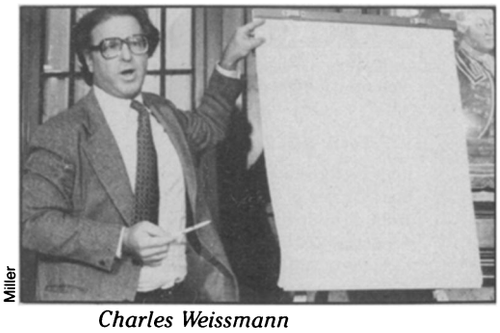
Interferon: Gene-Splicing Triumph


Miller

Charles Weissmann

More exciting than insulin, more promising than growth hormone — it's human interferon, a compound that can now be produced by laboratory bacteria. Charles Weissmann of the University of Zurich described the accomplishment last week at a seminar at the Massachusetts Institute of Technology and later at a news conference. "This is a tour de force, a demonstration of the power of the [recombinant DNA] technique," says Weissmann. "Interferon is one of the cases that has been considered to be among the most difficult."

The potential of interferon, along with its elusiveness, sparks much of the excitement. Since 1957, when interferon was discovered, experiments have indicated that the natural compound may be a broad-range killer of viruses and thus a cure for diseases ranging from hepatitis to the common cold. Limited experimental results even suggest that interferon might be useful in treating some types of cancer.

Although its appeal is great, research on interferon has been limited by the cost and short supply of the protein. Most interferon currently comes from Finland, where it is obtained from white blood cells induced with virus. The annual output of the world's major supply facility is only enough to treat about 600 cancer patients, at a cost of more than $10,000 apiece. The American Cancer Society has a clinical study underway using $2 billion worth of interferon to treat about 100 patients (SN: 10/28/78, p. 295) and the National Cancer Institute has another on the drawing board.

Animal interferon is no substitute for the human variety; it is also expensive to isolate and ineffective in human cells. On the other hand, scientists involved with the bacterially produced human interferon believe it will eventually be available in unlimited amounts and inexpensively — perhaps $10 per treatment. They expect moderate amounts to be ready for clinical trials within a year.

The procedure by which Weissmann coaxed bacteria to make biologically active interferon is a compendium of molecular biology techniques. Kari Cantell of the Finnish Red Cross Center first exposed human white blood cells to a virus and then the researchers extracted messenger RNA, the information that travels between active genes and a cell's protein-making machinery. The extracted RNA contains the message for interferon production, as well as instructions for producing other cellular proteins. Weissmann and collaborators then used enzymes to produce DNA copies of the messenger RNA most likely to represent interferon. With gene-splicing techniques, they inserted those DNA copies into rings, or plasmids, of bacterial DNA. They then moved the DNA plasmids into laboratory bacteria and grew each plasmid-containing bacterium into a colony.

The result was 15,000 colonies, some of which contained the human interferon gene. The scientists next examined the plasmid DNA from groups of more than 500 colonies, then examined the most promising batches in groups of 64, then of eight and finally as single colonies.

At this point, the problem was to find a needle in a haystack, without having an easy way to tell whether an object is a needle or not. Weissmann mixed the plasmid DNA with messenger RNA likely to contain the interferon sequence. When DNA-RNA hybrids formed, indicating a shared sequence, he checked to see whether the RNA really contained the interferon message. To do that, he injected the messenger RNA into a frog egg and determined whether any interferon was produced. In the test for interferon, a sample of material is placed on human cells growing in the laboratory and a virus is added. Healthy cells unaffected by the virus are the sign that active interferon is present.

A few bacterial colonies were chosen by these procedures as good candidates for interferon production. "To clinch it, the *E. coli* [bacteria] produce interferon-like compounds," Weissmann says.

The bacterial product is able to kill viruses and protect human cells growing in culture. In addition it is a protein, it withstands acid treatment, it reacts with antibodies that bind human white blood cell interferon and it is species specific — it does not effectively protect mouse and chicken cells against viruses. However, the bacterial product does not contain the sugars found on some human interferon and it is larger than the authentic human molecule. Weissmann believes that it contains a "signal" sequence that animal cells, but not bacteria, can cleave. In future experiments he hopes to tailor the bacterial product to include only the human length.

Another goal of further research is to substantially increase the bacterial yield. Currently each bacterium produces only a few molecules of interferon. Weissmann plans to relocate the gene within the plasmid and perhaps fuse it to a bacterial gene so it will be turned on more effectively.

The research was sponsored by Biogen, S.A., an international research concern based in Geneva. Biogen has applied for a patent on bacterial interferon production and the Shering-Plough Corp., a pharmaceutical company that is a major shareholder in Biogen, is the worldwide licensee of the patent rights.

"This procedure will obsolete all current processes," says Walter Gilbert of Harvard University, co-chairman of Biogen's board of directors. The uncertain parts of the procedure — obtaining the gene and showing that bacteria make the appropriate protein — have been achieved, according to Gilbert. Increasing the level of bacterial production and tailoring the protein are just matters of manipulating the DNA molecule, and the techniques for that are well known, he says. □

And progress on another front

One complication in mass production of interferon is that so little is known about the molecule itself. But scientists at California Institute of Technology, together with collaborators at E. I. du Pont de Nemours and Co., the National Institutes of Health and Yale University, have just announced that they have made a start at determining its sequence of amino acids. In a paper that will appear in the Feb. 1 SCIENCE, Michael Hunkapiller and Leroy Hood report the order of about one-sixth of the approximately 150 amino acid units of human and mouse types of interferon. They are now determining the remainder of the sequences.

The researchers are using a new "protein sequenator" that requires less than 1 percent the quantity of material needed in previous protein analyses. They predict that once the complete sequences of interferons are known, they can be chemically synthesized. Even the partial structures now available might make easier the construction of modified bacteria producing interferon. Hunkapiller and Hood see their highly sensitive sequenator as a biological equivalent of the physicist's high energy accelerator. They predict its use to characterize clinically important proteins that are available only in small quantities — such as neurohormones, membrane receptors and mediators of immunity. Such characterization should speed production of large quantities of the material with gene-splicing techniques.