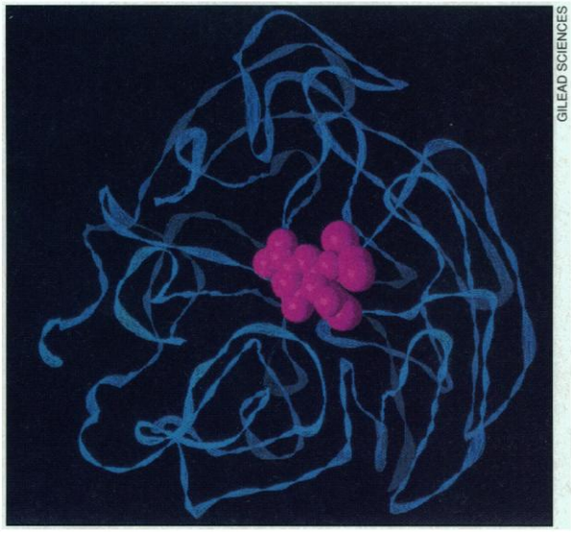
MEDICINE

Researchers Seek New Weapon Against the Flu

There may be a magic bullet after all, at least when it comes to the flu. Scientists at a California biotech company announced last week that they have developed a new compound that may ward off the flu by halting the spread of influenza viruses between cells in the mucous membranes of the nose and lungs. A report outlining the drug's design and synthesis appears in the 29 January issue of the *Journal of the American Chemical Society*.

So far, the drug, which is designed to be taken as a pill, has been tested only in animals. But if it proves to be safe and effective in human clinical trials set to begin this spring, it could have a huge impact: Each year, 20,000 to 40,000 Americans die from the flu, making it the deadliest infectious disease. And the illness costs as much as $12 billion a year in health care and lost productivity, according to the National Science and Technology Council. The new compound "is very exciting," says Robert Sidwell, a virologist with Utah State University in Logan who has tested it in his lab. Unlike vaccines, which only protect against certain strains of the virus, "it's effective against all types of influenza we've thrown at it."



GILEAD SCIENCES

Drug of choice. Compound (pink) blocks action of key viral enzyme (blue) by selectively binding with its active site.

Public health experts caution, however, that even if the new compound proves to knock out the virus in humans, it and a similar compound also under development shouldn't eclipse current vaccination programs. By preventing infection in the first place, vaccines limit the spread of seasonal epidemics. Also, if the drugs were used widely, drug-resistant strains of the virus could arise and become prevalent.

Developed by scientists at Gilead Sciences in Foster City, California, in conjunction with researchers at The Australian National University in Canberra and the University of California, Berkeley, the new compound blocks the activity of a viral enzyme called neuraminidase. The enzyme plays a vital role in the spread of infection between cells in the nasal and respiratory tracts by enabling newly formed viral particles to bud from an infected cell. The enzyme's task is to snip a chemical leash—a bond between a cell-bound sugar molecule and a surface protein on the virus called hemagglutinin—that tethers each particle to the cell. By mimicking the sugar, which is the neuraminidase's normal target, the drug binds to the enzyme and diverts it from its task.

Animal and cell culture studies reported at a conference last fall show that the new compound can shut down this process in many different influenza strains from both of the main A and B subtypes. That success stems from the fact that neuraminidase's active region "is almost identical for all the strains of the virus," says Gilead medicinal chemist Choung Kim. The drug, provisionally christened GS 4104, also quickly eliminated flu symptoms, such as fever and coughing, in ferrets, the best animal model of the illness.

Gilead's drug is actually the second of its kind. The first neuraminidase blocker, which has a similar chemical structure and binds to the same active site, was developed by researchers in Australia and the United Kingdom in 1993 and is now in clinical trials run by the British pharmaceutical giant Glaxo Wellcome. Called zanamivir, it must be given as a nasal spray or nose drops, or via an inhaler, largely because it cannot be absorbed by the gut. In early trials, zanamivir prevented the onslaught of flu symptoms when administered before infection, and it shortened their duration when given later. The compound also seems to be well tolerated and to have no adverse side effects, says Charles Penn, who heads medical strategy for Glaxo Wellcome in Uxbridge, United Kingdom. Glaxo researchers hope to begin final-stage clinical trials later this year.

Because patients often prefer the convenience of popping a pill, in 1994, Gilead scientists launched an effort to design a neuraminidase blocker that could be taken orally. To do so, they had to fashion a molecule with hydrophobic, or fatty, chemical groups, which would ease its passage through the gut wall. But they faced a challenge: The structure of the influenza neuraminidase enzyme, known since 1983, suggested that its active site prefers to bind "hydrophilic" chemical groups that have just the opposite character. Zanamivir's ability to bind the enzyme and inhibit it, in fact, seemed to depend largely on four hydrophilic groups.

In their search for a way around this roadblock, the researchers stripped the hydrophilic groups from a zanamivir-like starting molecule and then began adding back fat-friendly groups. After several failures, the team hit pay dirt. Adding a hydrocarbon-based alkyl group and a nitrogen-containing amine yielded a new molecule, GS 4104, that bound just as tightly to neuraminidase's active site as zanamivir but was hydrophobic enough to be absorbed by the gut.

GS 4104's binding ability "was completely unpredictable," says Kim's Gilead colleague Norbert Bischofberger. As it turns out, when GS 4104 binds to neuraminidase, the enzyme's conformation is changed slightly—forcing one amino acid near the active site to rotate. This shift opens up a small cleft in the enzyme, causing it to bind the drug's hydrophobic alkyl side chain.

Researchers believe that the tight binding of both GS 4104 and zanamivir to influenza neuraminidase should prevent the compounds from interfering with human enzymes and creating side effects. Cells in everything from the liver to the immune system depend on related versions of the enzyme, says Gilead molecular biologist Dirk Mendel, but lab tests with both neuraminidase blockers have shown that they bind to the influenza enzyme 1 million times more readily than to human versions. "I've looked at a lot of different antiviral drugs," says Utah's Sidwell of the Gilead compound. "This is one of the most selective drugs I've seen."

If the new drugs pass the rest of their tests, they would be a welcome addition to doctors' antiflu arsenal, says Frederick Ruben, who is the medical director of infection control at the University of Pittsburgh Medical Center. Two prescription drugs, amantadine and rimantadine, can shorten the duration of symptoms of type A strains of the flu. But

they don't work against type B strains, which account for about 30% of infections. And flu strains that are resistant to both drugs already have cropped up, says Ruben.

He and others caution that strains resistant to neuraminidase inhibitors also could arise if the drugs came into wide use. "If they were available over the counter year in and year out, that would [foster] the resistant strains," says Ruben. Gilead's Kim counters that the resistant strains are less likely to appear with the new antivirals because their target, neuraminidase's docking site, is virtually identical among different flu strains. To become resistant to the drugs, the virus would have to acquire genetic mutations that affect the docking site—changes that would be more likely to cripple the mutated strain than to give it a selective advantage. But Ruben points out that Glaxo researchers have already shown in the lab that flu viruses can develop resistance to their compound. "Resistance will be an issue, but nobody knows yet how important an issue it will be," says Alan Kendal, an epidemiologist and flu expert at the Emory School of Public Health in Atlanta.

Even if resistance doesn't develop, Kendal worries that the new drugs could end up boosting the overall number of flu sufferers by discouraging people from getting vaccinated prior to the flu season. Many of those infected might be able to limit the severity of their illness by taking antiviral compounds, but most would pass along the virus to other people before they experienced symptoms and took an antiviral. For that reason, he predicts, even if a magic bullet for the flu does make it to market, public health officials are likely to keep their focus on prevention.

–Robert F. Service

PLANT RESEARCH

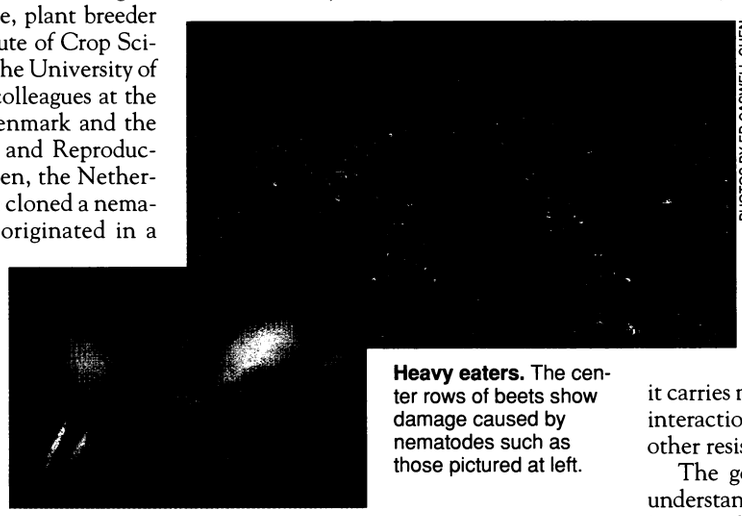
First Nematode-Resistance Gene Found

Nematodes are hearty eaters. Although barely visible to the human eye, these voracious threadworms annually destroy about $100 billion in crops worldwide. Some have a particular taste for sugar beets, while others also enjoy munching on a wide variety of other crops, including oilseed rape and cauliflower. And because classical plant breeding has so far failed to produce commercial crop varieties that resist the onslaught, protection relies largely on toxic chemical pesticides or crop rotation. But that could soon change.

On page 832 of this issue, plant breeder Christian Jung of the Institute of Crop Science and Plant Breeding at the University of Kiel, in Germany, and his colleagues at the University of Aarhus in Denmark and the Center for Plant Breeding and Reproductive Research in Wageningen, the Netherlands, report that they have cloned a nematode-resistance gene that originated in a wild beet plant. In the past 2 to 3 years, researchers have identified more than a dozen genes that make plants resistant to pathogens, including bacteria, viruses, and fungi (*Science*, 23 September 1994, p. 1804). But the new gene, called *Hs1*$^{pro\text{-}1}$, is the first one for resistance to an animal pest. The work "rounds out description of the range of plant genes that confer resistance to disease," says Purdue University plant biologist Gregory Martin.

The sequence of the protein encoded by the new gene provides a few clues to its function: Like the proteins made by other resistance genes, it may detect chemical signals made by the pest and then trigger an as-yet-unknown defensive reaction. But even before the resistance mechanism is known, says Jung, the gene might be engineered into major commercial crops, such as oilseed rape, to create resistant varieties.

In hunting down the gene over the last 8 years, the team transformed adversity into opportunity. Plant breeders have long tried to capitalize on the natural nematode resistance of plants such as wild beets by breeding them with susceptible crops such as the sugar beet, but the hybrids only had partial resistance and were unsuitable for commercial use. Out of that failure, however, came a clue to the location of the resistance gene. Jung's team and the Netherlands team found that some of the hybrids carried the nematode-resistance gene from wild beet on a tiny chromosomal translocation formed by two chromosomes breaking and joining abnormally. This was "a very, very fortunate find," says nematologist Valerie Williamson of the University of California, Davis, as it allowed researchers to focus their gene hunt on a small bit of the wild-beet genome that carried the breakpoint.



PHOTOS BY ED CASWELL-CHEN

Heavy eaters. The center rows of beets show damage caused by nematodes such as those pictured at left.

That lucky break, together with a particular marker sequence that was always inherited with nematode resistance, ultimately helped Jung and his colleagues home in on a candidate gene. They only found the suspect DNA in resistant plants, for example, and the predicted sequence of the protein it encoded revealed some motifs, such as repeated sequences rich in the amino acid leucine, previously found in other resistance genes. The team eventually confirmed its finding by transferring the gene into susceptible beet roots in culture and showing that the transformed roots resisted damage by nematodes.

The researchers found that nematodes trying to feed on the resistant roots were thwarted when their feeding structures degenerated. What causes that degeneration is unclear, but the products of other resistance genes have turned out to be receptors located in the plant cell membrane that set off a defensive reaction when tweaked by some product made by the pathogen. Similarly, the researchers suggest, the Hs1$^{pro\text{-}1}$ protein may serve as a membrane receptor for compounds injected into root cells by the nematode's piercing mouthpart. They note that the protein's overall structure suggests it resides in the membrane, and that the leucine-rich repeats it carries may be involved in protein-protein interactions between host and pathogen in other resistance proteins.

The goal now, besides getting a better understanding of the resistance mechanism, is to use the gene to create new lines of resistant sugar beets and other crops. Jung cautions that that may not be easy, mainly because it may be difficult to regenerate whole plants from the cells used for the gene transfers. "Sugar beet is a notoriously recalcitrant variety," he notes. Also, there are various strains of nematodes, and a gene that offers resistance to one may not work against others. Still, Jung has organized a large team of plant biologists to work on the problem and expects to tame nematodes' appetite: "We hope to have disease-resistant plants in the lab by the end of the year."

–Anne Simon Moffat