

Current Hypotheses for the Underlying Biology of Amyotrophic Lateral Sclerosis

Jeffrey D. Rothstein, MD, PhD

The mechanisms involved in selective motor neuron degeneration in amyotrophic lateral sclerosis remain unknown more than 135 years after the disease was first described. Although most cases have no known cause, mutations in the gene encoding Cu/Zn superoxide dismutase (*SOD1*) have been implicated in a fraction of familial cases of the disease. Transgenic mouse models with mutations in the *SOD1* gene and other ALS genes develop pathology reminiscent of the disorder, including progressive death of motor neurons, and have provided insight into the pathogenesis of the disease but have consistently failed to predict therapeutic efficacy in humans. However, emerging research has demonstrated that mutations and pathology associated with the TDP-43 gene and protein may be more common than *SOD1* mutations in familial and sporadic ALS. Putative mechanisms of toxicity targeting motor neurons include oxidative damage, accumulation of intracellular aggregates, mitochondrial dysfunction, defects in axonal transport, growth factor deficiency, aberrant RNA metabolism, glial cell pathology, and glutamate excitotoxicity. Convergence of these pathways is likely to mediate disease onset and progression.

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Amyotrophic lateral sclerosis (ALS), more familiarly known in the United States as Lou Gehrig's disease in honor of the famous baseball player afflicted with the disease in the 1930s, is the most common adult motor neuron disease.¹ With a typical age of onset between 50 and 60 years and a prevalence of 2 per 100,000 individuals, ALS is associated with a lifetime risk of approximately 1 in 2,000 individuals.^{1,2} First described by the French neurologist Jean-Martin Charcot in 1869, ALS is characterized by selective death of upper and lower motor neurons, causing progressive muscle atrophy, weakness, and spasticity. Denervation of the respiratory muscles is generally the fatal event and, in most cases, occurs within 5 years of disease onset.

Although no genetic component is apparent in 90 to 95% of cases of ALS,¹ referred to as sporadic, the remaining 5 to 10% of patients inherit the disease, typically in an autosomal dominant manner. Approximately 20% of familial cases are caused by mutations in the Cu/Zn superoxide dismutase (*SOD1*) gene, and the development of transgenic mouse models of human *SOD1* mutations opened an area of intense investigation into the pathogenesis of familial ALS.³ Because the sporadic and familial forms of ALS are clinically similar, progress in elucidating the mechanisms under-

lying familial ALS may provide insight into both forms of the disease.² Moreover, a recent study identified an aberrant *SOD1*-containing protein in spinal cord tissue from patients with sporadic ALS and from patients with familial ALS, suggesting a possible role for *SOD1* in both forms of the disease.⁴ However, the role of biochemically altered *SOD1* in sporadic ALS is highly speculative and not supported by strong human or animal data. Most recently, mutations of the RNA-metabolizing protein, TDP-43, have been uncovered by multiple groups worldwide in several rare autosomal dominant familial cases of ALS,^{5–8} and a pathological alteration of this protein is seen in most sporadic ALS cases, potentially making TDP-43 enormously significant in understanding sporadic and familial ALS. This review discusses the most recent hypotheses for the pathogenesis of ALS, lessons learned from in vitro and *SOD1* mouse models, and new hypotheses based on TDP-43 mutations.

Mutant *SOD1* Is Toxic to Motor Neurons Through Mechanisms Other Than the Loss of *SOD1* Activity

In 1993, the discovery of missense mutations in the *SOD1* gene in familial cases of ALS⁹ directed clinical

From the Department of Neurology and Neuroscience, Brain Science Institute, Johns Hopkins University, Baltimore, MD.

Address correspondence to Dr Rothstein, Department of Neurology and Neuroscience, Johns Hopkins University, 600 North Wolfe Street, Meyer 6-109, Baltimore, MD 21287. E-mail: jrothstein@jhmi.edu

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research efforts toward identifying the mechanisms by which mutant *SOD1* confers selective toxicity in motor neurons. Currently, more than 114 different mutations distributed throughout the 153-amino acid *SOD1* polypeptide have been linked to ALS³; the list of mutations is continually updated on the online database for ALS genetics (<http://www.alsod.org>).² A ubiquitously expressed cytoplasmic enzyme that functions normally to convert superoxide anions to hydrogen peroxide, *SOD1* was not an intuitive candidate for mediating motor neuron degeneration.¹

An initial hypothesis suggested that mutant *SOD1* reduced *SOD1* activity, promoting accumulation of toxic superoxide radicals.⁹ However, transgenic mice expressing familial ALS-linked mutant *SOD1*^{G93A} (ie, glycine substituted for alanine at position 93), *SOD1*^{G37R}, or *SOD1*^{G85R} demonstrated progressive loss of motor neurons despite unchanged or enhanced *SOD1* enzymatic activity.^{10–12} Moreover, ablation or overexpression of wild-type *SOD1* in mutant mice did not affect disease progression.¹³ Thus, mutant *SOD1* confers toxic properties without altering enzymatic activity. Current hypotheses for the underlying biology of ALS include oxidative damage, accumulation of intracellular aggregates, mitochondrial dysfunction, defects in axonal transport, growth factor deficiency, astroglial cell pathology, and glutamate excitotoxicity.

Oxidative Damage

The hypothesis that oxidative damage mediates mutant *SOD1* toxicity was based on the proposal that mutation-induced structural changes in the *SOD1* polypeptide exposed the active copper site to aberrant substrates.¹⁴ The peroxidase hypothesis suggested that *SOD1* mutations catalyzed copper-mediated conversion of hydrogen peroxide to reactive hydroxyl radicals, promoting a cascade of oxidative damage.¹⁵ Increased levels of oxidized products in parallel with disease progression were reported in *SOD1*^{G93A} mice¹⁶ but not in *SOD1*^{G37R} mice.¹⁷ Thus, the role of hydrogen peroxide as an aberrant substrate remains unclear. Peroxynitrite is another candidate substrate, with nitration of tyrosine residues in target proteins being the destructive end result.¹⁴ Increased levels of 3-nitrotyrosine, a marker for peroxynitrite-mediated oxidative damage, have been reported in *SOD1* mice¹⁷ and in ALS patients,¹⁸ but protein targets remain elusive.

A similarity between the peroxidase and the peroxynitrite hypotheses is the mediation of oxidative damage by copper. Delivery of copper to *SOD1* enzymes in motor neurons requires the copper chaperone for *SOD1* (CCS). Gene ablation of CCS had no effect on disease onset or progression in *SOD1* mice, suggesting that CCS-dependent copper loading does not mediate *SOD1* mutant toxicity.¹⁹ Although the possibility of a CCS-independent copper loading pathway cannot

be ruled out because residual *SOD1* activity is measurable (10–20%) in tissues from CCS-null mice, copper-mediated oxidative damage may play a limited role in ALS pathogenesis.

Intracellular Aggregates

A common feature of neurodegenerative disorders, intracellular aggregates represent another candidate mechanism by which mutant *SOD1* might confer toxicity in motor neurons.¹³ Insoluble *SOD1*-containing aggregates characteristic of familial ALS have been identified in motor neurons in *SOD1* mice. Moreover, aggregates appeared before or coincident with onset of disease symptoms and accumulated with disease progression, suggesting that *SOD1* aggregation may be an early event in disease pathogenesis. Intracellular aggregates may mediate motor neuron degeneration through several putative mechanisms, including sequestration of essential cellular components,¹³ reduced chaperone activity,²⁰ and impaired capacity of the ubiquitin-proteasome pathway.²¹ Ubiquitin-containing aggregates are characteristic of both familial and sporadic ALS, suggesting a shared pathogenic link.²¹

Mitochondrial Dysfunction

Mitochondrial dysfunction is common in many neurodegenerative disorders.³ Mitochondrial abnormalities such as swelling and vacuolization are apparent at early, presymptomatic stages in *SOD1*^{G37R} mice¹² but are not observed in *SOD1*^{G85R} mice.¹⁰ Nonetheless, several mechanisms of mutant *SOD1*-mediated damage to mitochondria have been proposed, including disruption of energy metabolism, clogging of protein import machinery, and impaired calcium buffering.³ Oral administration of creatine in *SOD1*^{G93A} mice delayed the onset of motor deficits and increased survival of motor neurons, supporting a role for mitochondrial energy dysfunction in disease progression.²² In addition, improper transport of mitochondria through long motor neuron axons (described later) could contribute to distal axon degeneration in ways not fully understood.

Caspase-Mediated Cell Death

Apoptosis is the mechanism by which motor neurons die in *SOD1* mice. The apoptotic signals caspase-1 and -3 are sequentially activated in *SOD1* mice, with chronic activation of caspase-1 occurring as an early event before symptom onset, and activation of caspase-3 occurring much later as the final effector of cell death.²³ Consistent with these reports, intracerebroventricular delivery of a broad caspase inhibitor in *SOD1*^{G93A} mutants decreased mRNA levels of caspase-1 and -3 in spinal cord tissue.²⁴ Moreover, motor neurons were spared in mice infused with the caspase inhibitor compared with vehicle-infused mice, and disease onset and progression were delayed. Addi-

tional evidence supporting an apoptotic mechanism of cell death includes reports that overexpression of the antiapoptotic protein Bcl-2²⁵ or deletion of the proapoptotic protein Bax²⁶ preserved motor function and extended the life span in *SOD1*^{G93A} mice. Thus, mutant *SOD1* toxicity appears to be mediated, at least in part, by caspases and other apoptotic factors.

Defects in Axonal Transport

Neurofilaments are the most abundant cytoskeletal proteins in motor neurons and play a key role in stimulating axonal growth and in determining axonal diameter.²⁷ Aberrant accumulation of neurofilaments in the cell body and proximal axons of motor neurons is a hallmark of ALS. Transgenic mice with point mutations or overexpression of neurofilament subunits display neurofilament accumulation and selective motor neuron dysfunction.^{27,28} Surprisingly, overexpression of neurofilament subunits in *SOD1*^{G37R} mice did not exacerbate disease progression, but instead ameliorated motor neuron degeneration and extended the life span.²⁹ Importantly, neurofilament accumulation was increased in motor neuron cell bodies and decreased in axons. Perikaryal accumulation of neurofilaments may counterbalance mutant *SOD1* toxicity by buffering against damaging intracellular events, such as excessive calcium levels²⁹ or hyperphosphorylation of neuronal substrates by cyclin-dependent kinase 5 (CDK5).³⁰ Furthermore, decreasing the axonal burden of neurofilaments may protect motor neurons, at least in part, by enhancing axonal transport, a hypothesis supported by the observation of defects in slow axonal transport in presymptomatic mutant *SOD1* mice.³¹ Also consistent with the view that impaired axonal transport may be involved in the degeneration of motor neurons, Puls and colleagues³² identified a point mutation in the gene encoding dynactin, a protein involved in retrograde transport, in a family with an autosomal dominant form of lower motor neuron disease characterized by vocal cord paralysis. The mechanisms by which axonal transport contributes to motor neuron death are unclear but could include inefficient delivery or removal of distal mitochondria and the retrograde transport of peripherally derived trophic factors.

Growth Factors

A putative role for vascular endothelial growth factor (VEGF) in the pathogenesis of ALS emerged from the discovery of motor deficits and ALS-like pathology in a transgenic mouse model bearing a targeted deletion of the hypoxia-response element in the VEGF gene.³³ These mice displayed reductions in baseline and hypoxic VEGF expression in neural tissue, and developed progressive motor deficits and degeneration of motor neurons beginning at 5 to 7 months of age. Hypotheses for the mechanism of action of VEGF include a

direct neuroprotective effect and an indirect ability to prevent ischemic damage by regulating vascular perfusion.³³ A neuroprotective role for VEGF is supported by studies demonstrating delayed motor neuron disease onset and progression in mutant *SOD1*^{G93A} models with genetic overexpression of VEGF,³⁴ intracerebroventricular administration of VEGF,³⁵ or intramuscular delivery of VEGF-expressing lentiviral vectors.³⁶ In addition to VEGF, insulin-like growth factor-1 and glial cell line-derived neurotrophic factor have been reported to delay disease onset and progression in *SOD1*^{G93A} mice.^{37,38}

Although preclinical studies elucidate possible pathogenic pathways and therapeutic options, human trials represent the best test of a hypothesis. Insulin-like growth factor-1 provided modest clinical efficacy in one of two trials in ALS patients.^{39,40} Clinical trials of VEGF are anticipated because three single nucleotide polymorphisms in the VEGF gene were identified as significant risk factors for ALS in a large European patient cohort.⁴¹

Glial Cell Pathology

A growing number of studies support the hypothesis that selective degeneration of motor neurons in ALS is not a cell-autonomous process; specifically, neighboring astrocytes contribute to disease progression (Fig).⁴² In the mid-1990s, defects in astroglia-specific glutamate transporters were identified in a large percentage of sporadic ALS patients,⁴³ and later in all *SOD1* rodent models.^{10,44} Astrocytic inclusions are early indicators of *SOD1* mutant toxicity, preceding symptom onset and increasing with disease progression.¹⁰ Unpublished data from Don Cleveland's group show that removal of mutant *SOD1* protein from astrocytes markedly delayed disease progression, indicating that nonneuronal cells are major contributors to disease progression.

Damage within more than one cell type appears to be required, because restricted expression of mutant *SOD1* in astrocytes⁴⁵ or motor neurons⁴⁶ alone failed to induce motor deficits in *SOD1* mice. The involvement of multiple cell types in ALS pathogenesis is supported by in vitro studies and analysis of chimeric mice made of mixtures of wild-type and *SOD1* mutant-expressing cells.⁴² These studies reported pathological abnormalities and cell death in wild-type motor neurons adjacent to mutant *SOD1*-expressing nonneuronal cells. Thus, the vulnerability of motor neurons to *SOD1*-mediated toxicity appears to be influenced by the cellular environment in general and by astrocytic damage in particular.

The role of astrocytes and other glial cells in the selective degeneration of motor neurons remains unclear. Astrocytes that express mutant *SOD1* secrete factors that are toxic to motor neurons, but specific factors have not yet been identified.⁴² Potential candidates in-

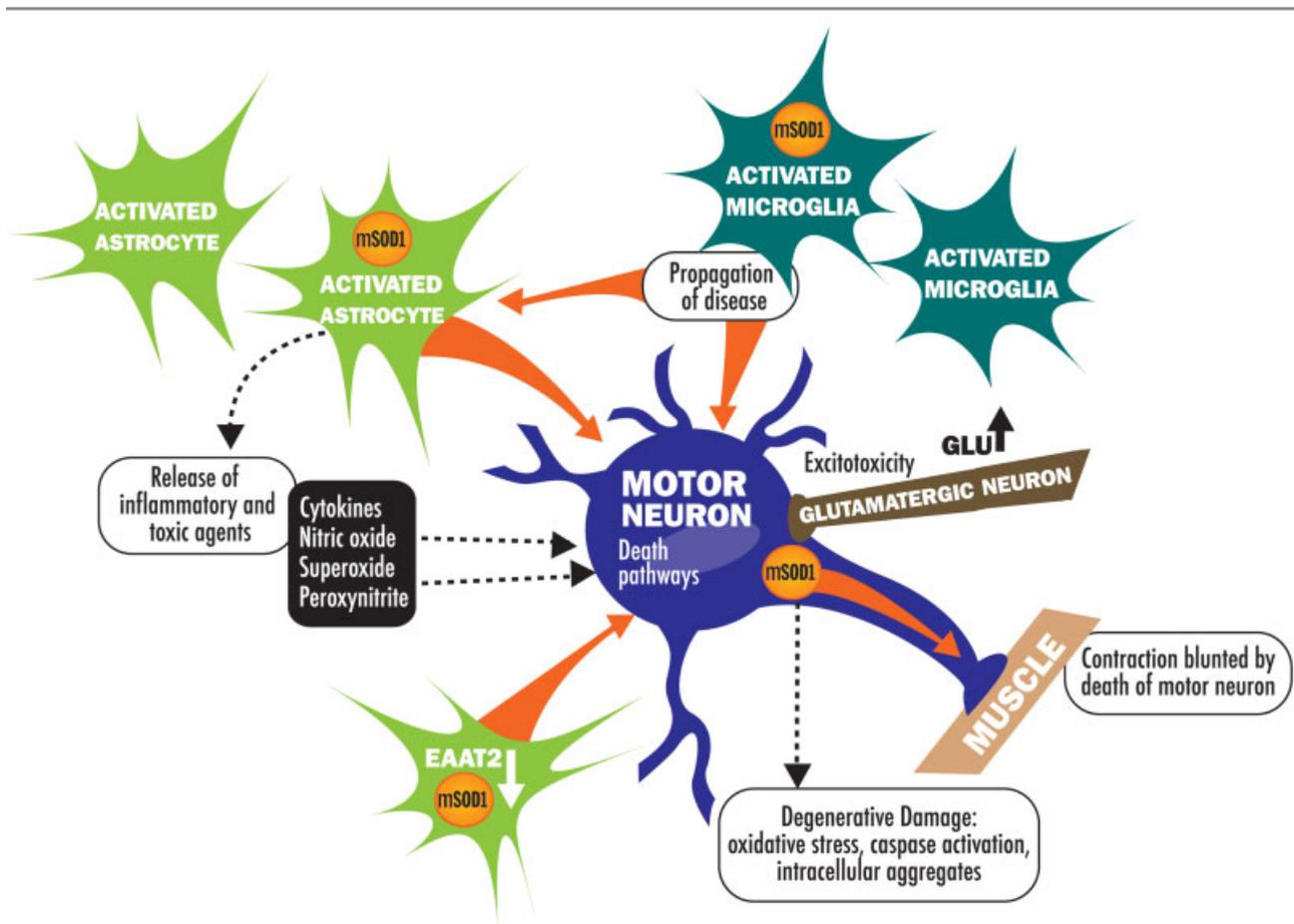
clude proinflammatory cytokines and chemokines, but genetic deletion of interleukin-1 β or tumor necrosis factor- α did not affect disease progression in *SOD1* mice. Thus, the role of proinflammatory mediators in the pathogenesis of ALS remains unsubstantiated, and astrocytes may confer toxicity to motor neurons via alternate mechanisms.

Glutamate Excitotoxicity

A well-recognized mechanism of neuronal death is glutamate excitotoxicity resulting from repetitive cell firing or the influx of excessive levels of calcium through permissive glutamate receptors.¹ Glutamate-mediated neurotoxicity was first proposed as a mechanism of motor neuron degeneration when increased levels of glutamate were discovered in the cerebrospinal fluid of ALS patients.⁴⁷ A follow-up study reported that increased cerebrospinal fluid glutamate levels were apparent in approximately 40% of nearly 400 patients with spo-

radic ALS and correlated with disease severity; this was one of the largest pathogenesis-oriented human studies in ALS.⁴⁸

Clearance of glutamate from the synaptic cleft by glutamate transporters is critical in preventing repetitive firing and subsequent excitotoxicity.¹ In motor neurons, rapid removal of synaptic glutamate is accomplished primarily by the astrocytic glutamate transporter EAAT2.⁴⁹ Depletion of EAAT2 in transgenic mice directly causes neuronal death.⁴⁹ In *SOD1*^{G85R} mutant mice at the end stage of disease, EAAT2 protein levels in the spinal cord are reduced to approximately 50% of normal.¹⁰ In *SOD1*^{G93A} transgenic rats, EAAT2 expression in the ventral horn is reduced presymptomatically and almost completely abolished by end-stage disease.⁴⁴ Trotti and colleagues⁵⁰ examined the function of EAAT2 in oocytes coexpressing *SOD1* proteins and found that mutant but not wild-type *SOD1* inactivated the transporter in the presence of hydrogen peroxide, further suggesting



*Fig. Mutant SOD1 toxicity in astrocytes promotes ALS pathogenesis.*⁴² Although mutant SOD1 inflicts cell-autonomous insults on motor neurons, these insults may be insufficient to mediate disease progression. Evidence that astrocytes promote motor neuron degeneration comes from reports that astrocytes expressing mutant SOD1 but not wild-type SOD1 decrease the survival of motor neurons by secreting several potentially toxic factor(s) into the cellular environment. In addition, mutant SOD1 selectively inactivates the glial glutamate transporter EAAT2, which is responsible for clearing synaptic glutamate from motor neurons, leading to excitotoxic death of motor neurons.

that EAAT2 is a target of mutant *SOD1* toxicity. Another study⁵¹ reported that overexpression of EAAT2 in mutant *SOD1*^{G93A} mice delayed the onset of motor deficits, decreased caspase-3 activation and aggregate formation, and partially increased neuroprotection compared with *SOD1* mutants in which EAAT2 expression was not manipulated. In addition, mutant *SOD1*^{G93A} mice treated with a β -lactam antibiotic that potently stimulates EAAT2 expression demonstrated delayed disease onset, slowed disease progression, and prolonged life span.⁵²

Analysis of motor cortex and spinal cord extracts from ALS patients demonstrated a nearly complete loss of EAAT2 protein in 25% of patients and some abnormality in EAAT2 protein expression in up to 80% of patients.⁴³ Collectively, these studies support the hypothesis that motor neuron degeneration is mediated by excitotoxic levels of extracellular glutamate resulting from selective impairment of the glial glutamate transporter EAAT2 and confirm that neuronal degeneration is not a cell-autonomous process.

TDP-43: Cellular Aggregation and RNA Metabolism

Recently, there has been extremely exciting progress in linking sporadic and familial ALS.⁵⁻⁸ An analysis of overlap between frontotemporal dementia and ALS showed a common pathology and protein, TDP-43. Cytoplasmic inclusions and altered nuclear-to-cytoplasmic immunolocalization of this poorly understood protein were discovered. Four separate reports identified gene mutations of TDP-43 in rare familial ALS cases, and putative polymorphisms of the protein have also been identified in sporadic ALS.⁵⁻⁸ The association of this mutant protein with both the rare familial form of ALS and sporadic ALS provides a strong parallel to the amyloid pathology observed in the brains of patients with Alzheimer's disease. Thus, the TDP-43 mutation and resulting alterations in protein localization and function may become the most important ALS-associated pathophysiology to date. Although the biology of TDP-43 is not likely to devalue the prior 15 years of mutant *SOD1* biology, it has the potential to supersede it and lead to an entirely new set of scientific observations and animal models, ultimately defining better therapeutics.

The discovery of these new mutations requires a better understanding of TDP-43 because little is understood about its normal biology. Analysis of the ALS-associated mutations in TDP-43 structure localize the putative protein abnormalities to the heterogeneous nuclear ribonucleoprotein binding domain of the C-terminal region of the protein, implicating abnormal RNA metabolism in the pathophysiology of mutated TDP-43.⁵³⁻⁵⁷ These new discoveries highlight reports from nearly 10 years ago that first showed aberrant

exon and intron splicing as the cause of abnormal RNA metabolism in sporadic ALS.⁵⁷ During the last decade, important genetic clues and tools for familial ALS have been elucidated. The next decade will almost certainly provide additional molecular and protein clues that will lead to better animal models, biomarker tools, and imaging ligands, all of which will significantly advance the discovery of new therapeutic agents.

Conclusion

Current hypotheses for the underlying biology of ALS represent noncompeting mechanisms that are likely to converge in various unfortunate patterns to mediate selective motor neuron degeneration.¹ Mutant *SOD1* toxicity has been linked to oxidative damage, accumulation of intracellular aggregates, mitochondrial dysfunction, defects in axonal transport, growth factor deficiency, glial cell pathology, and glutamate excitotoxicity. A convergence model of *SOD1* toxicity suggests that oxidative damage and mutant *SOD1* aggregates may increase the vulnerability of motor neurons by inhibiting chaperone and proteasome activity, at least in familial models of the disease. Intracellular aggregates may also impair mitochondrial function and disrupt neurofilament organization, which, in turn, may activate apoptotic factors and inhibit axonal transport, respectively. In addition to accumulating insults in motor neurons, similar damage in astrocytes may disrupt glutamate neurotransmission, leading to excitotoxic cell death. Putative pathogenic mechanisms mediated by mutant *SOD1* may be relevant in both familial and sporadic cases of ALS, given their clinical similarity, but the lack of concordance between *SOD1*-based animal models and human therapeutics is troubling. Newer emerging research on the biology of TDP-43 and its mutations in ALS may prove to be one of the most important advances in future ALS research.

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