, Hirano 1991) and familial ALS (Hirano et al. 1984), infantile spinal muscular atrophy, and hereditary sensory-motor neuropathy, first suggested that aberrant accumulation of neurofilaments may contribute to disease onset or progression. Multiple genetic manipulations involving either overexpression or deletion of various neurofilament subunits in mice expressing mutant SOD1 have confirmed the importance of neurofilaments in the ALS model. Reduction of axonal neurofilaments and contemporaneous increase either of assembled filaments or the neurofilament-M (NF-M) and neurofilament–H (NF-H) subunits within the perikarya slow the onset of SOD1-mediated disease (Supplemental Table 1: Follow the Supplemental Material link from the Annual Reviews home page at http://www.annualreviews.org). Increased expression of NF-H, which traps most neurofilaments within the neuronal cell bodies, has produced the most robust ameliorization of disease in SOD1 mutant mice thus far, extending life span by as much as six months in one SOD1[G37R] line (Couillard-Despres et al. 1998).

The results of these manipulations are counterintuitive. Perturbing the normal axonal and perikaryal architecture is expected to add insult to the already present SOD1-mediated injury. Two explanations for why this is not the case have been proposed: First, excess neurofilaments function as a buffer or sink for some other deleterious process. For example, increased perikaryal content of the NF-M and NF-H subunits, which carry multiphosphorylation domains that can be substrates for the prominent neuronal cyclin-dependent kinase 5 (CDK5), may serve as a buffer following disregulation of this kinase. Indeed, the CDK5 neuronal activator p35 is cleaved by proteolysis to p25, producing a mislocalized, constitutively activated form of CDK5 within the cell bodies of SOD1[G37R] mice (Nguyen et al. 2001b). Second, reducing the axonal burden of neurofilaments (for example, by deletion of the NF-L subunit that is required for filament assembly) may reduce the burden on axonal transport, thereby moderating the damage of mutant SOD1 (Williamson et al. 1998).

Are neurofilament mutations relevant to human disease? At least two studies suggest that they are. By examining the repetitive tail domain of NF-H, a set of small in-frame deletions or insertions has been identified in 1% of more than 1300 ALS patients (Al-Chalabi et al. 1999, Tomkins et al. 1998, Figlewicz

et al. 1994). Almost all these mutations appear in sporadic cases, implicating neurofilaments as important risk factors for sporadic disease. Furthermore, investigators (Mersiyanova et al. 2000, De Jonghe et al. 2001) found that a dominant mutation in NF-L is a primary cause of the motor neuropathy Charcot-Marie-Tooth disease (Type II). Taken together, these data suggest that neurofilament content and organization are important contributors and probable risk factors for disease.

PERIPHERIN AND MOTOR NEURON DEGENERATION

Another candidate contributor to motor neuron disease is peripherin, an intermediate protein expressed in spinal motor neurons, peripheral sensory neurons, and autonomic nerves. Corbo & Hays (1992) found peripherin with neurofilament proteins in the majority of axonal inclusions in motor neurons of ALS patients. Increasing expression of the major peripherin isoform (peripherin 58) in motor neurons of transgenic mice leads to late-onset motor neuron disease accompanied by disruption of neurofilament assembly and organization (Beaulieu et al. 1999). In humans, an even more toxic form, peripherin 61, is encoded by an unexpected splice variant in which a part of what is normally an intron provides an additional 3 kD of coding sequence. Expression of peripherin 61 in primary motor neurons is toxic, even at modest levels (Robertson et al. 2003). It is detectable in the lumbar spinal cord of sporadic ALS cases, but not in nondiseased controls (Robertson et al. 2003). Although induced in motor neurons of mutant SOD1 mice, peripherin's role in ALS has been called into question because neither elimination of all isoforms by gene deletion nor overexpression of it in an ALS model had any effect on survival in SOD1 mutant mouse models (Lariviere et al. 2003).

EXCITOTOXICITY

Glutamate-mediated excitotoxicity from repetitive firing and/or elevation of intracellular calcium by calcium-permeable glutamate receptors has long been implicated in neuronal death. In motor neurons, the bulk of the glutamate is actively cleared from the synapse by the glial glutamate transporter, EAAT2, presumably helping to prevent excitotoxicity (Rothstein et al. 1996, Tanaka et al. 1997). Evidence for abnormal glutamate handling in ALS first arose from the discovery that the cerebrospinal fluid of ALS patients had increased glutamate levels (Rothstein et al. 1990, Rothstein et al. 1991, Shaw et al. 1995), a finding now reported in 40% of sporadic ALS patients. Direct measurement of functional glutamate transport in ALS revealed a marked diminution in the affected brain regions, which was the result of pronounced loss of the astroglial EAAT2 protein (Rothstein et al. 1995). Although evidence for EAAT2 loss through RNA mis-splicing in astrocyes (Lin et al. 1998) has not been confirmed in subsequent studies, lowering EAAT2 levels with an antisense oligonucleotide has shown that loss of transport activity directly

induces neuronal death (Rothstein et al. 1996). In a patient with sporadic ALS, a mutation in EAAT2 involving a substitution of the putative N-linked glycosylation site asparagine 206 by a serine residue (N206S) was identified (Trotti et al. 2001). Further in vitro studies show that this mutation causes aberrant targeting to the membrane, which decreases glutamate uptake (Trotti et al. 2001), indicating that this may be an important risk factor in the disease. Finally, compared with motor neurons that are spared, the spinal motor neurons at risk are relatively reduced in intracellular calcium-binding components such as calbindin D28k and parvalbumin (Alexianu et al. 1994, Ince et al. 1993), consistent with an excitotoxic component to pathogenesis.

Excitotoxicity has provided one of the few mechanistic links between sporadic and SOD1 mutant–mediated ALS. SOD1 mutants in rodent disease models induce a focal loss (Figure 4) of EAAT2 selectively from the astrocytes within the portion of the spinal cord containing the motor neuron cell bodies (Howland et al. 2002). Thus, glutamate excitotoxicity is likely to be an important contributor to neuronal death. Indeed, the only FDA-approved therapy in ALS, Riluzole, functions by decreasing glutamate toxicity.

DYNEIN, DYNACTIN, AND RETROGRADE AXONAL TRANSPORT

A feature that distinguishes motor neurons from other cells is their extreme asymmetry (up to one meter long) and large volume (up to 5000 times that of a typical cell). This size places an enormous metabolic load on the more normally sized cell body whose job is to synthesize the components for this large cell. Components must be transported first into the extended axon. Then via both fast and slow transport, components move toward the synapse. Finally, fast, cytoplasmic dynein–mediated retrograde returns components, including multivesicular bodies and trophic factors such as nerve growth factor, back to the cell body. The finding that SOD1 mutants impair slow axonal transport months prior to disease onset (Williamson et al. 1998) led to the conclusion that diminished transport correlated with the development of motor neuron disease.

Although cytoplasmic dynein has many cellular roles, which include positioning the endoplasmic reticulum and Golgi as well as in assembly of the mitotic spindle, in neurons it is the only known motor for retrograde transport. Two dominant point mutations in it cause a progressive motor neuron disorder in mice (Hafezparast et al. 2003). Similarly, disruption in postnatal motor neurons of the dynactin complex, an activator of cytoplasmic dynein that makes it more processive (King & Schroer 2000), inhibits retrograde axonal transport (LaMonte et al. 2002), provoking a late-onset, progressive motor neuron disease. This is also true in human disease: A dominant point mutation in the p150 subunit of dynactin is the proximal cause of a lower motor neuron disorder that begins with vocal cord paralysis (Puls et al. 2003).

MICROGLIA AND INFLAMMATION

Microglia are the resident immune cells of the central nervous system. They resemble peripheral tissue macrophages and are the primary mediators of neuroinflammation (Kreutzberg 1996). In the healthy adult brain, microglia exist as "resting" microglia, characterized by a small cell body and fine, ramified processes and minimal expression of surface antigens. Upon injury to the central nervous system, these cells become rapidly activated and exert their effects on neurons and macroglia (astrocytes and oligodendrocytes) through the release of cytotoxic and inflammatory substances such as oxygen radicals, NO, glutamate, cytokines, and prostaglandins (Kreutzberg 1996, Hanisch 2002). Microglial involvement has been described in many acute and chronic neurological diseases (Kreutzberg 1996, McGeer & McGeer 1999), although little is known about the role of microglia in ALS. In ALS tissues, there is strong activation and proliferation of microglia in regions of motor neuron loss (Kawamata et al. 1992, Ince et al. 1996). Recent studies showed that expression of proinflammatory mediators [TNF-alpha, Interleukin-1B, and cyclooxygenase 2 (COX-2)] is an early event in mouse models of ALS (Alexianu et al. 2001, Elliott 2001, Nguyen et al. 2001a, Almer et al. 2002, Hensley et al. 2002). Additional evidence for microglial involvement in ALS is provided by pharmacological studies aimed at delaying ALS progression. Minocycline, an antibiotic that also blocks microglial activation (Yrjanheikki et al. 1999), slows disease in ALS mice (Kriz et al. 2002, Van Den Bosch et al. 2002, Zhu et al. 2002). Similarly, inihibition of a key enzyme in prostaglandin synthesis (COX-2) with celecoxib prolongs survival by 25% (Drachman et al. 2002). Both minocylcine and celecoxib are currently being studied in human clinical trials.

GROWTH FACTORS

Vascular Endothelial Growth Factor

A completely unexpected contributor to motor neuron survival emerged from the discovery that ALS-like symptoms and neuropathology can be produced in mice bearing a targeted deletion that eliminates the ability of the vascular endothelial cell growth factor (VEGF) gene to respond to tissue hypoxia (Oosthuyse et al. 2001). VEGF has long been recognized as a crucial factor in controlling the growth and permeability of blood vessels. Hypoxia-induced expression of VEGF through transcription factors that respond to low oxygen tension is crucial for maintaining or restoring the vascular perfusion of normal tissues and for triggering the growth of blood vessels to supply the extraordinary metabolic demands of tumors. Targeted deletion of the hypoxia-response element in the VEGF gene resulted in mice with a normal baseline expression of VEGF but with a pronounced deficit in the ability to induce VEGF in response to hypoxia. Motor neuron deficits first appeared in mice between five and seven months of age and gradually progressed. All the classic features of ALS were observed: accumulation of neurofilaments in spinal cord

and brainstem motor neurons, degeneration of motor axons, and the characteristic denervation-induced muscle atrophy.

In a large European study to determine whether alterations in the VEGF gene may be linked to human ALS (Lambrechts et al. 2003), three single nucleotide polymorphisms in the promoter region of the VEGF gene were identified, implicating VEGF as a risk factor in the disease. However, no variations were seen in the hypoxia-response element. The promoter variants in the VEGF gene in these patients coincided with reduced levels of plasma VEGF. In addition, expression of VEGF carrying these single base-pair changes in a culture system lowered the levels of VEGF and resulted in cell death. Decreasing the levels of VEGF in transgenic mice expressing SOD1^{G93A} decreased the age at onset of disease and decreased their life span.

Although these data strongly implicate VEGF as a risk factor for ALS, further validation is required to confirm that VEGF, and not other more important genes in close association with variations in VEGF, is the key gene involved. A sub-population in England showed no association with these variations (Lambrechts et al. 2003), suggesting that genetic background, the presence of additional modifiers, environment, or lifestyle may also be important. Further studies to determine whether VEGF has a direct function as a neurotrophic factor for motor neurons or a more indirect function in motor neuron survival are under way.

Neurotrophic Factors

Neurotrophic factors selectively regulate the growth and survival of certain populations of neurons in the central and peripheral nervous systems. They have an essential role in neuronal development and in the maintenance of differentiated neurons. Because astrocytes and microglia are important sources of neurotrophic factors, damage to those cells as occurs pathologically in ALS implicates that loss of trophic support is one of the underlying factors causing motor neuron degeneration. Anand et al. (1995) detected decreased levels of ciliary neurotrophic factors (CNTF) in the postmortem tissue of ALS patients, indicating that trophic support may be impaired. Earlier onset of disease occurred in a patient with an SOD1 mutation and a homozygous mutation in the CNTF gene, supporting the idea that neurotrophic factors may play a role in ALS (Giess et al. 2002). However, the discovery that some individuals who lack the CNTF gene do not develop motor neuron disease argues against a strong association between ALS and CNTF (Takahashi et al. 1994). Furthermore, a recent study demonstrated that lack of CNTF does not affect age of onset, clinical presentation, rate of progression, or disease duration in sporadic or familial (SOD1^{D90A}) ALS (Al-Chalabi et al. 2003). Other trophic factors that may be involved in ALS pathogenesis include brain-derived neurotrophic factor, glial cell line–derived neurotrophic factor (GDNF), and insulin-like growth factor-I (IGF-1), as they all support the survival of motor neurons in vivo and in vitro (Elliott & Snider 1996, Oppenheim 1996).

Despite the suggestion that trophic factors may be important in ALS, human trials with neurotrophic factors have been disappointing and studies in the mouse

models have been relatively unimpressive (Supplemental Table 1). Previous trials based on neurotrophic factors are problematic because the neurotrophin may not have been delivered effectively to the target neurons. In a recent study, Kaspar et al. (2003) used a gene therapy approach to deliver trophic factors directly to neurons, as outlined in Figure 5. Adeno-associated virus (AAV) expressing GDNF or IGF-1 was injected into the hindlimb and intercostal muscles of SOD1^{G93A} mice. IGF-1-containing AAV, which is retrogradely transported back to the neurons of the spinal cord, robustly prolonged survival, although GDNF delivered in a similar manner did not. It is unknown whether the IGF-1, which is probably produced and secreted by the motor neurons, had an effect on neurons producing the IGF-1, neighboring neurons, astrocytes, or all of the above.

MODELING TOXICITY IN ALS

In considering what lessons have now been learned about the mechanisms underlying ALS, the evidence at first glance seems to support a discouraging series of divergent possibilities (Figure 6). However, the timing and selectivity for motor neuron killing may arise from the unfortunate convergence of a series of factors—all of which are necessary to place motor neurons at risk—rather than from a single alternative (Figure 6).

For familial ALS, the disease results from an acquired toxicity of mutant SOD1 rather than from any loss of function of the protein. The acquired toxic property affects both neurons and glia, and SOD1 expression in either cell type alone is insufficient to result in disease. The exact nature of this toxicity is uncertain, but in neurons it likely disrupts several basic cellular functions including protein breakdown by the ubiquitin-proteasome system, slow anterograde transport, fast retrograde axonal transport, calcium homeostasis, mitochondrial function, and maintenance of the cytoskeletal architecture. Aggregates are clearly identifiable but thus far have not been directly linked to any of these disruptions in cellular function and could, in theory, be protective rather than pathogenic. Although these changes are occurring in the motor neurons, there is concurrent damage to the astrocytes and activation of microglial cells. The striking loss of glutamate transporters in the astroglial cells likely results in increased levels of glutamate and causes an excitotoxic stress to the struggling motor neurons. Activated microglial cells induce many inflammatory cytokines that may contribute to the death of motor neurons. In the moribund motor neuron, activated caspase-3 deals the final blow.

What about the 98% of disease that does not arise from SOD1 mutations? The convergence model, including a common set of risk factors, may prove correct here too. The key difference is the substitution of an additional combination of initiating genetic or environmental modifiers in lieu of mutant SOD1. Environment can contribute to ALS, as demonstrated by several clusters of disease, e.g., the 50-fold increased risk for ALS in Guam in the 1950s (Mulder & Kurland 1987). More recently, a twofold increased risk of ALS has been recognized among veterans of the Gulf War (Haley 2003, Horner et al. 2003). All the individual events described

above likely contribute to the selective loss of motor neurons, thus each represents a target for therapy. Yet despite, or perhaps because of, the multiple possible targets, ALS therapy in both mice and humans has thus far yielded disappointing results.

CHALLENGES: DEVELOPING THERAPIES FOR ALS

ALS is a complex disease with multiple causes, making the discovery of effective pharmacologic therapies challenging. Despite the impressive list of therapies attempted in both mouse and humans (Supplemental Table 1), Riluzole is currently the only FDA-approved compound that may slow disease progression and extend survival, although its effect on both is generously described as modest. Nonpharmacologic therapies such as maintaining nutrition and attending to respiratory function have more significant effects than does any currently available medication, but none of these therapeutic efforts significantly slows disease progression. Many neurologists recommend vitamin supplements and antioxidants, and many discuss with patients medications such as minocycline and celecoxib that have shown some efficacy in mouse models. Clinical trials for several of these compounds are ongoing. As in other neurodegenerative diseases, ALS therapies are advancing in three overlapping areas: (*a*) small molecules including "off-the-shelf" compounds; (*b*) delivering protein, DNA, or RNA; and (*c*) novel gene therapy approaches including viral vectors and stem cells.

Therapies that could be quickly translated to clinical practice may be found among the group of small molecules already available, some of which have been approved by the FDA for other uses. The ALS community has recently embarked on such an approach: In a joint effort by the ALS Association and the National Institute of Neurologic Disorders and Stroke, and involving many different laboratories, researchers have screened 1040 compounds; the results have not been published.

In addition to traditional pharmacologic molecules, proteins, DNA, or RNA may affect disease. In previous ALS trials, growth factors were delivered subcutaneously or directly into the cerebral spinal fluid (Supplemental Table 1). None of these trials were particularly effective. As discussed above, one of the major problems may have been failure to deliver the protein to the target tissue. A recent viral-mediated approach may have solved this problem. When injected into muscles, AAV expressing IGF-1 is retrogradely transported to the spinal cord and prolongs survival in ALS mice. A clinical trial using a similar approach in humans is anticipated. Other novel strategies include the development of antisense and small interfering RNA that block the synthesis of SOD1 (Ding et al. 2003).

Gene therapy and stem cell approaches are being actively and enthusiastically considered for ALS. Delivery of proteins by viral vectors (as discussed above) represents one such gene therapy approach. Stem cells could be engineered to produce a growth factor and then injected or placed within the central nervous

system. Both mouse and human embryonic stem cells, when cultured in vitro to produce embroid bodies, are able to differentiate into various types of brain cells (Anderson et al. 2001). The ability to push these cells toward a motor neuron lineage was recently demonstrated (Wichterle et al. 2002). Remarkably, when motor neurons that were created in vitro were introduced into a lesioned chick spinal cord, some stem cell–derived motor neurons extended axons and innervated muscle (Wichterle et al. 2002). Other sources of stem cells such as bone marrow (Brazelton et al. 2000) and muscle (Goodell et al. 2001) are being investigated, but it remains controversial whether these stem cells will provide an abundant source of neurons.

Neural cell replacement therapies are based on the idea that neurological function lost to injury or neurodegeneration can be improved by introducing new cells that can form appropriate connections and replace the function of lost neurons. In ALS, it is hard to imagine that many transplanted motor neurons would form appropriate connections with target muscles, where motor axons need to extend distances up to a meter in length to reach the target. An alternative strategy to the Herculean task of replacing the long motor neurons would be to replace non-neurons because normal nonneuronal cells can sharply extend survival of motor neurons that express SOD1 (Clement et al. 2003).

Another approach could be to stimulate endogenous stem cells in the brain or spinal cord to generate new neurons. Contrary to earlier belief, neurogenesis does occur in the adult nervous system, particularly in the hippocampus and olfactory bulb (Alvarez-Buylla 1992, Alvarez-Buylla & Garcia-Verdugo 2002). Studies to understand the molecular determinants and cues to stimulate endogenous stem cells are under way (Gage 2002, Clarke et al. 2000, Magavi & Macklis 2001). Although promising, we are only beginning to learn the potentials and challenges of these cells, especially for use in neurodegenerative diseases such as ALS.

Given the convergence of multiple pathways leading to disease, various therapies targeting different processes may be the most effective. Although the exact combination of therapies is not yet known, new insights into disease mechanisms and anticipated discoveries of new genes responsible for ALS foster hopes that therapies to significantly slow this disease are within reach.

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LITERATURE CITED

Abalkhail H, Mitchell J, Habgood J, Orrell R, de Belleroche J. 2003. A new familial amyotrophic lateral sclerosis locus on chromosome 16q12.1-16q12.2. *Am. J. Hum. Genet.* 73:383–89

Al-Chalabi A, Andersen PM, Nilsson P, Chioza B, Andersson JL, et al. 1999. Deletions of the heavy neurofilament subunit tail in amyotrophic lateral sclerosis. *Hum. Mol. Genet.* 8:157–64

Al-Chalabi A, Scheffler MD, Smith BN, Parton MJ, Cudkowicz ME, et al. 2003. Ciliary neurotrophic factor genotype does not influence clinical phenotype in amyotrophic lateral sclerosis. *Ann. Neurol.* 54:130–34

Alexianu ME, Ho BK, Mohamed AH, La Bella

V, Smith RG, Appel SH. 1994. The role of calcium-binding proteins in selective motoneuron vulnerability in amyotrophic lateral sclerosis. *Ann. Neurol.* 36:846–58

Alexianu ME, Kozovska M, Appel SH. 2001. Immune reactivity in a mouse model of familial ALS correlates with disease progression. *Neurology* 57:1282–89

Almer G, Teismann P, Stevic Z, Halaschek-Wiener J, Deecke L, et al. 2002. Increased levels of the pro-inflammatory prostaglandin PGE2 in CSF from ALS patients. *Neurology* 58:1277–79

Alvarez-Buylla A. 1992. Neurogenesis and plasticity in the CNS of adult birds. *Exp. Neurol.* 115:110–14

Alvarez-Buylla A, Garcia-Verdugo JM. 2002. Neurogenesis in adult subventricular zone. *J. Neurosci.* 22:629–34

Anand P, Parrett A, Martin J, Zeman S, Foley P, et al. 1995. Regional changes of ciliary neurotrophic factor and nerve growth factor levels in post mortem spinal cord and cerebral cortex from patients with motor disease. *Nat. Med.* 1:168–72

Andersen PM. 2000. Genetic factors in the early diagnosis of ALS. *Amyotroph. Lateral Scler. Other Motor Neuron Disord.* 1 (Suppl. 1):S31–42

Andersen PM, Spitsyn VA, Makarov SV, Nilsson L, Kravchuk OI, et al. 2001. The geographical and ethnic distribution of the D90A CuZn-SOD mutation in the Russian Federation. *Amyotroph. Lateral Scler. Other Motor Neuron Disord.* 2:63–69

Anderson DJ, Gage FH, Weissman IL. 2001. Can stem cells cross lineage boundaries? *Nat. Med.* 7:393–95

Andreassen OA, Ferrante RJ, Klivenyi P, Klein AM, Shinobu LA, et al. 2000. Partial deficiency of manganese superoxide dismutase exacerbates a transgenic mouse model of amyotrophic lateral sclerosis. *Ann. Neurol.* 47:447–55

Andrus PK, Fleck TJ, Gurney ME, Hall ED. 1998. Protein oxidative damage in a transgenic mouse model of familial amyotrophic lateral sclerosis. *J. Neurochem.* 71:2041–48

Beal MF, Ferrante RJ, Browne SE, Matthews RT, Kowall NW, Brown RH Jr. 1997. Increased 3-nitrotyrosine in both sporadic and familial amyotrophic lateral sclerosis. *Ann. Neurol.* 42:644–54

Beaulieu JM, Nguyen MD, Julien JP. 1999. Late onset death of motor neurons in mice overexpressing wild-type peripherin. *J. Cell Biol.* 147:531–44

Beckman JS, Carson M, Smith CD, Koppenol WH. 1993. ALS, SOD and peroxynitrite. *Nature* 364:584

Beckman JS, Chen J, Crow JP, Ye YZ. 1994. Reactions of nitric oxide, superoxide and peroxynitrite with superoxide dismutase in neurodegeneration. *Prog. Brain Res.* 103:371–80

Blaauwgeers HG, Anwar Chand M, van den Berg FM, Vianney de Jong JM, Troost D. 1996. Expression of different metallothionein messenger ribonucleic acids in motor cortex, spinal cord and liver from patients with amyotrophic lateral sclerosis. *J. Neurol. Sci.* 142:39–44

Blair IP, Bennett CL, Abel A, Rabin BA, Griffin JW, et al. 2000. A gene for autosomal dominant juvenile amyotrophic lateral sclerosis (ALS4) localizes to a 500-kb interval on chromosome 9q34. *Neurogenetics* 3:1–6

Borchelt DR, Lee MK, Slunt HS, Guarnieri M, Xu ZS, et al. 1994. Superoxide dismutase 1 with mutations linked to familial amyotrophic lateral sclerosis possesses significant activity. *Proc. Natl. Acad. Sci. USA* 91:8292–96

Bowling AC, Barkowski EE, McKenna-Yasek D, Sapp P, Horvitz HR, et al. 1995. Superoxide dismutase concentration and activity in familial amyotrophic lateral sclerosis. *J. Neurochem.* 64:2366–69

Brazelton TR, Rossi FM, Keshet GI, Blau HM. 2000. From marrow to brain: expression of neuronal phenotypes in adult mice. *Science* 290:1775–79

Bruening W, Roy J, Giasson B, Figlewicz DA, Mushynski WE, Durham HD. 1999. Up-regulation of protein chaperones preserves viability of cells expressing toxic

Cu/Zn-superoxide dismutase mutants associated with amyotrophic lateral sclerosis. *J. Neurochem.* 72:693–99

Bruijn LI, Beal MF, Becher MW, Schulz JB, Wong PC, et al. 1997a. Elevated free nitrotyrosine levels, but not protein-bound nitrotyrosine or hydroxyl radicals, throughout amyotrophic lateral sclerosis (ALS)-like disease implicate tyrosine nitration as an aberrant in vivo property of one familial ALS-linked superoxide dismutase 1 mutant. *Proc. Natl. Acad. Sci. USA* 94:7606–11

Bruijn LI, Becher MW, Lee MK, Anderson KL, Jenkins NA, et al. 1997b. ALS-linked SOD1 mutant G85R mediates damage to astrocytes and promotes rapidly progressive disease with SOD1-containing inclusions. *Neuron* 18:327–38

Bruijn LI, Cleveland DW. 1996. Mechanisms of selective motor neuron death in ALS: insights from transgenic mouse models of motor neuron disease. *Neuropathol. Appl. Neurobiol.* 22:373–87

Bruijn LI, Houseweart MK, Kato S, Anderson KL, Anderson SD, et al. 1998. Aggregation and motor neuron toxicity of an ALS-linked SOD1 mutant independent from wild-type SOD1. *Science* 281:1851–54

Bush AI. 2002. Is ALS caused by an altered oxidative activity of mutant superoxide dismutase? *Nat. Neurosci.* 5:919

Carpenter S. 1968. Proximal axonal enlargement in motor neuron disease. *Neurology* 18:841–51

Chou SM, Fakadej AV. 1971. Ultrastructure of chromatolytic motoneurons and anterior spinal roots in a case of Werdnig-Hoffmann disease. *J. Neuropathol. Exp. Neurol.* 30:368–79

Clarke DL, Johansson CB, Wilbertz J, Veress B, Nilsson E, et al. 2000. Generalized potential of adult neural stem cells. *Science* 288:1660–63

Clement AM, Nguyen MD, Roberts EA, Garcia ML, Boillee S, et al. 2003. Wild-type nonneuronal cells extend survival of SOD1 mutant motor neurons in ALS mice. *Science* 302:113–17

Corbo M, Hays AP. 1992. Peripherin and neurofilament protein coexist in spinal spheroids of motor neuron disease. *J. Neuropathol. Exp. Neurol.* 51:531–37

Corson LB, Strain JJ, Culotta VC, Cleveland DW. 1998. Chaperone-facilitated copper binding is a property common to several classes of familial amyotrophic lateral sclerosis-linked superoxide dismutase mutants. *Proc. Natl. Acad. Sci. USA* 95:6361–66

Couillard-Despres S, Zhu Q, Wong PC, Price DL, Cleveland DW, Julien JP. 1998. Protective effect of neurofilament heavy gene overexpression in motor neuron disease induced by mutant superoxide dismutase. *Proc. Natl. Acad. Sci. USA* 95:9626–30

Culotta VC, Klomp LW, Strain J, Casareno RL, Krems B, Gitlin JD. 1997. The copper chaperone for superoxide dismutase. *J. Biol. Chem.* 272:23469–72

Dal Canto MC, Gurney ME. 1994. Development of central nervous system pathology in a murine transgenic model of human amyotrophic lateral sclerosis. *Am. J. Pathol.* 145:1271–79

Dawson TM. 2000. New animal models for Parkinson's disease. *Cell* 101:115–18

De Jonghe P, Mersivanova I, Nelis E, Del Favero J, Martin JJ, et al. 2001. Further evidence that neurofilament light chain gene mutations can cause Charcot-Marie-Tooth disease type 2E. *Ann. Neurol.* 49:245–49

Delisle MB, Carpenter S. 1984. Neurofibrillary axonal swellings and amyotrophic lateral sclerosis. *J. Neurol. Sci.* 63:241–50

Ding H, Schwarz DS, Keene A, Affar el B, Fenton L, et al. 2003. Selective silencing by RNAi of a dominant allele that causes amyotrophic lateral sclerosis. *Aging Cell* 2:209–17

Drachman DB, Frank K, Dykes-Hoberg M, Teismann P, Almer G, et al. 2002. Cyclooxygenase 2 inhibition protects motor neurons and prolongs survival in a transgenic mouse model of ALS. *Ann. Neurol.* 52:771–78

Elliott JL. 2001. Cytokine upregulation in a murine model of familial amyotrophic lateral

sclerosis. *Brain Res. Mol. Brain Res.* 95:172–78

Elliott JL, Snider WD. 1996. Motor neuron growth factors. *Neurology* 47:S47–53

Estevez AG, Crow JP, Sampson JB, Reiter C, Zhuang Y, et al. 1999. Induction of nitric oxide-dependent apoptosis in motor neurons by zinc-deficient superoxide dismutase. *Science* 286:2498–500

Field LS, Furukawa Y, O'Halloran TV, Culotta VC. 2003. Factors controlling the uptake of yeast copper/zinc superoxide dismutase into mitochondria. *J. Biol. Chem.* 278:28052–59

Figlewicz DA, Krizus A, Martinoli MG, Meininger V, Dib M, et al. 1994. Variants of the heavy neurofilament subunit are associated with the development of amyotrophic lateral sclerosis. *Hum. Mol. Genet.* 3:1757–61

Gage FH. 2002. Neurogenesis in the adult brain. *J. Neurosci.* 22:612–13

Garcia ML, Lobsiger CS, Shah SB, Deerinck TJ, Crum J, et al. 2003. NF-M is an essential target for the mylein-directed "outside-in" signaling cascade that mediates radial axonal growth. *J. Cell Biol.* 163:1011–20

Gaudette M, Hirano M, Siddique T. 2000. Current status of SOD1 mutations in familial amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler. Other Motor Neuron Disord.* 1:83–89

Giess R, Holtmann B, Braga M, Grimm T, Muller-Myhsok B, et al. 2002. Early onset of severe familial amyotrophic lateral sclerosis with a SOD-1 mutation: potential impact of CNTF as a candidate modifier gene. *Am. J. Hum. Genet.* 70:1277–86

Gonatas NK, Stieber A, Mourelatos Z, Chen Y, Gonatas JO, et al. 1992. Fragmentation of the Golgi apparatus of motor neurons in amyotrophic lateral sclerosis. *Am. J. Pathol.* 140:731–37

Gong YH, Elliott JL. 2000. Metallothionein expression is altered in a transgenic murine model of familial amyotrophic lateral sclerosis. *Exp. Neurol.* 162:27–36

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.* 20:660–65

Goodell MA, Jackson KA, Majka SM, Mi T, Wang H, et al. 2001. Stem cell plasticity in muscle and bone marrow. *Ann. NY Acad. Sci.* 938:208–18

Guegan C, Vila M, Rosoklija G, Hays AP, Przedborski S. 2001. Recruitment of the mitochondrial-dependent apoptotic pathway in amyotrophic lateral sclerosis. *J. Neurosci.* 21:6569–76

Gurney ME. 1994. Transgenic-mouse model of amyotrophic lateral sclerosis. *N. Engl. J. Med.* 331:1721–22

Hadano S, Hand CK, Osuga H, Yanagisawa Y, Otomo A, et al. 2001. A gene encoding a putative GTPase regulator is mutated in familial amyotrophic lateral sclerosis 2. *Nat. Genet.* 29:166–73

Hafezparast M, Klocke R, Ruhrberg C, Marquardt A, Ahmad-Annuar A, et al. 2003. Mutations in dynein link motor neuron degeneration to defects in retrograde transport. *Science* 300:808–12

Haley RW. 2003. Excess incidence of ALS in young Gulf War veterans. *Neurology* 61:750–56

Hall ED, Andrus PK, Oostveen JA, Fleck TJ, Gurney ME. 1998. Relationship of oxygen radical-induced lipid peroxidative damage to disease onset and progression in a transgenic model of familial ALS. *J. Neurosci. Res.* 53:66–77

Hand CK, Khoris J, Salachas F, Gros-Louis F, Lopes AA, et al. 2002. A novel locus for familial amyotrophic lateral sclerosis, on chromosome 18q. *Am. J. Hum. Genet.* 70:251–56

Hanisch UK. 2002. Microglia as a source and target of cytokines. *Glia* 40:140–55

Hensley K, Floyd RA, Gordon B, Mou S, Pye QN, et al. 2002. Temporal patterns of cytokine and apoptosis-related gene expression in spinal cords of the G93A-SOD1 mouse model of amyotrophic lateral sclerosis. *J. Neurochem.* 82:365–74

Hentati A, Ouahchi K, Pericak-Vance MA, Nijhawan D, Ahmad A, et al. 1998. Linkage of a commoner form of recessive amyotrophic lateral sclerosis to chromosome 15q15-q22 markers. *Neurogenetics* 2:55–60

Higgins CM, Jung C, Ding H, Xu Z. 2002. Mutant Cu, Zn superoxide dismutase that causes motoneuron degeneration is present in mitochondria in the CNS. *J. Neurosci.* 22:RC215

Hirano A. 1991. Cytopathology of amyotrophic lateral sclerosis. *Adv. Neurol.* 56:91–101

Hirano A, Nakano I, Kurland LT, Mulder DW, Holley PW, Saccomanno G. 1984. Fine structural study of neurofibrillary changes in a family with amyotrophic lateral sclerosis. *J. Neuropathol. Exp. Neurol.* 43:471–80

Horner RD, Kamins KG, Feussner JR, Grambow SC, Hoff-Lindquist J, et al. 2003. Occurrence of amyotrophic lateral sclerosis among Gulf War veterans. *Neurology* 61:742–49

Hosler BA, Siddique T, Sapp PC, Sailor W, Huang MC, et al. 2000. Linkage of familial amyotrophic lateral sclerosis with frontotemporal dementia to chromosome 9q21-q22. *JAMA* 284:1664–69

Howland DS, Liu J, She Y, Goad B, Maragakis NJ, et al. 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. USA* 99:1604–9

Ince P, Stout N, Shaw P, Slade J, Hunziker W, et al. 1993. Parvalbumin and calbindin D-28k in the human motor system and in motor neuron disease. *Neuropathol. Appl. Neurobiol.* 19:291–99

Ince PG, Shaw PJ, Slade JY, Jones C, Hudgson P. 1996. Familial amyotrophic lateral sclerosis with a mutation in exon 4 of the Cu/Zn superoxide dismutase gene: pathological and immunocytochemical changes. *Acta Neuropathol. (Berlin)* 92:395–403

Jaarsma D, Haasdijk ED, Grashorn JA, Hawkins R, van Duijn W, et al. 2000. Human Cu/Zn superoxide dismutase (SOD1) overexpression in mice causes mitochondrial vacuolization, axonal degeneration, and premature motoneuron death and accelerates motoneuron disease in mice expressing a familial amyotrophic lateral sclerosis mutant SOD1. *Neurobiol. Dis.* 7:623–43

Jaarsma D, Rognoni F, van Duijn W, Verspaget HW, Haasdijk ED, Holstege JC. 2001. CuZn superoxide dismutase (SOD1) accumulates in vacuolated mitochondria in transgenic mice expressing amyotrophic lateral sclerosis-linked SOD1 mutations. *Acta Neuropathol. (Berlin)* 102:293–305

Jacob C, Maret W, Vallee BL. 1998. Control of zinc transfer between thionein, metallothionein, and zinc proteins. *Proc. Natl. Acad. Sci. USA* 95:3489–94

Johnston JA, Dalton MJ, Gurney ME, Kopito RR. 2000. Formation of high molecular weight complexes of mutant Cu, Zn-superoxide dismutase in a mouse model for familial amyotrophic lateral sclerosis. *Proc. Natl. Acad. Sci. USA* 97:12571–76

Jonsson PA, Ernhill K, Andersen PM, Bergemalm D, Brannstrom T, et al. 2004. Minute quantities of misfolded mutant superoxide dismutase-1 cause amyotrophic lateral sclerosis. *Brain* 127:73–88

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* 301:839–42

Kato S, Takikawa M, Nakashima K, Hirano A, Cleveland DW, et al. 2000. New consensus research on neuropathological aspects of familial amyotrophic lateral sclerosis with superoxide dismutase 1 (SOD1) gene mutations: inclusions containing SOD1 in neurons and astrocytes. *Amyotroph. Lateral Scler. Other Motor Neuron Disord.* 1:163–84

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

Kawamura Y, Dyck PJ, Shimono M, Okazaki H, Tateishi J, Doi H. 1981. Morphometric comparison of the vulnerability of peripheral motor and sensory neurons in amyotrophic lateral sclerosis. *J. Neuropathol. Exp. Neurol.* 40:667–75

King SJ, Schroer TA. 2000. Dynactin increases

the processivity of the cytoplasmic dynein motor. *Nat. Cell Biol.* 2:20–24

Klivenyi P, Ferrante RJ, Matthews RT, Bogdanov MB, Klein AM, et al. 1999. Neuroprotective effects of creatine in a transgenic animal model of amyotrophic lateral sclerosis. *Nat. Med.* 5:347–50

Kong J, Xu Z. 1998. Massive mitochondrial degeneration in motor neurons triggers the onset of amyotrophic lateral sclerosis in mice expressing a mutant SOD1. *J. Neurosci.* 18:3241–50

Kostic V, Jackson-Lewis V, de Bilbao F, Dubois-Dauphin M, Przedborski S. 1997. Bcl-2: prolonging life in a transgenic mouse model of familial amyotrophic lateral sclerosis. *Science* 277:559–62

Kreutzberg GW. 1996. Microglia: a sensor for pathological events in the CNS. *Trends Neurosci.* 19:312–18

Kriz J, Nguyen MD, Julien JP. 2002. Minocycline slows disease progression in a mouse model of amyotrophic lateral sclerosis. *Neurobiol. Dis.* 10:268–78

Kunst CB, Messer L, Gordon J, Haines J, Patterson D. 2000. Genetic mapping of a mouse modifier gene that can prevent ALS onset. *Genomics* 70:181–89

Lambrechts D, Storkebaum E, Morimoto M, Del-Favero J, Desmet F, et al. 2003. VEGF is a modifier of amyotrophic lateral sclerosis in mice and humans and protects motoneurons against ischemic death. *Nat. Genet.* 34:383–94

LaMonte BH, Wallace KE, Holloway BA, Shelly SS, Ascano J, et al. 2002. Disruption of dynein/dynactin inhibits axonal transport in motor neurons causing late-onset progressive degeneration. *Neuron* 34:715–27

Lariviere RC, Beaulieu JM, Nguyen MD, Julien JP. 2003. Peripherin is not a contributing factor to motor neuron disease in a mouse model of amyotrophic lateral sclerosis caused by mutant superoxide dismutase. *Neurobiol. Dis.* 13:158–66

Lee MK, Cleveland DW. 1996. Neuronal intermediate filaments. *Annu. Rev. Neurosci.* 19:187–217

Leigh PN, Swash M. 1991. Cytoskeletal pathology in motor neuron diseases. *Adv. Neurol.* 56:115–24

Leigh PN, Whitwell H, Garofalo O, Buller J, Swash M, et al. 1991. Ubiquitin-immunoreactive intraneuronal inclusions in amyotrophic lateral sclerosis. Morphology, distribution, and specificity. *Brain* 114:775–88

Li M, Ona VO, Guegan C, Chen M, Jackson-Lewis V, et al. 2000. Functional role of caspase-1 and caspase-3 in an ALS transgenic mouse model. *Science* 288:335–39

Lin CL, Bristol LA, Jin L, Dykes-Hoberg M, Crawford T, et al. 1998. Aberrant RNA processing in a neurodegenerative disease: the cause for absent EAAT2, a glutamate transporter, in amyotrophic lateral sclerosis. *Neuron* 20:589–602

Lino MM, Schneider C, Caroni P. 2002. Accumulation of SOD1 mutants in postnatal motoneurons does not cause motoneuron pathology or motoneuron disease. *J. Neurosci.* 22:4825–32

Liochev SI, Fridovich I. 2000. Copper- and zinc-containing superoxide dismutase can act as a superoxide reductase and a superoxide oxidase. *J. Biol. Chem.* 275:38482–85

Liu H, Zhu H, Eggers DK, Nersissian AM, Faull KF, et al. 2000. Copper(2+) binding to the surface residue cysteine 111 of His46Arg human copper-zinc superoxide dismutase, a familial amyotrophic lateral sclerosis mutant. *Biochemistry* 39:8125–32

Magavi SS, Macklis JD. 2001. Manipulation of neural precursors in situ: induction of neurogenesis in the neocortex of adult mice. *Neuropsychopharmacology* 25:816–35

Mather K, Martin JE, Swash M, Vowles G, Brown A, Leigh PN. 1993. Histochemical and immunocytochemical study of ubiquitinated neuronal inclusions in amyotrophic lateral sclerosis. *Neuropathol. Appl. Neurobiol.* 19:141–45

Mattiazzi M, D'Aurelio M, Gajewski CD, Martushova K, Kiaei M, et al. 2002. Mutated human SOD1 causes dysfunction of oxidative

phosphorylation in mitochondria of transgenic mice. *J. Biol. Chem.* 277:29626–33

McGeer EG, McGeer PL. 1999. Brain inflammation in Alzheimer disease and the therapeutic implications. *Curr. Pharm. Des.* 5:821–36

Mersiyanova IV, Perepelov AV, Polyakov AV, Sitnikov VF, Dadali EL, et al. 2000. A new variant of Charcot-Marie-Tooth disease type 2 is probably the result of a mutation in the neurofilament-light gene. *Am. J. Hum. Genet.* 67:37–46

Mulder DW, Kurland LT. 1987. Motor neuron disease: epidemiologic studies. *Adv. Exp. Med. Biol.* 209:325–32

Mulder DW, Kurland LT, Offord KP, Beard CM. 1986. Familial adult motor neuron disease: amyotrophic lateral sclerosis. *Neurology* 36:511–17

Nagai M, Aoki M, Miyoshi I, Kato M, Pasinelli P, et al. 2001. Rats expressing human cytosolic copper-zinc superoxide dismutase transgenes with amyotrophic lateral sclerosis: associated mutations develop motor neuron disease. *J. Neurosci.* 21:9246–54

Nagano S, Satoh M, Sumi H, Fujimura H, Tohyama C, et al. 2001. Reduction of metallothioneins promotes the disease expression of familial amyotrophic lateral sclerosis mice in a dose-dependent manner. *Eur. J. Neurosci.* 13:1363–70

Nguyen MD, Julien JP, Rivest S. 2001a. Induction of proinflammatory molecules in mice with amyotrophic lateral sclerosis: no requirement for proapoptotic interleukin-1beta in neurodegeneration. *Ann. Neurol.* 50:630–39

Nguyen MD, Lariviere RC, Julien JP. 2001b. Deregulation of Cdk5 in a mouse model of ALS: toxicity alleviated by perikaryal neurofilament inclusions. *Neuron* 30:135–47

Niwa J, Ishigaki S, Hishikawa N, Yamamoto M, Doyu M, et al. 2002. Dorfin ubiquitylates mutant SOD1 and prevents mutant SOD1-mediated neurotoxicity. *J. Biol. Chem.* 277:36793–98

Oosthuyse B, Moons L, Storkebaum E, Beck H, Nuyens D, et al. 2001. Deletion of the hypoxia-response element in the vascular endothelial growth factor promoter causes motor neuron degeneration. *Nat. Genet.* 28:131–38

Oppenheim RW. 1996. Neurotrophic survival molecules for motoneurons: an embarrassment of riches. *Neuron* 17:195–97

Otomo A, Hadano S, Okada T, Mizumura H, Kunita R, et al. 2003. ALS2, a novel guanine nucleotide exchange factor for the small GTPase Rab5, is implicated in endosomal dynamics. *Hum. Mol. Genet.* 12:1671–87

Palmiter RD. 1998. The elusive function of metallothioneins. *Proc. Natl. Acad. Sci. USA* 95:8428–30

Pasinelli P, Houseweart MK, Brown RH, Jr., Cleveland DW. 2000. Caspase-1 and -3 are sequentially activated in motor neuron death in Cu, Zn superoxide dismutase-mediated familial amyotrophic lateral sclerosis. *Proc. Natl. Acad. Sci. USA* 97:13901–6

Pramatarova A, Laganiere J, Roussel J, Brisbois K, Rouleau GA. 2001. Neuron-specific expression of mutant superoxide dismutase 1 in transgenic mice does not lead to motor impairment. *J. Neurosci.* 21:3369–74

Puls I, Jonnakuty C, LaMonte BH, Holzbaur EL, Tokito M, et al. 2003. Mutant dynactin in motor neuron disease. *Nat. Genet.* 33:455–56

Rae TD, Schmidt PJ, Pufahl RA, Culotta VC, O'Halloran TV. 1999. Undetectable intracellular free copper: the requirement of a copper chaperone for superoxide dismutase. *Science* 284:805–8

Raoul C, Estevez AG, Nishimune H, Cleveland DW, deLapeyriere O, et al. 2002. Motoneuron death triggered by a specific pathway downstream of Fas: potentiation by ALS-linked SOD1 mutations. *Neuron* 35:1067–83

Reaume AG, Elliott JL, Hoffman EK, Kowall NW, Ferrante RJ, et al. 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

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. USA* 92:689–93

Robertson J, Doroudchi MM, Nguyen MD, Durham HD, Strong MJ, et al. 2003. A neurotoxic peripherin splice variant in a mouse model of ALS. *J. Cell Biol.* 160:939–49

Rosen DR, Siddique T, Patterson D, Figlewicz DA, Sapp P, et al. 1993. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* 362:59–62

Rothstein JD, Dykes-Hoberg M, Pardo CA, Bristol LA, Jin L, et al. 1996. Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. *Neuron* 16:675–86

Rothstein JD, Kuncl R, Chaudhry V, Clawson L, Cornblath DR, et al. 1991. Excitatory amino acids in amyotrophic lateral sclerosis: an update. *Ann. Neurol.* 30:224–25

Rothstein JD, Tsai G, Kuncl RW, Clawson L, Cornblath DR, et al. 1990. Abnormal excitatory amino acid metabolism in amyotrophic lateral sclerosis. *Ann. Neurol.* 28:18–25

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

Ruddy DM, Parton MJ, Al-Chalabi A, Lewis CM, Vance C, et al. 2003. Two families with familial amyotrophic lateral sclerosis are linked to a novel locus on chromosome 16q. *Am. J. Hum. Genet.* 73:390–96

Sapp PC, Hosler BA, McKenna-Yasek D, Chin W, Gann A, et al. 2003. Identification of two novel loci for dominantly inherited familial amyotrophic lateral sclerosis. *Am. J. Hum. Genet.* 73:397–403

Selkoe DJ. 2001. Presenilin, Notch, and the genesis and treatment of Alzheimer's disease. *Proc. Natl. Acad. Sci. USA* 98:11039–41

Shaw PJ, Forrest V, Ince PG, Richardson JP, Wastell HJ. 1995. CSF and plasma amino acid levels in motor neuron disease: elevation of CSF glutamate in a subset of patients. *Neurodegeneration* 4:209–16

Shibata N, Asayama K, Hirano A, Kobayashi M. 1996. Immunohistochemical study on superoxide dismutases in spinal cords from autopsied patients with amyotrophic lateral sclerosis. *Dev. Neurosci.* 18:492–98

Shinder GA, Lacourse MC, Minotti S, Durham HD. 2001. Mutant Cu/Zn-superoxide dismutase proteins have altered solubility and interact with heat shock/stress proteins in models of amyotrophic lateral sclerosis. *J. Biol. Chem.* 276:12791–96

Son M, Fathallah-Shaykh HM, Elliott JL. 2001. Survival in a transgenic model of FALS is independent of iNOS expression. *Ann. Neurol.* 50:273

Steffan JS, Bodai L, Pallos J, Poelman M, McCampbell A, et al. 2001. Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in Drosophila. *Nature* 413:739–43

Subramaniam JR, Lyons WE, Liu J, Bartnikas TB, Rothstein J, et al. 2002. Mutant SOD1 causes motor neuron disease independent of copper chaperone-mediated copper loading. *Nat. Neurosci.* 5:301–7

Takahashi R, Yokoji H, Misawa H, Hayashi M, Hu J, Deguchi T. 1994. A null mutation in the human CNTF gene is not causally related to neurological diseases. *Nat. Genet.* 7:79–84

Takeuchi H, Kobayashi Y, Ishigaki S, Doyu M, Sobue G. 2002. Mitochondrial localization of mutant superoxide dismutase 1 triggers caspase-dependent cell death in a cellular model of familial amyotrophic lateral sclerosis. *J. Biol. Chem.* 277:50966–72

Tanaka K, Watase K, Manabe T, Yamada K, Watanabe M, et al. 1997. Epilepsy and exacerbation of brain injury in mice lacking the glutamate transporter GLT-1. *Science* 276:1699–702

Tomkins J, Usher P, Slade JY, Ince PG, Curtis A, et al. 1998. Novel insertion in the KSP region of the neurofilament heavy gene in amyotrophic lateral sclerosis (ALS). *NeuroReport* 9:3967–70

Trotti D, Aoki M, Pasinelli P, Berger UV, Danbolt NC, et al. 2001. Amyotrophic lateral sclerosis-linked glutamate transporter

mutant has impaired glutamate clearance capacity. *J. Biol. Chem.* 276:576–82

Van Den Bosch L, Tilkin P, Lemmens G, Robberecht W. 2002. Minocycline delays disease onset and mortality in a transgenic model of ALS. *NeuroReport* 13:1067–70

Vukosavic S, Stefanis L, Jackson-Lewis V, Guegan C, Romero N, et al. 2000. Delaying caspase activation by Bcl-2: A clue to disease retardation in a transgenic mouse model of amyotrophic lateral sclerosis. *J. Neurosci.* 20:9119–25

Wang J, Slunt H, Gonzales V, Fromholt D, Coonfield M, et al. 2003. Copper-binding-site-null SOD1 causes ALS in transgenic mice: aggregates of non-native SOD1 delineate a common feature. *Hum. Mol. Genet.* 12:2753–64

Wang J, Xu G, Gonzales V, Coonfield M, Fromholt D, et al. 2002. Fibrillar inclusions and motor neuron degeneration in transgenic mice expressing superoxide dismutase 1 with a disrupted copper-binding site. *Neurobiol. Dis.* 10:128–38

Wichterle H, Lieberam I, Porter JA, Jessell TM. 2002. Directed differentiation of embryonic stem cells into motor neurons. *Cell* 110:385–97

Wiedau-Pazos M, Goto JJ, Rabizadeh S, Gralla EB, Roe JA, et al. 1996. Altered reactivity of superoxide dismutase in familial amyotrophic lateral sclerosis. *Science* 271:515–18

Wilhelmsen KC. 1997. Disinhibition-dementia-parkinsonism-amyotrophy complex (DDPAC) is a non-Alzheimer's frontotemporal dementia. *J. Neural Transm. Suppl.* 49:269–75

Williamson TL, Bruijn LI, Zhu Q, Anderson KL, Anderson SD, et al. 1998. Absence of neurofilaments reduces the selective vulnerability of motor neurons and slows disease caused by a familial amyotrophic lateral sclerosis-linked superoxide dismutase 1 mutant. *Proc. Natl. Acad. Sci. USA* 95:9631–36

Williamson TL, Corson LB, Huang L, Burlingame A, Liu J, et al. 2000. Toxicity of ALS-linked SOD1 mutants. *Science* 288:399

Wong PC, Pardo CA, Borchelt DR, Lee MK, Copeland NG, et al. 1995. An adverse property of a familial ALS-linked SOD1 mutation causes motor neuron disease characterized by vacuolar degeneration of mitochondria. *Neuron* 14:1105–16

Wong PC, Waggoner D, Subramaniam JR, Tessarollo L, Bartnikas TB, et al. 2000. Copper chaperone for superoxide dismutase is essential to activate mammalian Cu/Zn superoxide dismutase. *Proc. Natl. Acad. Sci. USA* 97:2886–91

Yamanaka K, Vande Velde C, Bertini E, Boespflug-Tanguy O, Cleveland DW. 2003. Unstable mutants in the peripheral endosomal membrane component ALS2 cause early onset motor neuron disease. *Proc. Natl. Acad. Sci.* 100:16041–46

Yang Y, Hentati A, Deng HX, Dabbagh O, Sasaki T, et al. 2001. The gene encoding alsin, a protein with three guanine-nucleotide exchange factor domains, is mutated in a form of recessive amyotrophic lateral sclerosis. *Nat. Genet.* 29:160–65

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. USA* 96:13496–500

Zhu S, Stavrovskaya IG, Drozda M, Kim BY, Ona V, et al. 2002. Minocycline inhibits cytochrome c release and delays progression of amyotrophic lateral sclerosis in mice. *Nature* 417:74–78
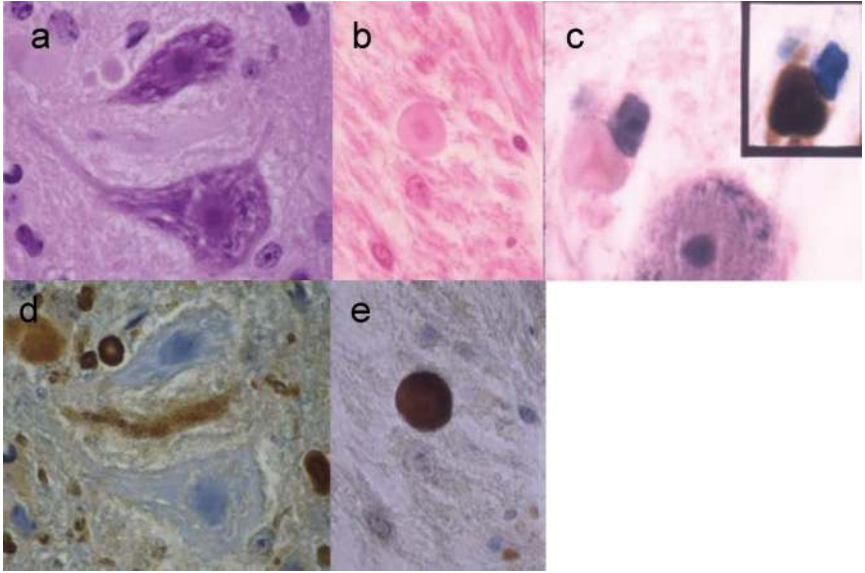
Figure 1   SOD1 reactive inclusions in both motor neurons and astrocytes. (*A*) Motor neuron from an SOD1^{G85R} ALS mouse. (*B*) Motor neuron from an ALS patient with SOD1 frameshift mutation at position 126. (*C*) Astrocyte from a SOD1^{G85R} mouse. Top row (*A*, *B*, *C*) is stained with hematoxylin and eosin. Bottom row (*D*, *E*) and the insert in *C* represent the same sections stained with an antibody to SOD1. These inclusions are also immunoreactive with antibodies to ubiquitin (not shown). Modified with permission from Bruijn et al. 1997b, 1998.
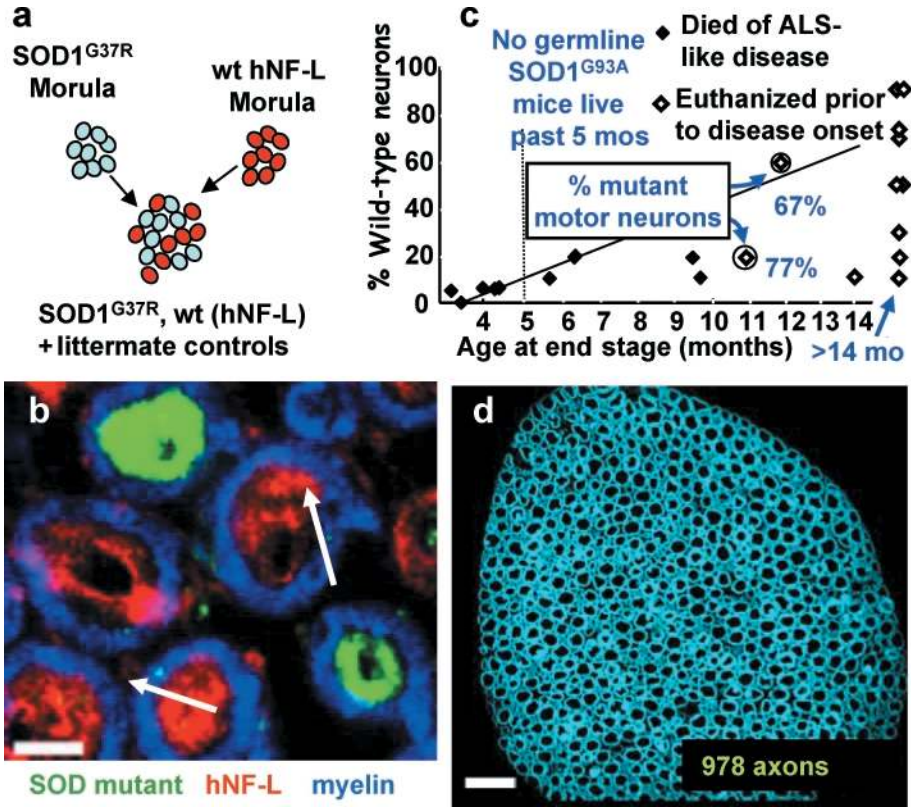
Figure 2 Noncell autonomous toxicity of ALS-causing SOD1 mutants. (*A*) Morula aggregation to produce chimeric mice in which the wild-type neurons were marked by a trace level of human neurofilament-L (NF-L), and mutant neurons and nonneurons were marked by mutant human SOD1. (*B–C*) Even though 30% of mutant motor neurons expressed SOD1[G37R], none were killed in one chimeric animal even six months after all mice that expressed the mutant systemically had died from motor neuron loss. (*B*) Immunofluorescent localization of mutant SOD1 (*green*), all axons [with an antibody specific for human NF-L (*red*) and myelin (*blue*)] in a lumbar motor root. (*C*) Robust extension of the life span of chimeric mice with a high proportion of mutant neurons. Chimeras were constructed similar to the scheme in (*A*) except using SOD1[G93A] mutant morulas. (*D*) There were no signs of degeneration or axon loss, with 978 axons present (normal animals have 927, +/– 99, axons in this root). Scale bar is 40 microns. Reproduced with permission from Clement et al. (2003).
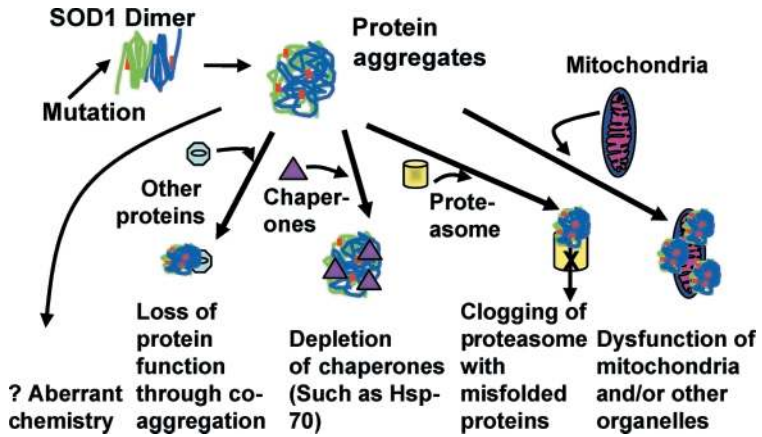
Figure 3 Putative toxicities of protein aggregates. Aberrant chemistry, loss of protein function, depletion of chaperones, loss of proteasome function, and dysfunction of mitochondria are all putative toxicities of protein aggregates.
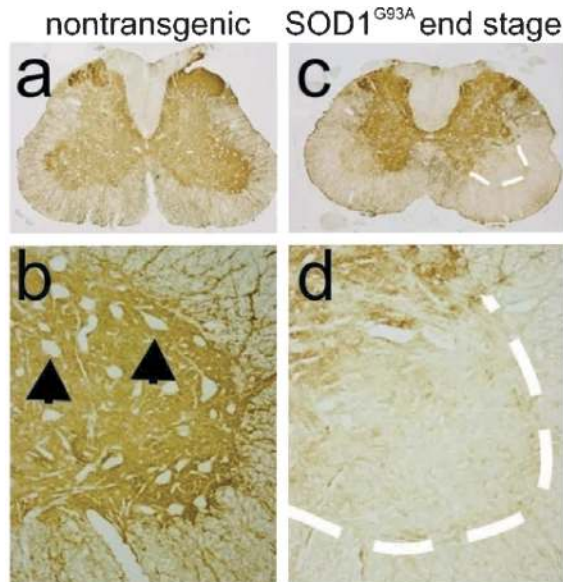

Figure 4 Excitoxicity in ALS. Selective loss of the glial glutamate transporter EAAT2 in the anterior horn during disease within an ALS-linked SOD1 mutant. (*A*) Nearly ubiquitous expression (*brown*) of the glutamate transporter EAAT2 in the gray matter of the spinal cord in nontransgenic animals. (*B*) Higher magnification view, showing EAAT2 staining surrounding the motor neurons (*arrows*). (*C–D*) Striking loss of EAAT2 staining in the anterior horn of end-stage SOD1^{G93A} rats. Reproduced with permission from Howland et al. (2002).
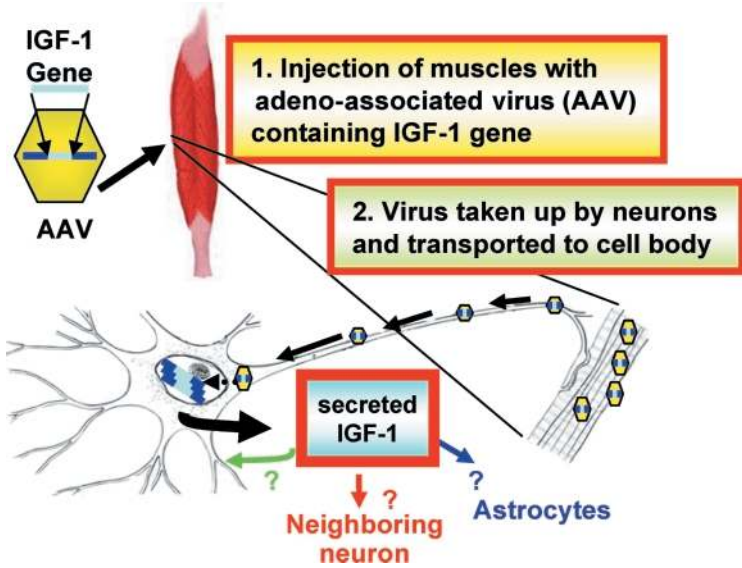
Figure 5 Viral delivery of neurotrophins to motor neurons slows ALS-like disease in an SOD1 mutant model, presumably by forcing the local production by motor neurons within the spinal cord.
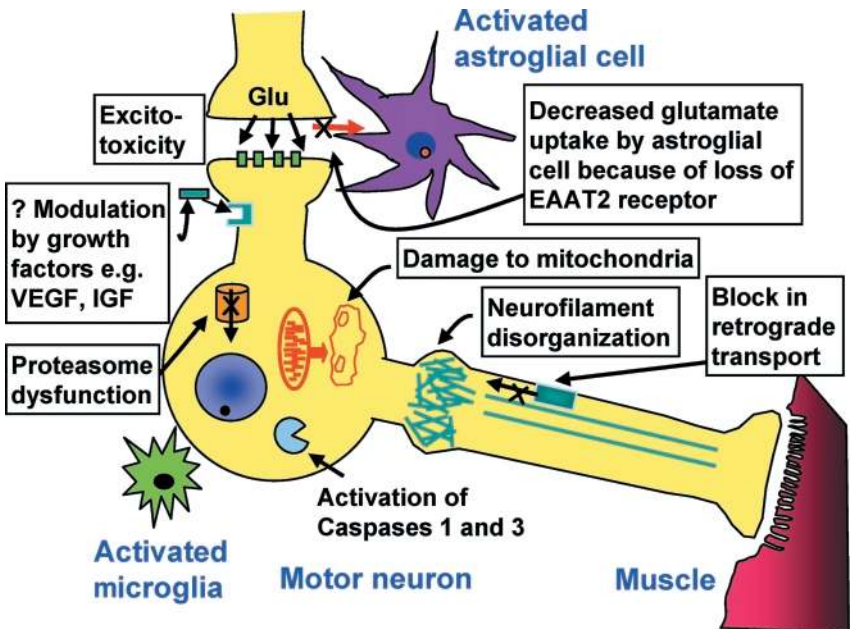

Figure 6 Convergence of multiple pathways that may damage the motor neuron.