The authors went on to show that VLA-1 is crucial for protective immunity to heterologous influenza virus infection. That was made clear by using a blocking antibody to VLA-1 and by analyzing mice genetically lacking VLA-1. Interestingly, although VLA-1 knockout mice had more influenza A–specific $CD8^+$ memory T cells in the spleen than normal mice, they had no protective immunity.

What is not yet clear is exactly how VLA-1 enhances cell survival and maintains the effector phenotype. Does VLA-1 initiate an intracellular signaling process, or does attachment to collagen somehow protect these cells from other, potentially lethal, cellular interactions? The mechanism behind the decline of memory T cells over time also remains to be identified. Memory T cells may ultimately lose VLA-1 expression and return to the circulation, or the VLA-1/collagen interaction may provide only limited protection from apoptosis.

This study could enhance efforts to develop immunotherapy against T-cell-mediated diseases and create new vaccines. Treatments that interfere with the interaction of T-cell VLA-1 with collagen may diminish the immunopathological symptoms of diseases like asthma and rheumatoid arthritis.

Using the newly developed, intranasally administered, live attenuated influenza vaccine that also stimulates cellular immune responses directly in the mucosa of the respiratory tract could have added benefits over more conventional vaccines. The conventional killed influenza vaccine is administered intramuscularly and relies on the induction of humoral immune responses; the vaccine does not provide protection against new heterosubtypic influenza strains.

The live attenuated vaccine, only recently introduced into clinics, could make the difference between life and death for some individuals. A live vaccine has the benefit of more conserved, cross-protective $CD8^+$ T cell responses that could provide at least partial protection against new emerging strains. The new avian influenza is one such strain; it has the potential to cause the next influenza pandemic, and is the focus of intensive vaccine development efforts.

A note of caution is in order: repeated doses of a vaccine that maintains high numbers of memory T cells in the lung could also amplify immunopathology after repeated infection by heterologous viruses[15].

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Putting the heat on ALS

Susanna C Benn & Robert H Brown, Jr

An enhancer of the heat shock response alleviates symptoms of neurodegeneration and prolongs lifespan in a mouse model of amyotrophic lateral sclerosis—even when administered after onset (pages 402–405).

In patients with amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig disease, degeneration of motor neurons causes progressive muscular weakness, atrophy and paralysis. Respiratory failure inexorably leads to death, typically within four or five years. There is no definitive treatment for this disease; riluzole, the only drug approved for ALS, is at best palliative and prolongs survival by only 10–15%. The cause of most cases of ALS is not defined, but a small subset of cases—about 2–3%—are caused by missense mutations in the gene encoding cytosolic Cu/Zn superoxide dismutase-1 (SOD-1).

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In this issue, Kieran *et al.*[1] provide evidence that a new category of molecules may ameliorate the disease. These investigators show that daily treatments with arimoclomol, a coinducer of heat shock proteins, slows the process of motor neuron death and thereby extends survival in a transgenic $SOD1^{G93A}$ mouse model of ALS (**Fig. 1**).

Several indices of motor neuron viability, including cell counts and measurements of motor unit number and muscle fatigue index (a measure of early motor neuron dysfunction), confirm the benefit of arimoclomol, particularly for large motor neurons. The treatment is remarkable both for its degree of effectiveness and because it works on mice that are already symptomatic.

Despite intensive investigations, the salient pathogenic mechanisms have not been identified with certainty for any type of ALS. It is clear in transgenic rodents that high levels of mutant SOD-1 protein kill motor neurons in a dose-dependent manner. In these models, multiple processes contribute to the demise of motor neurons, including early misfolding and aggregation of mutant SOD-1 protein[2], excitotoxicity mediated by failing synaptic transport of glutamate into glial cells, diminished energy generation due to mitochondrial dysfunction, and impaired axonal transport.

In both rodents and humans, afflicted motor neurons prompt proliferation of surrounding astrocytes and microglial cells in affected spinal gray matter. Gene expression and biochemical studies reveal hallmarks of inflammation, such as activation of cyclooxygenase. These findings are evident in both sporadic ALS cases and those associated with mutant SOD-1. The motor neuron death process is not cell-autonomous. Rather, it is intimately related to the context of surrounding cells; cell death of motor

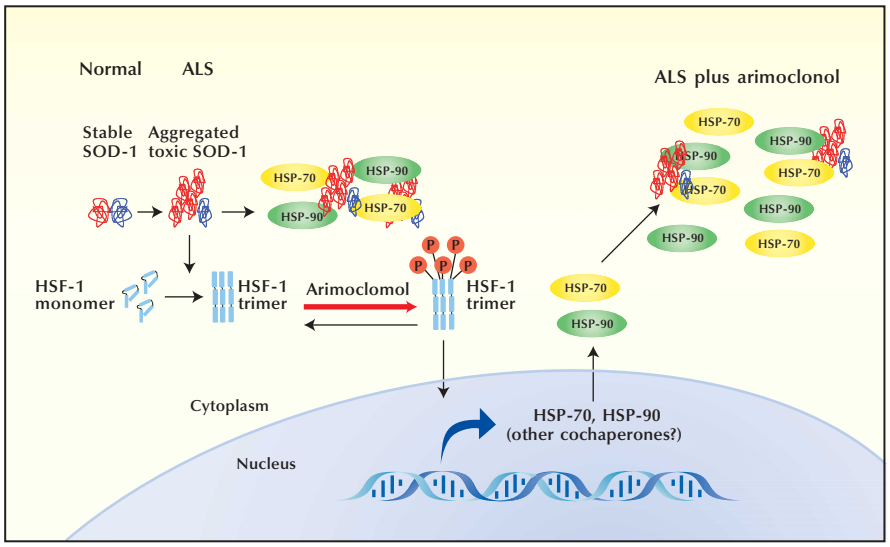


Figure 1 Stressors such as heat, oxidants, toxins and mutations trigger misfolding of mutant (red) and wild-type (blue) SOD-1; this initiates trimerization of HSF-1 monomers in the cytoplasm. HSF-1 trimers become phosphorylated and translocate to the nucleus, where they induce *Hsp* gene transcription. In ALS, aggregates of SOD-1 sequester HSP-70 and HSP-90, inhibiting the protective functions of the HSPs in normal cellular homeostasis. Kieren *et al.* show that arimoclomol promotes HSF-1 phosphorylation, enhancing the induction of HSPs, which become more numerous and available to fulfill chaperone and antiapoptotic HSP functions, including the chaperoning of misfolded SOD-1.

neurons expressing mutant SOD-1 protein can be rescued by surrounding non-neuronal, wild-type cells[3].

Several aspects of the new findings are notable. First, arimoclomol prolongs survival when administered after disease onset. To date, more than 70 drugs have been tested in these mice, but there have been no published reports of compounds that are beneficial when started after disease onset. Moreover, few compounds prolonged survival by more than 10%, even when started presymptomatically.

Second, this report provides a new therapeutic approach to this disease—enhancement of the heat shock response (**Fig. 2**). Indirectly, this supports the hypothesis that the cellular Achilles heel in hereditary (and possibly also sporadic) ALS is the accumulation of misfolded SOD-1 protein and any associated binding proteins. Such accumulations could have dire downstream consequences, including direct toxicity to subcellular organelles and proteosomal dysfunction. In this sense, Kieran *et al.* have validated earlier *in vitro* studies showing that expression of heat shock protein-70 (HSP-70) blunts the neurotoxic effects of acutely expressed mutant SOD-1 (refs. 4–6).

Third, this study suggests that manipulating the whole heat shock pathway achieves more profound neuroprotection than altering levels of individual HSP molecules. Transgenic expression of HSP-70 alone does not slow disease progression in these ALS mice. By contrast, arimoclomol does, presumably because it acts physiologically. The authors document that arimoclomol induces the phosphorylation of a heat shock protein–inducing factor (HSF-1), and thereby upregulates expression of HSP-70 and HSP-90 (ref. 7). The molecule may also upregulate cochaperones such as HSP-40, CHIP and Bag.

Fourth, the study suggests that other activators of the heat shock response may also prove beneficial in the *SOD1*^{G93A} mice and related diseases. Examples include carbenoxolone[8], an antiulcer drug that directly activates the HSP-70 promoter; the herbal compound celastrol[9]; and the anti-inflammatory drug indomethacin[10], which, among other mechanisms, activates DNA binding of HSF-1.

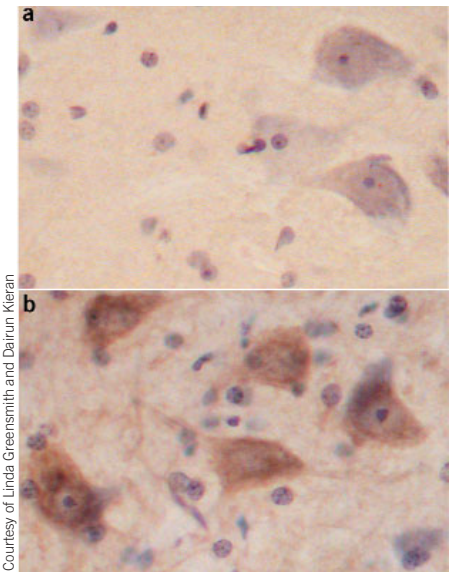
This report should generate several follow-up investigations. Most important, it will be crucial to show that these observations are robust. The present findings are predicated on a relatively small number of mice in each treatment arm, so replication is of paramount importance.

It will also be important to study further how arimoclomol works. For instance, which kinases and phosphorylases impinge on HSF-1, and are they also drug targets? Of interest is the possibility that substances that coordinately upregulate HSPs and their cochaperones will be therapeutic in other neurodegenerative diseases, many of which are a consequence of protein instability (for example, amyloid-β in Alzheimer disease, α-synuclein in Parkinson disease, huntingtin in Huntington disease, and prion protein in Creutzfeld-Jacob disease).

It will be important to establish whether enhancement of heat shock and protein chaperone responses is beneficial in cases of ALS that do not arise from *SOD1* gene mutations. Because the cellular and biochemical phenotypes of sporadic and familial ALS overlap considerably, one anticipates that HSF-1 induction will be beneficial for all types of ALS. In the absence, however, of an animal model for sporadic ALS (see page 347), this will only be resolved with human clinical trials.

The neuroprotective effects of HSP activation may also extend to conditions such as stroke, or trauma of the brain and spinal cord. Bimoclomol, an analog of arimoclomol, induces HSP-70 expression and prevents neuronal degeneration in a neonatal model of motor[11] and sensory[12] neuron death, and in models of diabetic neuropathies[13], cardiovascular injury[14], ischemia and reperfusion injury[15].

Knowledge of the genetic defects causing neurodegeneration in ALS have provided



Courtesy of Linda Greensmith and Dairun Kieran

Figure 2 Upregulating HSPs. (**a**) The lumbar spinal cord of a SOD1^{G93A} mouse immunostained for HSP-90 and lightly counterstained to recognize cells. (**b**) The same region of a SOD1^{G93A} mouse treated with arimoclomal. The region contains more neurons and has more intense HSP90 staining.

insight into molecular pathogenesis, and have led to the development of disease models in transgenic mice and simple *in vitro* systems. The ultimate hope for these powerful tools is that they will facilitate the discovery of new drugs and therapies. The present report by Kieran *et al.* is an important step in this direction.

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Sporadic ALS: blame it on the editor

Stuart A Lipton

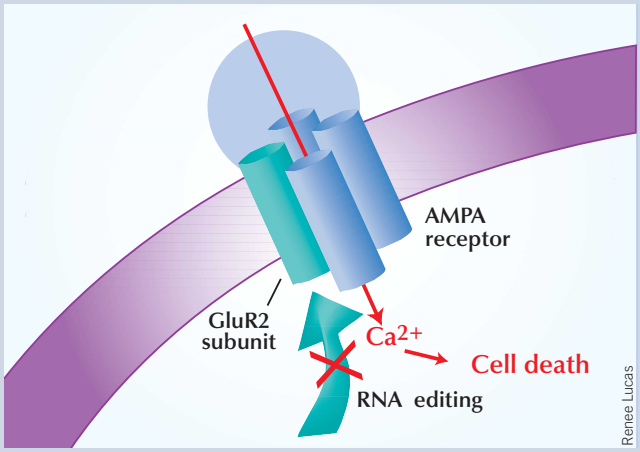
The sporadic form of amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig disease, accounts for approximately 95% of all cases. Although mice with mutations in the gene encoding superoxide dismutase-1 (SOD-1) are used to study hereditary ALS, there is no universally accepted model for sporadic ALS. Circumstantial evidence, however, has pointed to glutamate, the major excitatory transmitter of the brain, as a culprit in sporadic cases of ALS[1,2]. Excessive activation of glutamate receptors initiates excessive calcium influx, activation of cell death pathways and production of free radicals.

This excitotoxic hypothesis now has a molecular underpinning in individuals with ALS. In a recent issue of *Nature*, Kawahara *et al.*[3] provide evidence that abnormal post-transcriptional modification of mRNA encoding a glutamate receptor subunit may promote sporadic ALS. This abnormal RNA editing may lead to the exaggerated glutamate receptor activity and calcium influx that promotes excitotoxicity.

During RNA editing, gene-specified codons are altered by RNA-dependent deaminases (such as adenosine deaminase). In the case of the GluR2 glutamate receptor subunit, a crucial glutamine to arginine conversion in the putative second membrane domain affects the properties of the ion channel associated with the AMPA-type glutamate receptor[4]; among the effects are a drastic reduction in the permeability of this channel to calcium. In the absence of RNA editing, AMPA channels with GluR2 subunits are much more permeable to calcium.

The authors observed abnormal editing of GluR2 in spinal motoneurons from five individuals with ALS. But they saw no abnormal editing in any of the control postmortem specimens, including those with normal nervous systems and those with other neurologic disorders. Because of this abnormal editing, large calcium influxes can occur through AMPA-type glutamate receptors in these motoneurons, rendering them susceptible to cell death. This deficiency in RNA editing could potentially contribute to or even explain many cases of sporadic ALS.

The findings also point the way to mouse models of sporadic disease. An existing transgenic mouse with calcium-permeable GluR2 subunits develops a motoneuron disease late in life[5]. This mouse may be a reasonable model with which to study potential therapeutic drug responses in sporadic ALS. In the future, expressing unedited GluR2 subunits specifically in spinal motoneurons might generate an even better animal model.



One caveat of the study is that Kawahara *et al.* found that only spinal motoneurons were affected by aberrant RNA editing, and not upper motoneurons in the cerebral cortex; yet ALS affects both populations of neurons. Abnormal RNA editing, therefore, cannot explain all motoneuron loss in ALS. This caveat also raises the possibility that we are looking at the effect rather than the cause. Could another cause of the disease, such as oxidative or nitrosative stress, result in abnormal RNA editing and thus contribute to the demise of spinal motoneurons? Another question is whether other normally edited RNAs are also affected in sporadic cases of ALS, such as the GluR6 subunit of kainite-type glutamate receptors.

Whatever the mechanism, the new work raises the hope that studying dysfunction of RNA-editing enzymes may produce a new therapeutic target for sporadic ALS. Counteracting overly active, calcium-permeable glutamate receptors may also offer a new form of therapeutic intervention for the disease.

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