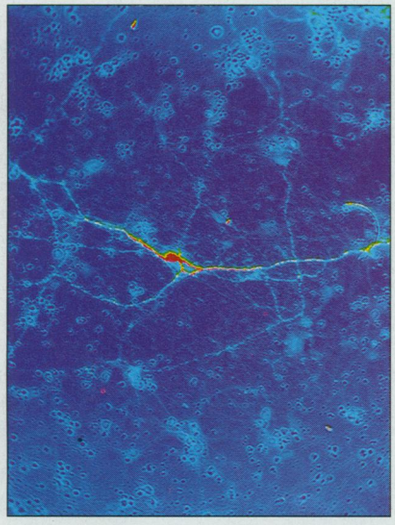
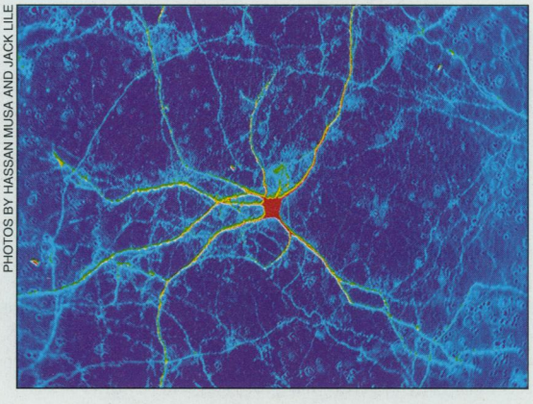
Promising Protein for Parkinson's

A new neurotrophic factor promotes the survival of dopamine-producing neurons, the type of nerve cell that deteriorates in the brains of Parkinson's patients

For decades, the protein known as nerve growth factor (NGF) reigned as the only agent known to spur neuronal development and repair. But in the past several years, a steady stream of discoveries has filled journals with reports of at least a half-dozen more such nerve-nurturing agents with acronyms like BDNF, CNTF, and IGF-1. Now a research team at the biotech company Synergen, in Boulder, Colorado, has added an exciting new element to this neurotrophic alphabet soup by isolating and cloning what could be the most potent and specific nerve growth factor yet. Most tantalizing, the newcomer's biological properties suggest that it may have potential as a treatment for Parkinson's disease, a serious brain degenerative condition estimated to afflict about 1 million people in the United States.





PHOTOS BY HASSAN MUSA AND JACK LILE

Fired-up nerve cell. The nerve cell treated with GDNF *(below)* has more branches than the control. The deeper red color also indicates that it makes more of a key enzyme for dopamine synthesis.

On page 1130, neuroscientist Frank Collins and his colleagues at Synergen report isolating the new protein, which they call "glial cell line-derived neurotrophic factor" (GDNF). In tests done on cultured rat brain neurons, GDNF selectively promoted survival of a specific class of neurons: those that secrete the neurotransmitter dopamine. These are the neurons that typically degenerate in Parkinson's disease. And though many questions must be answered before clinical trials are possible, the hope is that GDNF might be able to prevent or even reverse the tremors, muscle rigidity, and other characteristic symptoms of the disease. "The work is very exciting and I'm very enthusiastic," says neuroscientist Darwin Berg of the University of California, San Diego (UCSD). "We can only hope it will do what we need it to do in the cells that are dying in Parkinson's disease."

The Synergen feat started with observations by others that glial cells, the cells that provide support services for neurons, secrete substances that help dopaminergic neurons survive. In order to isolate those unidentified substances, the Synergen team focused on a line of rat glial cells that seemed to secrete them in great quantity. After 6 months of painstaking biochemical purifications, they managed to isolate the neuronal nutrient, or neurotrophic factor. "Protein purification is an almost intuitive process, requiring a great deal of finesse," Collins says, giving credit to the team's principal biochemist, Leu-Fen H. Lin. "God knows how little might be present in your sample, and if you don't do everything right you can get hung up for years."

With the purified rat protein in hand, the team analyzed a stretch of its amino acid sequence and back-translated to the language of DNA, enabling them to construct a genetic probe. They then used the probe to pull out the GDNF gene from a human gene library. From there it was a relatively straightforward task to insert the gene into bacterial cells and mass produce human GDNF. A final bit of biochemical flair was demonstrated, Collins says, by Jack Lile, who induced the newborn protein to fold up into its correct, biologically active conformation.

And active it is. GDNF is not a growth factor in the sense of stimulating nerve cell division, but it does have a marked effect in keeping them alive. Nerve cells maintained in culture normally die off. But in one series of experiments, embryonic dopaminergic rat neurons treated with human recombinant GDNF maintained their numbers for 3 weeks, while their untreated counterparts decreased by 70%. GDNF also dramatically speeded up maturation of embryonic neurons and their apparent ability to communicate with neighbors, stimulating extensive sprouting of new neural branches in as short a time as 7 days. And it did so at concentrations on the order of one picomolar—far lower than the concentrations required for other nerve growth factors to function.

Specificity a good sign

How GDNF works is still unclear. Its sequence reveals it to be a new member of the transforming growth factor *beta* superfamily. Other superfamily members are known to regulate embryonic development and cell growth by binding to cell surface receptors and influencing gene transcription, but GDNF's receptor is so far undiscovered.

Just as exciting as GDNF's potency, Collins says, is its exquisite specificity in tests so far: It had no effect on neurons that release the neurotransmitters GABA or serotonin rather than dopamine. No other growth factor for dopaminergic neurons has exhibited such specificity, he says, which increases the chances that it could be an effective Parkinson's treatment. That would be good news indeed, since current drugs like levodopa and deprenyl can slow the progression of Parkinson's but lose their effectiveness over months or years.

The new protein may have some advantages not only over existing drug treatments, but also over another experimental Parkinson's therapy that is much discussed these days: brain transplantation of human fetal neurons from aborted fetuses. So far, transplantation has met with only modest success in a handful of patients, and even that has been overshadowed by highly charged debates over the therapy's link to abortion. "There are a lot of difficulties with these cell transplants, both ethical and practical," says

neuroscientist Lou Reichardt of the University of California, San Francisco (UCSF). "My own feeling is that anything that even resembles a drug, like some of these neurotrophic factors, will be an improvement over these difficult and dicey surgeries and transplantations."

A long road to the clinic

Much as clinicians long for such success, however, it's a long way from tests on cultured rat neurons to clinical trials in humans. For one thing, notes UCSD neuroscientist Fred Gage, the Synergen workers have so far tested GDNF on only a few types of neurons. They haven't, for example, ruled out the possibility that it might also act on cholinergic neurons and on those that release the many regulatory peptides in the midbrain. Overstimulation of such nerve cells could lead to a kind of neurochemical babble in the brain. "Its effects on dopamine neurons are impressive," Gage says, "but to call it a dopaminergic-specific growth factor at this early stage strikes me as a little premature."

With the stakes so high, the Synergen group is already moving ahead with further studies. To find out whether GDNF is as specific as it appears, they've begun testing GDNF on a broader mix of cultured human neurons. And to learn more of its potential for Parkinson's, they've launched trials in rodents and primates. Collins is mostly close-mouthed about these important animal studies, but he says that in animals with neural deficits resembling Parkinson's, GDNF has had "a marked effect in reducing behavioral deficiencies."

Even if this therapeutic promise is borne out, however, another large problem would still have to be solved: that of delivering the drug to the right place. As proteins, neurotrophic factors are too big to cross the blood-brain barrier, and none of the options for getting them into the brain is especially attractive. The simplest approach would be to deliver GDNF directly into the gray matter via a permanently placed cannula. Swedish researchers have tried this technique in a few patients in trials of NGF for Alzheimer's and Parkinson's. "Technically, drilling a hole in the head is not that hard," says Ronald Lindsay, vice president for neurobiology at Tarrytown, New York-based Regeneron. "It's actually a lot easier than doing surgery on the heart." But there are other problems to worry about, he notes, not least of which is the potential for fatal brain infection from a permanently open site.

Researchers are also attempting to use carrier molecules to sneak proteins across the blood-brain border. Another possibility is to transplant cells genetically engineered to secrete GDNF into the brains of Parkinson's patients, either alone or with fetal neurons. But neither of these approaches is ready for the clinic.

Some skeptics, doubting that neurotrophic factors will ever make their way into the central nervous system, are setting their sights on a newly discovered class of smaller molecules that have many of the same properties as neurotrophic factors but with a lot less baggage. "The beauty of these small molecules is that they cross the blood-brain barrier," says Frank Baldino, president of Cephalon in Westchester, Pennsylvania, one of the companies taking the mini-molecule tack. "People who keep focusing on growth factors for central nervous system diseases are missing the boat."

But neuroscientist William Mobley of UCSF argues that neurotrophic factors, because they are already well characterized, are in a much better position to make a clinical difference in the next few years. As for GDNF, Mobley says, "It's really important and they've done a very good job. Best of all, it says there are still more factors out there to be found, and things are going to get incredibly interesting." And for the people suffering from neurodegenerative diseases, that word interesting can only translate as hope.

–Rick Weiss

Rick Weiss is a staff writer at Health *magazine in San Francisco.*

IMMUNOLOGY

Imanishi-Kari Says Her New Data Shows She Was Right

While the 15 April *Journal of Immunology* will never find itself on *The New York Times* bestseller list, it is capturing far more attention than might be expected for a regular issue of a specialized scientific journal. Is that because it contains a new method of attacking the AIDS virus, a novel strategy for cancer immunotherapy, or the discovery of an important autoimmune gene? Not at all. What has garnered the unusual level of interest are two papers addressing a small, complicated area of immunology that concerns few researchers today.

The curiosity about these manuscripts stems not just from the data they contain, but also from the presence of an author's name on both papers: Thereza Imanishi-Kari. She is the Tufts University immunologist who coauthored what may be the most controversial journal article ever written, a disputed (and since retracted) 1986 paper in *Cell* for which Imanishi-Kari has been accused of fabricating data. Now, she says, these new publications "confirm many of the original claims." And interviews by *Science* show that many in the immunology community are impressed. "These papers would suggest that what was observed in the first paper is reproducible. This is a very thorough piece of work, extremely well-documented," says immunologist J. Donald Capra of the University of Texas Southwestern Medical Center.

"These papers suggest that what was observed in the first paper is reproducible."
—J. Donald Capra

But even so, the papers won't put an end to a long-running federal investigation into the paper or to the charges of misconduct. The new papers "deal with many of the scientific questions with the *Cell* paper, but they don't speak to fraud," admits Tufts immunologist and Imanishi-Kari supporter Henry Wortis. The official verdict in the case, which has also marred the career of Noble laureate David Baltimore, a coauthor on the 1986 paper, should come this summer, when the Public Health Service Office of Research Integrity (ORI) issues its final report.

Even in the absence of misconduct allegations, the 1986 manuscript would have been controversial to the small cadre of immunologists familiar with its topic because its observations were used to argue for conclusions about the immune system that most researchers were unwilling to accept. Imanishi-Kari (who was then at MIT), Baltimore, and their four coauthors created transgenic mice by introducing into the animals a gene encoding a particular form of an antibody protein, a version the mice did not normally produce.

Active antibody genes have to be assembled from separate bits and pieces of DNA in the genome, and to ensure that the foreign gene would be functional, the group introduced it in its "arranged" version. The expectation was that the added gene would prevent the arrangement, and thus the expression, of the animal's own gene, which produces a slightly different variation of the an-