Neurotrophic Factors Enter the Clinic

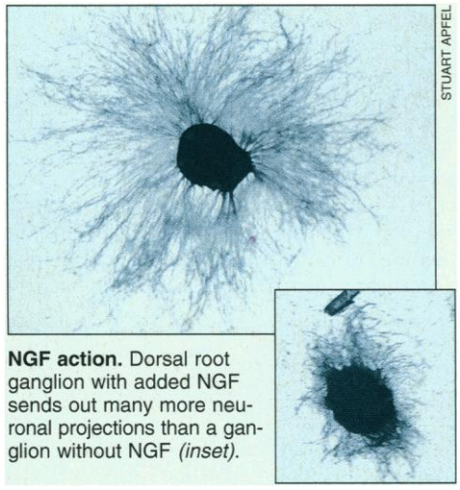
The biotech industry launches a new class of nerve-nurturing drugs with high hopes of toppling stubborn neurological diseases such as Lou Gehrig's disease

Neurons are among our most precious cells. They play vital roles in our lives, governing everything from the recoil of a finger from a hot stove to the understanding of Dante. Unlike the vast majority of other cells, however, most neurons must perform these all-important tasks for an entire lifetime, as they aren't replaced if destroyed by injury or disease. Given that neurons are so precious, it's not surprising that one of the hottest areas in neuroscience today is the study of neurotrophic factors: naturally occurring proteins that keep neurons alive and healthy during embryonic development and later in normal adult life.

This interest in neurotrophic factors is not merely academic. In fact, it has already generated a "wave of excitement" in the biotech world, says Jeff Vaught, senior vice president for research at Cephalon, a biotech company in West Chester, Pennsylvania. A half-dozen or more companies have clinical trials planned or under way for testing neurotrophic factors against debilitating neurodegenerative diseases, including Alzheimer's, Parkinson's, and amyotrophic lateral sclerosis (ALS), or Lou Gehrig's disease.

"Most of the diseases for which these neurotrophic factors are being developed are diseases for which current therapies are virtually nonexistent," says biotech stock analyst Margaret McGeorge of Hancock Institutional Equity Services in San Francisco. That lack of treatments, she says, is a driving force behind the current enthusiasm. But McGeorge also points out that "it is much too early to say" which factors will pan out as useful drugs. One highly touted neurotrophic factor has already stumbled in clinical trials—possibly a result of being rushed into large-scale trials. That experience hints that bringing these nerve-nurturing drugs to market could pose bigger challenges than companies originally thought. Still, that setback has only slightly dimmed the excitement that surrounds neurotrophic factors in the research and biotech communities.

That excitement has been building to its current crescendo for a long time, beginning more than 30 years ago with the Nobel prize–winning discovery of the first neurotrophic factor—nerve growth factor (NGF)—by Rita Levi-Montalcini, Stanley Cohen, and Viktor Hamburger at Washington University in St. Louis. They showed that NGF ensures the survival of certain peripheral neurons as they grow toward and connect with target tissues during the development of the nervous system. Later work showed that NGF is needed for the survival of some brain neurons as well.



STUART APFEL

NGF action. Dorsal root ganglion with added NGF sends out many more neuronal projections than a ganglion without NGF *(inset)*.

But many types of nerve cells didn't respond to NGF, raising the possibility that they are supported by other factors. The search for those factors has so far uncovered roughly a dozen proteins that help neurons survive throughout the body and brain. Some, like NGF, work only on some types of nerve cells. Others, such as insulin-like growth factor (IGF) and fibroblast growth factor (FGF), were originally identified as growth factors for other tissues, such as muscle, or the immune system, and were only later found to nurture neurons as well. As the field has grown, the definition of a neurotrophic factor "has been expanded a lot," says Washington University neuroscientist Eugene Johnson, "and it's clear that there are a lot more players."

ALS: A cautionary tale

As this cast of characters expanded, researchers quickly realized that some of the newly discovered growth factors might serve as therapeutic agents for neurological diseases. The first disease to be targeted by neurotrophic factors in a major clinical trial was ALS, a deadly condition in which the motor neurons—neurons that control the skeletal muscles—progressively degenerate, eventually depriving patients of all movements, even the ability to breathe.

ALS was a good starting point for several reasons. For one thing, there's no effective treatment for the disorder. In addition, motor neurons, unlike neurons of the brain, are accessible to protein drugs introduced into the bloodstream. Says Cephalon's Vaught, diseases like ALS should be "very amenable to growth-factor application because the projections from the affected neurons lie outside the blood-brain barrier," the physiological wall that keeps large molecules like proteins from entering the brain.

A number of growth factors had already been shown to support the growth of motor neurons in laboratory cultures, and three of those factors became early candidates for treating ALS: brain-derived neurotrophic factor (BDNF) and insulin-like growth factor I (IGF-I), both made by muscles controlled by motor neurons, and ciliary neurotrophic factor (CNTF), made by the Schwann cells that form an insulating sheath around the neurons.

In addition to the cell-culture work, all three factors also turned out to aid the healing of injured motor neurons in rats. Moreover, the factors improved the condition of mice with hereditary motor-neuron diseases similar to ALS. That was enough for two companies, Regeneron Pharmaceuticals of Tarrytown, New York, and Synergen of Boulder, Colorado, to begin clinical trials of CNTF and for a third, Cephalon, to begin trials with IGF-I. Regeneron is also developing BDNF, in collaboration with Amgen of Thousand Oaks, California, although they have not yet begun large-scale trials.

But the high hopes with which these trials began quickly turned into what many observers view as a cautionary lesson for the field. The first publicly reported results with CNTF looked promising enough: Last September, at the World Congress of Neurology in Vancouver, British Columbia, Regeneron announced that 12 ALS patients who had been receiving CNTF injections in a small, preliminary trial showed less of a decline in muscle strength than did 14 patients receiving a placebo. The results were not statistically significant, Jesse Cedarbaum, vice president for clinical affairs at Regeneron, told *Science* in an interview last November, but they were sufficiently encouraging to justify a full-scale trial of CNTF's effectiveness. Last year Regeneron began that trial, which includes 720 patients at 36 clinical sites.

But even as Regeneron trumpeted its ear-

ly results, optimism about CNTF had already begun to erode. Rumors were circulating that patients in a preliminary safety trial of CNTF carried out by Synergen had suffered side effects, including coughing, fever, weight loss, and activation of herpes virus, which can hide in latent form in some neurons.

Similar side effects were also rumored to be plaguing Regeneron's large-scale trial—rumors that were soon to be borne out. In March, Regeneron researchers announced that they had unblinded the data on 550 patients who had completed 6 months of the trial and found that a substantial number of those receiving CNTF had not only had serious side effects, but had actually fared worse on measures of muscle strength than did patients receiving placebos. Patients who didn't experience side effects showed modest improvement as compared to controls, but investors weren't mollified by that scrap of good news, and Regeneron's stock dropped 50%. The company has tried to salvage the trial by applying to the Food and Drug Administration to continue it with lower doses aimed at reducing the side effects.

But the question remains whether anything more than modest effects can be expected of CNTF. Synergen has a large trial in progress, using lower drug doses than Regeneron, based on the findings of its safety trials. The trial has not been unblinded, but insiders say the prospects don't look very exciting. "Although all the numbers haven't been added up [for either large-scale trial], the clinical results in ALS are less than we had hoped for," says Tufts University neurologist Theodore Munsat, who has participated in the Synergen trials. The best that can be expected, says Munsat, is "a modest slowing of the deterioration rate." He questions whether such modest effects would improve the quality of a patient's life enough to justify use of the drug.

But some researchers think the first round of trials is not a fair test of CNTF. Neurologist Michael Sendtner of the Max Planck Institute for Psychiatry in Martinsried, Germany, argues that the way the drug is administered could be key to reducing its side effects and improving its effectiveness. He recently found, for example, that CNTF has a half-life of only 3 minutes in the bloodstream of a rat. That means the injected drug has little time to reach the motor neurons. While in the bloodstream, it encounters tissues such as the liver and lungs and also interacts with blood-borne immune cells. CNTF's interaction with these cells and tissues, which don't usually encounter the factor, may account for its side effects, says Sendtner. He suggests there is still hope for better results if CNTF were delivered directly to the cerebrospinal fluid, which bathes the spinal roots of the motor neurons.

Even if CNTF doesn't pan out, biotech researchers hope IGF-I will have fewer drawbacks as an ALS therapy. That drug has already been used in large-scale drug trials for conditions including diabetes, dwarfism, and osteoporosis without showing serious side effects. "The good news about IGF-I is that it certainly appears so far to be fairly benign," says biotech analyst Timothy Wilson of the New York securities firm Hambrecht & Quist. "But the big question mark is whether or not it will have efficacy in ALS." The Cephalon trial aims to answer that question.

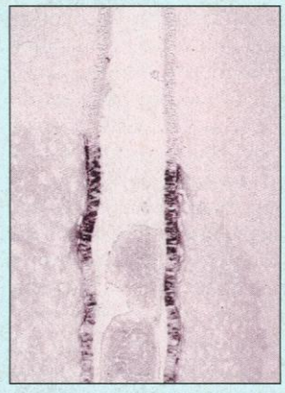
But even if other companies do hit pay dirt with an ALS treatment, close observers of the field think Regeneron's experience holds some lessons for all the companies involved. Some argue, for example, that Regeneron moved precipitously, relying on results from early trials that were too small for adequate analysis of optimal dosing. "When companies have tried to move too quickly through these clinical trials, they usually find disappointment," says R. Brandon Fradd, a biotech stock analyst with Montgomery Securities in San Francisco. But Fradd and others emphasize that, despite the first disappointing results, the field of neurotrophic factors still shows promise.

Peripheral neuropathy: Better odds

Some of that promise may be realized in tackling a more straightforward problem. One reason finding ALS therapies is so difficult is that the cause of the motor neuron degeneration remains unknown, so there is no good animal model for the disease. The story is different for peripheral neuropathies: the deterioration of sensory or motor neurons

Solving the Delivery Puzzle

Neurobiologists have high hopes that neurotrophic factors, naturally occurring proteins that help keep nerve cells healthy, may one day provide effective therapies for a wide variety of neurodegenerative diseases (see main text). But before that day comes, they will have to solve a problem: how to administer the factors safely and effectively. It's unlikely that the factors will ever be available as pills that can be washed down with a gulp of water, because they are proteins and thus would be rapidly destroyed in the digestive tract before they could be absorbed. For diseases of the peripheral nerves, the drugs may be injected into the patient's skin, but for brain conditions such as Alzheimer's or Parkinson's disease they may need to be pumped directly into the brain to circumvent the blood-brain barrier, which keeps proteins carried by the bloodstream from reaching the brain.



CYTOTHERAPEUTICS

Growing in. GDNF in capsule attracts growing neurons.

But even though these approaches are being used in the early clinical trials, each has inherent disadvantages. Injecting a trophic factor into the skin sends it into the bloodstream, allowing it to influence many tissues, possibly causing unwanted side effects. And a catheter pumping a trophic factor directly into the brain could be a conduit for dangerous infections. That's why researchers are working to develop more sophisticated delivery systems.

One possibility, under development at CytoTherapeutics Inc. in Providence, Rhode Island, involves enclosing neurotrophic factor–producing cells in tiny, semipermeable capsules that can be implanted anywhere in the body. The capsules—which have been tested in animals and humans—can be implanted in the brain or in the cerebrospinal fluid at the base of the spine. They let nutrients and cellular products pass in and out, but they keep the cells contained and isolated from the recipients' immune defenses. "You can almost think of it as a biological pump," says biotech analyst Margaret McGeorge of Hancock Institutional Equity Services in San Francisco. "You have got a little factory in there, churning out [trophic factor]."

Another strategy is to engineer a person's own skin cells to produce a neurotrophic factor and then tranplant the engineered cells into the brain—a method being tested in rats and monkeys by Fred Gage at the University of California, San Diego. Or the protein factors might be joined with molecules that help them slip across the blood-brain barrier. That approach, being developed by scientists at Alkermes Inc. in Cambridge, Massachusetts, would allow trophic factors injected into the skin to still reach the brain. With these and other approaches in the pipeline, researchers have a rich supply of potential tools to get these nourishing factors to diseased neurons that need them to survive.

–M.B.

caused by diabetes or by treatment with cancer chemotherapeutic agents such as vincristine, cisplatin, and taxol. For those conditions, there are good models. "You can cause peripheral neuropathy with vincristine in a rat and in man," says Wilson, "and if IGF-I prevents it in the rat, there is a very good chance it will do so in man, because it's the same chemical insult."

And this condition has already been met, since IGF-I does prevent drug-induced peripheral neuropathies in animals, as does NGF. Jack Kessler, Stuart Apfel, and their colleagues at Albert Einstein College of Medicine in New York have collaborated with Cephalon and with Genentech of South San Francisco to show the effectiveness of these two factors against drug-induced neuropathies in mice.

As in the rest of this field, clinical trials are following very quickly behind laboratory results; Cephalon is planning to begin clinical trials of IGF-I for peripheral neuropathy within a few months. Genentech has already completed a small multicenter dosing and safety trial of NGF in human patients with peripheral sensory neuropathy, and expects to begin a larger trial by this summer, which would include diabetics with sensory neuropathy. They are tackling diabetes first, says Kessler, who is participating in the studies, because "diabetic neuropathy is a much more prevalent and disabling medical problem" than neuropathy from cancer chemotherapy. As a result of neuropathy, diabetics often experience ulceration of their feet and legs, which can eventually require amputation.

But those two neurotrophic factors aren't the only ones being tried out against neuropathy. Genentech scientist Gao Wei-Qiang recently demonstrated that a factor called neurotrophin-3 (NT-3) protects mice from neuropathy caused by the anti-cancer drug cisplatin, says Franz Hefti, director of Genentech's neuroscience department. The upshot, says Hefti, is that "for peripheral neuropathy a combination of neurotrophins is likely to do the trick."

Parkinson's and Alzheimer's: A fresh approach

Most of the clinical trials now underway have concentrated on testing neurotrophic factors as treatments for conditions such as ALS and peripheral neuropathy in which peripheral nerve cells deteriorate. But that kind of therapy doesn't exhaust the possible uses of the trophic factors. One other important area where these proteins are now beginning to play a role is as experimental therapies for neurodegenerative diseases of the brain.

Alzheimer's is the most prevalent of those diseases, and the leading candidate for Alzheimer's therapy is NGF. An early clue that NGF might be of benefit came from experiments on one of the major groups of neurons that degenerate in Alzheimer's—the cholinergic neurons. These neurons (so called because they release acetylcholine as a neurotransmitter) send their projections into a memory center in a brain structure called the hippocampus. Several research groups have shown that when cholinergic neurons in rat brains are damaged by having their projections cut, they can be saved from dying by infusions of NGF.

Further experiments suggest that saving those neurons may have consequences for mental function. When Fred Gage and coworkers at the University of California, San Diego, infused NGF into the brains of aging rats that showed memory impairment, they found what Gage describes as "very good improvement in [learning] behavior." On a test of memory retention, he adds, the NGF-treated rats "were in the range of the aged nonimpaired group."

Encouraging as these results are, it's far too soon to imagine that NGF will do the same thing for Alzheimer's patients that it did for aging rats. For one thing, the rats are not a perfect animal model for Alzheimer's. Furthermore, it isn't clear whether the decay of the cholinergic neurons is in fact a cause or an effect of human Alzheimer's. Nevertheless, the animal results suggest that keeping the cholinergic neurons alive can improve mental function, says Genentech's Hefti.

Given that possibility, Alzheimer's researchers are ready to see what effects the neurotrophic factor might have in human beings. Lars Olson and his colleagues at the Karolinska Institute in Stockholm have already tried NGF infusion in one Alzheimer's patient; they report that the patient showed improvement on a memory test. Both Genentech and Synergen, the latter in collaboration with Syntex, are planning clinical trials of NGF for Alzheimer's disease as soon as next year. "We don't expect NGF to cure the disease," says Hefti, "but we expect NGF to have a significant behavioral effect."

Researchers also have high hopes for neurotrophic factors in the treatment of Parkinson's disease, another common and intractable neurodegenerative disease, which is characterized by the degeneration of certain dopamine-producing neurons in the brain. Last year, Frank Collins and his colleagues at Synergen purified a protein they call glial cell-line derived neurotrophic factor (GDNF), which supports the survival, in cell culture, of the neurons that die in Parkinson's disease. Using GDNF, "we are hoping that we can reverse the disease," says Synergen chief executive officer Larry Soll. GDNF won't bring a dead neuron back to life, he notes, but it might do something almost as good: Experiments in rats and mice by Synergen researchers suggest GDNF can coax surviving neurons to send out new projections to replace those from lost neurons. If it has similar effects in primates, Soll says Synergen hopes to have GDNF in clinical trials by 1995.

Although the future of any single therapeutic approach with neurotrophic factors remains uncertain, the possibilities are numerous enough to fuel several generations of clinical trials, using increasingly sophisticated delivery systems (see box). And even if the first round comes up short of expectations, observers say it is only a matter of time until our besieged neurons find new pharmaceutical allies in the form of proteins designed by nature to keep neurons alive.

–Marcia Barinaga

Some Neurotrophic Factors Moving Toward Clinical Trials

Factor	Diseases	Companies developing
Nerve growth factor (NGF)	Peripheral neuropathy Alzheimer's disease Spinal-cord injury	Genentech Synergen/Syntex
Brain-derived neurotrophic factor (BDNF)	Amyotrophic lateral sclerosis (ALS) Parkinson's disease	Amgen/Regeneron
Neurotrophin-3 (NT-3)	Peripheral neuropathy	Genentech Amgen/Regeneron Takeda
Ciliary neurotrophic factor (CNTF)	ALS Motor neuron diseases	Regeneron Synergen
Glial cell-line derived neurotrophic factor (GDNF)	Parkinson's disease	Synergen
Insulin-like growth factor I (IGF-I)	ALS Peripheral neuropathy Motor neuron diseases Post-polio syndrome	Cephalon

Additional Readings

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