Mouse Model Found for ALS

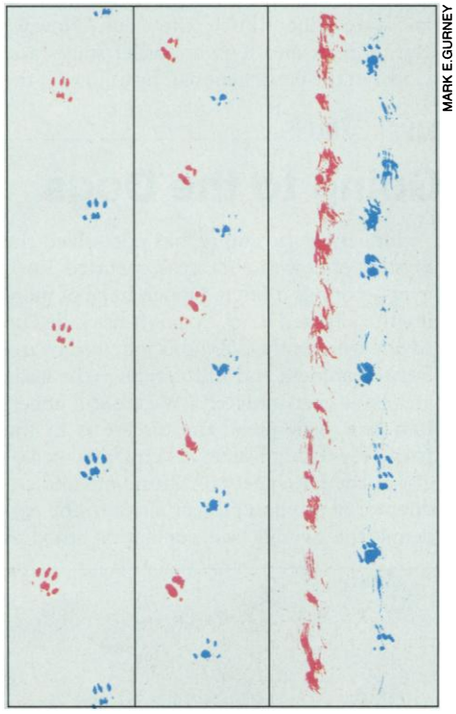
Mice carrying a mutant gene associated with a hereditary form of ALS develop motor neuron degeneration much like that of the human disease

Of all the chronic diseases that afflict humankind, those causing degeneration of the brain and nervous system have been among the most difficult to study—partly because there are few small-animal models that accurately mimic their pathology and clinical features. Now, a research team led by Mark Gurney of Northwestern University Medical School in Chicago may have filled that gap for one of the most devastating neurodegenerative diseases: amyotrophic lateral sclerosis (ALS). The existence of an animal model offers a ray of hope for understanding the mechanism underlying development of the always-fatal condition, which also goes by the name Lou Gehrig's disease.

Gurney and his colleagues, including Northwestern's Haifeng Pu and Arlene Chiu of the Beckman Research Institute of the City of Hope Medical Center in Duarte, California, produced the model by introducing into mice a mutant gene that has been linked to a hereditary form of ALS (see p. 1772). Mice that acquired the gene, which carries the genetic code for an enzyme known as Cu,Zn superoxide dismutase (SOD), developed weakness and neuronal degeneration similar to that of ALS patients.

Neurobiologist Donald Price of Johns Hopkins University School of Medicine, who has seen a videotape of Gurney's transgenic mice, says their clinical signs are just what "you would think they should be" for an ALS model. "I'm on such a high about Mark's work," enthuses Price. That high is augmented by work by Price's colleagues, including Donald Cleveland at Johns Hopkins and Nancy Jenkins of the National Cancer Institute who introduced a different SOD gene mutant into mice; one line of their transgenic mice is beginning to show clinical features similar to those of Gurney's. And at least one additional group, led by Jon Gordon of Mount Sinai Medical Center in New York City, has found that giving mice a mutant SOD gene can cause them to develop ALS-like features. Neither the Price nor the Gordon groups have yet published their work.

Robert Abendroth, who has chaired the research committee of the ALS Association for the past 15 years, describes the Gurney paper as an "important development." Not only does it provide direct evidence that a mutant SOD protein can cause ALS, as the genetic studies indicated, but, Abendroth says, it also provides a much-needed system for determining just how the mutant enzyme brings about the neuronal degeneration of ALS. Indeed, Gurney's work already points to one important conclusion: The mutations apparently confer some new and deleterious activity on SOD rather than causing a loss of the enzyme's activity, as some researchers had suspected.



MARK E. GURNEY

Tracking ALS. The footprints show how mice carrying the mutant SOD gene become progressively more paralyzed.

What that new, harmful activity might be remains unknown. But researchers hope that if they can learn how SOD mutations cause the hereditary form of ALS, the information will also provide clues to what causes the much larger number of cases of "sporadic," or noninherited, ALS. Another hope is that the mouse model will help in the development and testing of effective therapies for ALS of all types. "Use of a mouse would be a tremendous advancement in terms of safety, expense, and speed" over tests conducted in humans, Abendroth says.

The development of this mouse model has come remarkably quickly. The SOD gene was implicated in hereditary ALS only about 18 months ago, through a genetic linkage study conducted by a large multilab team, including Robert Brown Jr. of Harvard University's Massachusetts General Hospital, Robert Horvitz of the Massachusetts Institute of Technology, and Teepu Siddique of Northwestern, a co-author of the current paper. The discovery was intriguing because of SOD's known role in the cell: It converts superoxide radicals (highly reactive molecules produced by a variety of normal cellular activities) to less reactive products and thus presumably protects cells from harm. But the genetic studies didn't reveal how the mutations cause SOD to go awry in hereditary ALS. The situation was complicated, for example, by the fact that, while SOD breaks down superoxide radicals, it may actually form other types of dangerous free radicals.

One possibility was that people who inherit a mutant SOD gene might have less-than-normal amounts of the active enzyme. And since superoxide radicals could be very injurious to cells, decreased SOD activity might lead to an accumulation of nerve damage over time. Last summer, Siddique's group, in collaboration with crystallographers John Tainer and Elizabeth Getzoff of the Scripps Research Institute in La Jolla, California, provided support for this hypothesis (*Science*, 20 August 1993, pp. 986 and 1047). Their work suggested that the SOD mutations might reduce the stability, and thereby the activity, of the enzyme. And consistent with this, the researchers found that the activity of mutant SODs in red blood cells from patients with hereditary ALS averaged about 50% of normal values.

Still, those experiments didn't rule out a different possibility: that the mutations might confer some new and damaging function on the SOD enzyme. And that's exactly what the mouse models suggest. "We now have genetic proof for the mechanism. It's a dominant gain of function," Gurney says. His group found, for example, that one line of mice carrying a mutant human SOD gene developed degeneration in their motor neurons—the same class of nerve cells that degenerate in human ALS—and became paralyzed, even though copies of the animals' own normal SOD gene remained intact. In fact, the Gurney group's findings, as well as studies done by the Hopkins group in cultured cells, indicate that the particular mutation in the SOD gene that causes the animals' paralysis has little effect on the en-

zyme's ability to break down superoxide, although it does reduce the enzyme's stability.

What's more, Gurney, who has been comparing notes with Gordon and Price about their transgenic animals, says all three groups find that it's the animals making the highest amounts of the mutant SOD proteins that become paralyzed, a finding that runs counter to the idea that decreased SOD activity is at fault in ALS. Instead, Gordon says, "there must be some critical alteration in how SOD is handling free radicals."

Exactly what that critical alteration is remains to be established, but some clues may lie in evidence that SOD does more than break down superoxide radicals. Joseph Beckman's group at the University of Alabama, Birmingham, has found, for example, that the enzyme reacts with peroxynitrite, forming a product that may damage proteins by adding nitrate groups to them, and Irwin Fridovich of Duke University has shown that the enzyme is also a nonspecific peroxidase, an enzyme that might damage many cell constituents. The mutations might therefore lead to a shift favoring one or another of these reactions—or they might have some other, as-yet-to-be-determined effect on the enzyme.

ALS researchers are optimistic that the mouse models will not only help pin down exactly what these mutations do, but also provide a better understanding of the pathological changes underlying ALS. Gurney examined the motor neurons of his transgenic animals only after their paralysis became so advanced that they had to be euthanized because they could no longer forage for food and water. But as Price points out, "Now we can go back and look at earlier stages and work out the pathogenesis." By following the course of the disease in the animals, researchers might be able to detect early degenerative changes in the motor neurons even before they cause paralysis. If so, the results might provide clues to therapies that can slow or prevent the debilitating symptoms of ALS.

If there is one caveat that could dampen enthusiasm for the new ALS models, it's the worry that the sporadic form of the disease, which accounts for 90% of the cases, might develop differently from the hereditary form. But since the symptoms in the two forms of the disease are virtually indistinguishable, there's reason to believe the underlying mechanisms are also similar. If so, the new models could provide a universal test bed for ALS. And that, says Price, will "open up a whole window of challenge and opportunity that wasn't there before."

–Jean Marx

CANINE DISTEMPER VIRUS

Serengeti's Big Cats Going to the Dogs

A mysterious ailment that has killed at least 60 lions in Tanzania's Serengeti National Park has been identified. The culprit: canine distemper virus. Until last year, this virus, which, as the name suggests, infects dogs and wolves, had never been known to infect a feline population. Yet "there is no doubt about it," says Max J.G. Appel, a virologist at Cornell University's College of Veterinary Medicine and a specialist on morbilli viruses, which include canine distemper.

Researchers have been trying to identify the killer since the first of the Serengeti's lions succumbed on 3 February (*Science*, 3 June, p. 1404). Suspicions centered on canine distemper virus because the clinical symptoms of uncontrollable twitching and convulsions "are exactly the symptoms one sees in dogs with canine distemper," says Appel. Those suspicions intensified when telltale viral inclusions were found in the lions' tissue. And in the past 2 weeks, Appel has come up with the clinching evidence: At least 75% of the blood samples taken from 60 lions had a high count of antibodies to the virus. His lab also found viral antigens in tissue taken from two dead lions.

Fingering the culprit has not solved the mystery of how the outbreak occurred, however—nor has it offered much hope of helping the afflicted lions. So far, it has killed up to one third of the 250 lions followed by the lion researchers, and all 16 prides in the study area have been afflicted. "We are still uncertain how widespread the disease is in the park," says Craig Packer, a behavioral ecologist at the University of Minnesota and co-director of the lion project. Prior to the epidemic the overall lion population stood at 3000, an all-time high.

PHOTOS BY ANNE HILBORN

Canine convulsion. Male lion displaying symptoms typical of canine distemper virus infection.

Researchers are wary of using standard canine distemper vaccines on the big cats because the vaccines are made from weakened live viruses, "and there's a small chance of actually causing the disease," according to Packer. "There's nothing we can do for this current outbreak [among the lions] except let it run its course," says Packer. There is one bright spot: The virus has not killed every lion it has infected, and researchers hope that the survivors will be left with some immunity against future distemper outbreaks.

Most puzzling to Appel is why canine distemper virus is showing up in lions at all. Named for canines, it has also afflicted skunks as well as raccoons and nearly wiped out black-footed ferrets in Wyoming a decade ago. But Appel notes that "canines and felines have lived together for hundreds of years, and until last year we had never seen the disease in large cats."

The first known outbreak in big cats occurred in two wild animal parks in southern California last year. Nineteen animals—lions, leopards and tigers—died. A black leopard also succumbed at a zoo in Illinois. Appel and his colleagues' study of these events will appear in the July issue of the *Journal of Veterinary Diagnostic Investigations*. He speculates that the captive animals "may have picked it up from raccoons, which carry the virus and are always around zoos." Scientists with the Serengeti lion project suspect that the felines contracted the disease from domestic dogs living near the park.

But that doesn't answer the question of how the virus suddenly acquired the capacity to cross the species barrier. One way for it to do so might be the acquisition of new genetic capabilities, but the strain responsible for last year's outbreak in exotic zoo cats appears to match the strain carried by raccoons. To determine whether the Serengeti lions' version is a mutated strain that has overcome the cats' previous immunity, Appel hopes to isolate and clone the virus later this summer. He'll also test blood samples collected from lions over the past 10 years to determine whether they have suffered from the disease in the past. "It may be that the virus has been around for some time," Appel says, noting that there have been outbreaks of other morbilli viruses in seal and dolphin populations in the past few years. "It may be that we have better tools and that past epidemics like these were simply never properly diagnosed."

–Virginia Morell