NEUROBIOLOGY

Mutant Enzyme Provides New Insights Into the Cause of ALS

Neurobiologists had two reactions to the 1993 discovery of a genetic mutation at fault in some hereditary cases of amyotrophic lateral sclerosis (ALS). The first was joy, because the gene's normal function—encoding an enzyme that breaks down certain tissue-damaging molecules—promised insights into the causes of this devastating neurodegenerative disease, which usually kills in 3 to 5 years. But the second, delayed reaction was frustration. Figuring out how mutations in the gene, called *SOD1*, cause ALS has proved much tougher than expected. Now that situation may be changing for the better.

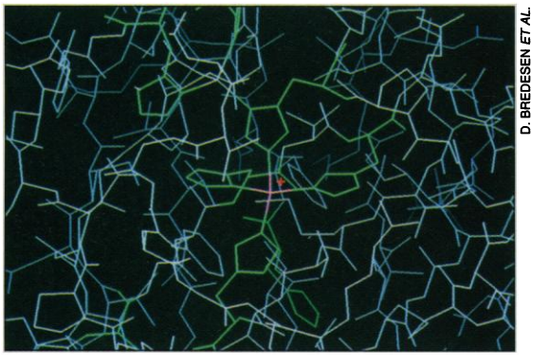
One reason for the change is new evidence from a research team led by chemist Joan Valentine of the University of California, Los Angeles, and neurobiologist Dale Bredesen of the La Jolla Cancer Research Foundation. On page 515, they report that ALS-causing mutations in *SOD1* may injure nerve cells by enhancing the ability of its encoded enzyme to act as a peroxidase, oxidizing—and thereby damaging—lipids and other cell components. Because lipids are important constituents of cell membranes, this could lead to membrane degeneration and the consequent death of nerve cells.

Further support for this idea comes from new studies of mice with an experimental form of ALS. Mark Gurney of Pharmacia-Upjohn's lab in Kalamazoo, Michigan, and his colleagues have found, among other things, that feeding the animals moderate amounts of the antioxidants vitamin E and selenium delays the onset of their ALS symptoms. Bredesen says that these results, which will appear in the February *Annals of Neurology*, are "very compatible with the chemistry we are publishing."

After years of puzzling over *SOD1*'s role in ALS, other researchers studying the disease welcome the two reports. "I think they're both very interesting. At this point we need all the fresh ideas we can get," says neurologist Robert Brown of Harvard's Massachusetts General Hospital, who was a member of the team that first showed that mutations in *SOD1* can cause ALS. And more ideas about how an altered *SOD1* product could cause disease are on the way: Recent work suggests that the mutant enzyme—copper/zinc-dependent superoxide dismutase (CuZnSOD)—may also produce dangerous peroxynitrite radicals.

Understanding the biochemical changes underlying familial ALS (FALS), Brown and others say, may improve the understanding of the much greater number of noninherited "sporadic" cases. And that in turn may lead to better ALS therapies, targeted at the mutated CuZnSODs.

The new view of *SOD1*'s role in ALS is a marked turnaround from the original thinking. The main job of CuZnSOD is to break down the superoxide radicals produced as side products of the cell's energy-producing reactions. These radicals, oxygen molecules with an unpaired electron, can do a lot of damage to cell constituents. Early results suggested that the *SOD1* mutations reduce CuZnSOD's activity in destroying superoxide radicals, thereby allowing them to harm neurons. There were some problems with this "loss of function" idea, however.



D. BREDESEN *ET AL.*

On site. To react with CuZnSOD, hydrogen peroxide *(red x)* has to slip into the active site near the copper ion *(magenta)*.

One of them was that it runs counter to the genetic behavior of FALS, which is a dominant disease, caused by a single bad copy of the gene. Such conditions are almost always caused by the gain of some toxic activity. And researchers knew they had to consider that CuZnSOD mutations might boost the potentially toxic side reactions the enzyme was known to carry out, including peroxidation and peroxynitrite production.

Then, in mid-1994, evidence for a gain of function began to emerge. Researchers, including Gurney's team, which was then at Northwestern University Medical School in Chicago, put copies of the mutated human *SOD1* gene into mice, which developed symptoms very much like those of human ALS (*Science*, 17 June 1994, pp. 1663 and 1772). Because the animals have their own normal *SOD1* gene copies, the work "pretty well established that [FALS] can't be due to a decrease in SOD activity," says biochemist Irwin Fridovich of Duke University, who discovered that CuZnSOD can act as a peroxidase.

And that's where the new work comes in. With decreased SOD activity apparently out of the running as an FALS cause, Valentine, Bredesen, and their colleagues decided to find out what else the enzyme might be doing, starting with a look at its peroxidase activity. In test tube studies comparing the mutant and wild-type (normal) enzymes, they found, Bredesen says, that the peroxidase reaction "was catalyzed at a low level by wild-type SOD, but at a higher level by the mutant SODs."

Because the copper atoms in CuZnSOD are needed for its enzymatic activity, the researchers next decided to look at the effect a copper chelator, which ties copper ions up in chemical complexes, might have on cultured neurons containing either wild-type or mutant *SOD1* genes. The scientists had previously found that neurons with mutant genes had increased susceptibility to apoptosis (programmed cell death). But at the lower concentrations added to the neurons, the copper chelator reduced the apoptosis induced by the mutant genes by 30% to 70%. The chelator had no effect on cells with the normal gene. The observation suggests that the increased apoptosis is the result of CuZnSOD's activity as a peroxidase enzyme, with membrane lipids as a particularly susceptible target because the double bonds they contain are readily attacked by peroxidation reactions.

While the Bredesen-Valentine team was investigating enzyme pathology, Gurney's team had begun studying the biochemical changes that take place during ALS development in the brains and spinal cords of their genetically engineered mice. They also began looking for drugs that might prevent or slow the course of the disease. And their findings seemed to strike a chord with the enzyme observations.

Gurney and his colleagues found, for example, that vitamin E concentrations go down with age in the ALS animals, while they go up in control animals. That, he says, suggests that the vitamin "is used up defending against reactions" of the type that Bredesen described. Still more support comes from the Gurney team's finding that vitamin E and selenium delayed the onset of the animals' symptoms by about 2 weeks, from an average of about 92 days to about 108 days. The supplements did not, however, extend the animals' life-spans.

But even though both new sets of results point to peroxidation reactions as an important part of the mechanism by which *SOD1* mutations cause ALS, there may be other parts as well. About 3 years ago, Joseph Beckman of the University of Alabama, Birmingham, suggested another culprit: increased generation of peroxynitrite radicals by the mutated enzyme. And recent evidence is also beginning to support this idea.

Peroxynitrite radicals can damage the cell's proteins by adding nitrate groups to the amino acid tyrosine. In work that has not yet been published, several groups, including Beckman's and Gurney's, have found such nitrated tyrosines in cell proteins in tissue samples from both ALS patients and mice. The targets include the neurofilaments, protein bundles needed for neuronal structure and function. Indeed, neurobiologists suspect that both peroxynitrite and peroxidation may be involved in damaging neurons. The two ideas are "absolutely not competing," says neurobiologist Sam Sisodia of Johns Hopkins University. "They're both within the realm of possibility."

Despite the recent progress, researchers still have a long way to go to understand what causes ALS. Bredesen notes, for example, that no one has demonstrated directly that membrane lipids are attacked by CuZnSOD peroxidase. And then there is the biggest question of all: whether the information being gleaned from the studies will lead to effective ALS therapies. Bredesen's and Gurney's results suggest, for example, that treatment with antioxidants might delay development of ALS in individuals carrying SOD1 mutations, although a great deal more work will be needed to prove that. As Brown says, "This is a story that is still very much in its infancy. It's still evolving." But, he adds, "That's what's exciting."

–Jean Marx

IMMUNOLOGY

Modified Microbe May Boost TB Vaccine

BOSTON—In medicine's war against bacterial infections, one of its longtime allies has been a member of the enemy: bacille Calmette-Guérin (BCG). The relatively benign mycobacterium has been widely used as a vaccine against its more malicious cousin, the tuberculosis (TB) organism, because the similarity between BCG and TB proteins appears to arm the body's immune system against a subsequent TB attack. But BCG's protective effect varies greatly among different populations and wanes as people age. Now reinforcements may be on the way, in the form of the intercellular messengers known as cytokines, which can rally the immune system.

A group of researchers in Cambridge, Massachusetts, reports engineering BCG to express several mouse cytokines that stimulate immune cells such as macrophages and T cells to begin an all-out immunological assault on the invader. In this week's issue of the *Proceedings of the National Academy of Sciences*, molecular biologist Richard Young of the Whitehead Institute for Biomedical Research and his colleagues say that inoculating mice with the recombinant bacterium greatly strengthened their cells' immune responses to tuberculosis antigens.

The accomplishment raises the prospect of an improved vaccine against the worldwide TB epidemic and is winning accolades from other researchers. "A tremendous achievement," says Barry Bloom, an immunologist at the Albert Einstein College of Medicine in New York who specializes in the study of TB and who collaborated with Young on earlier studies of BCG. Moreover, the research could lead to more effective cancer therapies, because conventional BCG spurs the immune system to attack some bladder tumors, and scientists believe a recombinant form could provide an even greater stimulus. The technique, however, hasn't yet proven itself against TB or cancer, even in animals. Still, Kenneth Stover, a molecular microbiologist at the Seattle firm PathoGenesis, says that "it's potentially exciting. There is definitely room for an improved BCG vaccine, and this may be a much cheaper, safer way to do it than adding cytokines as drugs."

Slow-growing BCG has been used as a live, attenuated TB vaccine for about 50 years outside the United States, where it is not approved because the organism's resemblance to TB produces false positives in TB screening tests. Three years ago, Young's team, including immunologist Peter Murray of the Whitehead Institute and molecular virologist Anna Aldovini of Boston's Children's Hospital, hit on the idea of enhancing its effects on "cell-mediated" immunity—the immune response that calls on cells such as T cells and macrophages to strike back at pathogens. Manipulating BCG to produce its own mammalian cytokines, they realized, might enable it to stimulate a stronger response by these immune system cells.

But the researchers were not sure that the mycobacterium could be made to produce and secrete cytokines, complex proteins that are biologically inactive unless their multiple units are bound and folded in the proper configuration. There was a safety issue as well, says Young. The bacterium might overproduce the cytokines, which could be toxic and actually compromise immune system functioning.

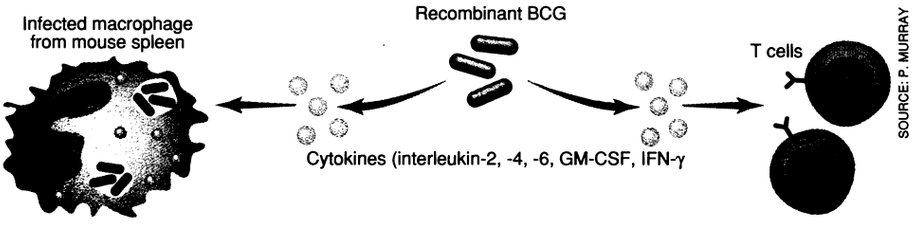
The group designed a two-part experiment to test these questions. They first inserted mouse genes encoding many different cytokines behind bacterial promoters, or "on switches," and signal sequences that ferry proteins through the bacterial cell wall. Progress was slow—mycobacterial strains such as BCG are notoriously difficult to grow—but the genetically engineered bacteria delivered the goods. Says Young, "We were able to secrete in active form a number of the cytokines we tried," including interferon-γ, granulocyte-macrophage colony-stimulating factor (GM-CSF), and interleukin-2, -4, and -6.

The researchers then injected these strains into mice. Not only did the mice remain healthy, but their immune systems still functioned properly—and showed a strengthened response to TB antigens. T cells later isolated from their spleens responded to purified tuberculosis proteins by proliferating and producing cytokines at rates up to 10 times higher than normal. The researchers now plan to test whether the cytokine-producing BCG strains are capable of strengthening immunity to the actual tuberculosis bacterium in experimental animals.

The Whitehead team's advance is also encouraging cancer researchers such as Michael O'Donnell, a urologist at Boston's Beth Israel Hospital, who hopes that the recombinant bacterium will prove to be a more powerful immunotherapeutic agent against cancer. In body cavities where BCG can be injected and confined, such as the bladder, the bacterium has been shown to eliminate superficial tumors, possibly by provoking an antibacterial response that also purges tumor cells. Recombinant BCG may be able to augment this process, O'Donnell says.

He is already testing the new BCG strains' effectiveness against tumor cells in vitro, with promising preliminary results. "Suddenly you have an organism that can stimulate a response in itself, and can be directed and focused by the incorporation of ... cytokines," says O'Donnell. "It's an incredible asset."

–Wade Roush



Booster bug. Strains of bacille Calmette-Guérin (BCG), engineered to express mammalian immune system messengers called cytokines, stimulate an enhanced response to tuberculosis proteins.