Commentary

Amyotrophic lateral sclerosis: Human challenge for neuroscience

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For an uncommon disease, amyotrophic lateral sclerosis (ALS) is the object of disproportionate attention these days. Synonyms include Lou Gehrig’s disease, Charcot disease, and motor neuron disease. ALS is a research focus partly because it shares features with two more common public health problems—Alzheimer disease and Parkinson disease. All three affect the aging nervous system. All three are cruelly disabling and ultimately fatal. No therapy has stemmed the progression of any of them, and ALS advances most rapidly. All three are marked histologically by degeneration of specific sets of neurons and the changes are not denoted by any indication of chronic ischemia, inflammation, or vascular inadequacy. In all three, the neuronal degeneration is unexplained. Although the clinical manifestations differ, excitotoxicity and peroxidation have been implicated in theories of pathogenesis for all of them. In all three diseases, fewer than 10% of cases are familial, usually in an autosomal dominant pattern. Sometimes two of the three disorders occur in the same family or even the same person (1, 2). Dementia is encountered ultimately in about 25% of people with Parkinson disease, often enough to be considered an integral manifestation of the condition. ALS and dementia are also paired with disproportionate frequency (1).

ALS has proved to be a fertile field of research that covers a broad reach of modern neuroscience, drawing upon nerve growth factors, excitotoxicity (especially glutamate toxicity), free radical neurotoxicology, aberrant neuronal accumulation of neurofilaments, autoimmunity, paraneoplastic syndromes, persistent viral infection, molecular genetics, and transgenic mice (3). Even though the disease is still irreversible, these theories have led to therapeutic trials that sustain hope for patients and their families.

A new chapter started in 1991, when a multinstitutional team led by neurologists Tippu Siddique and Robert Brown mapped familial ALS to chromosome 21 (4). After a few false starts, the gene product was identified as the copper/zinc superoxide dismutase type 1 (SOD1) (5). These landmark discoveries opened new opportunities to unravel the murky genetics and pathogenesis of the disease. The findings provide the background for the paper of Ripps et al. (6) in this issue.

Of all cases of ALS, 5–10% are familial. Of the familial cases, fewer than 20% map to the SOD1 gene, so there is locus heterogeneity; the other 80% map to some other gene. There is also allelic heterogeneity; at least 24 missense mutations (7–15) and one deletion (16) have been found in the SOD1 gene. The most common point mutation is an Ala → Val substitution at codon 4 (A4V); this mutation accounts for 38% of all familial ALS (14).

Although new mutations must occur, remarkably few have been identified in people with the sporadic disease (17) and the SOD mutation does not account for the unusually high prevalence of the disease in Guam (18).

The association with SOD1 makes it possible, for the first time, to generate credible theories of the pathogenesis of familial ALS. The enzyme catalyzes the dismutation of superoxide, the cytoplasmic free radical generated in normal oxidative reactions. The products are hydrogen peroxide (H2O2) and oxygen. If SOD were inactive, the superoxide radical would accumulate and might itself be cytotoxic because it can act as either an oxidant or a reductant (19). Superoxide can also react with nitric oxide to form peroxynitrite (20), which nitrates tyrosine residues to form a nitronium-like intermediate and might, thereby, interfere with the functions of SOD or other proteins.

The amino acid sequence of SOD is known and crystallographic studies have formulated a precise picture of the normal structure. Inactivation of the enzyme could result from mutations that affect either the active site or the process of dimerization. Although the known mutations do not affect the active site, the conformation or stability might be altered. If the mutant enzyme were totally inactive, residual activity should be about 50% of normal because only one allele is abnormal in a dominant disease; a loss of that magnitude might double the concentration of superoxide. In the first studies, enzyme activity was actually about 50% of normal in circulating erythrocytes (21, 22), lymphoblastoid cell lines (23), cerebrospinal fluid of patients with familial ALS (24, 25), and the frontal cortex of deceased patients with the disease (21). Erythrocyte SOD activity was decreased in patients with any of six mutations (20).

As a consequence of the low SOD1 activity, the consensus viewed peroxidation and free radical damage as the agent of cytotoxicity. This theory of pathogenesis was reinforced when it was found that, in contrast to the familial form, SOD1 activity was normal in sporadic ALS by the same assays (21, 26). In addition, biochemical studies of brain suggested that oxidative damage was more likely in the nonfamilial disease, as assessed by protein carbonyl content (21).

However, there were reservations about this interpretation. (i) Most enzymes are normally present in excess amounts. Half-normal activity is encountered in the heterozygous carriers of autosomal recessive genes, and these carriers are typically asymptomatic. Half-normal enzyme activity is compatible with normal life. It would therefore be surprising if half-normal activity resulted in an autosomal dominant disease (although an analogy is provided by half-normal activity of the key enzyme in patients with another autosomal dominant disease, acute intermittent porphyria). (ii) Erythrocyte and cerebrospinal fluid enzyme activities are sometimes difficult to reproduce and, in two studies of familial ALS, SOD activity was normal in red blood cells (27, 28). Also, in contrast to the first reports that differentiated familial and sporadic ALS on the basis of SOD1 activity in the central nervous system (21), later investigators (29) found low SOD1 activity in the brain and spinal cord of patients with sporadic ALS.

To analyze the problem, Gurney et al. (30) used transgenic mice, introducing either of two human mutant SOD1 enzymes (A4V or G93A); the human enzyme was expressed, but at 50% of normal activity. The mouse genes also continued to function and, in one line of mice, there was overexpression of total SOD1 in affected animals. Nevertheless, the animals developed a clinical syndrome of hind limb paralysis, with histological signs of degeneration and loss of motor neurons in spinal cord. Therefore, the neurological disease could not be attributed to loss of SOD1 function; instead, there must have been a gain of some function.

Because the disease was expressed in only one line of mice, Gurney et al. (30) stated that they could not exclude the possibility that the “site of integration of...
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the mechanism, SOD is

widely distributed in diverse organs, and it

is not clear why motor neurons are so

selectively vulnerable in either the human
disease or the transgenic mouse disease. Pardo
et al. (38) found immunocytochemical evidence
of SOD in mouse motor neurons and in other central nervous
system cells that are not affected in ALS; they concluded that
"high, rather than limiting levels of SOD1 may place motor
neurons selectively at risk" in familial ALS.

On the other hand, Rothstein et al. (39) found that
apoptotic death of cultured spinal neuronal neurons was induced by
inhibition of SOD activity. Troy and Shelanski (40) found that
down-regulation of SOD induced apoptotic cell death in another
neuronal cell line. But apoptosis is not characteristic of the pathology of human ALS
(41); instead, ubiquitinated neuronal inclusions
and accumulation of neurofilaments in proximal axonal swellings (43) are
more commonly seen. Transgenic animals may prove important in
analyzing the role of neurofilaments because over-
expression of the mouse genes for neurofilament proteins leads to
degeneration of motor neurons, accumulation of neurofilaments,
and a paralytic disease (44–47). Figlewicz et al. (48) found mutations in the
C-terminal region of the human gene for
the neurofilament heavy subunit in five patients with sporadic ALS, but
they noted that no ALS families have been
mapped to 22q21, the locus for that gene.
Therefore, there have not yet been any
documented mutations of human genes
for neurofilament proteins in human ALS, even though the human SOD
mutation can lead to accumulation of neurofilaments (ref. 49 and G. A. Rouleau,
A. Clark, K. Roeke, A. Pramatarova, A. Kri-

zus, O. Suchowsky, J.-P. Julienn, & D.
Figlewicz, unpublished data) and
neurofilament mRNA levels are decreased in
the central nervous system, perhaps in a
compensatory response (50). In mice
bearing the human G37R mutation, Wong et al.
(P. C. Wong, C. A. Pardo, D. R.
Borchelt, M. K. Lee, N. G. Copeland,
N. A. Jenkins, S. S. Sisodia, D. W. Cleve-
dland, & D. L. Price, unpublished data) found
vacuolar degeneration in motor
neurons, a change attributed to mi-
tochondrial pathology.

No spontaneous animal model of ALS
is completely analogous to the human dis-
ease (27), so we can expect increasing
attention to transgenic animals. It would
be a tremendous boon if transgenic mice
provide the vehicle for the discovery of an
effective therapy (8). The transgenic mice
have already had a cautionary effect; if
the mouse disease results from a toxic
gain-of-function effect, it may be necessary
to reconsider plans already in motion to
administer SOD as a treatment (51).

I am indebted to Drs. D. L. Price and Guy
Rouleau for access to manuscripts prior to pub-
lication. E. Kandel, S. Przedborski, A. P. Hays,
N. Latov, and D. J. Lange made helpful sug-

gestions.

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