Interferon (I): On the Threshold of Clinical Application

Last September, the American Cancer Society (ACS) announced that it was allocating $2 million for the purchase of interferon. Several lines of research, including studies on a very small number of human cancer patients, suggested that this naturally occurring material, best known for its ability to inhibit viral reproduction, might also prevent tumor growth. Thus, the ACS agreed to supply the interferon, which is currently a very expensive commodity, for clinical trials in a limited number of cancer patients.

The ACS announcement has stimulated even further the already active area of interferon research. In this article and another to follow, Science *will examine what is known about interferon—its chemistry, mode of action, and potential clinical applications.*

Twenty-two years have passed since interferon was discovered at the National Institute of Medical Research in London by the late Alick Isaacs and Jean Lindenmann, who is now at the Institute for Medical Virology in Zurich. During that time, the antiviral activity of the substance has been well established; interferon appears to be the body's first line of defense against infection by many different viruses. Now, there are indications that the agent may have another potential clinical role—the treatment of some cancers in human patients.

In addition, clinical trials indicate that interferon may control serious viral infections in cancer patients, many of whom are extremely susceptible to infections because their immune systems have been suppressed, either by their disease or by the therapies used in treatment. Interferon may also help patients whose immune systems have been suppressed because they are undergoing organ transplantation.

None of this means that interferon is ready for widespread clinical application, however. The studies performed thus far have included only very small numbers of patients, and there is a need for expanded clinical trials. The ACS has therefore agreed to provide $2 million for the purchase of some 40 billion units of interferon. This amount will only be enough to treat about 150 patients, but the ACS is seriously considering raising the ante by spending an additional $2 million to $3 million for interferon, according to Frank Rauscher, the society's vice president for research.

Most investigators cite lack of interferon as the major handicap to their research. Cells make the material, which is very potent, in extremely small quantities. Preparing enough to carry out basic biochemical and physiological studies, let alone clinical trials, has been difficult. Moreover, interferon is generally considered to be species-specific in its action. Human patients should respond only to human interferon, if this is the case. The scarcity of the human material could be a major hindrance to its widespread use in patients, even if expanded trials show it to be a valuable therapeutic agent. (Potential ways of increasing interferon production are outlined in the accompanying box.)

All cells can produce interferon when they are appropriately stimulated, usually by viral infection or by treatment with double-stranded RNA. Human cells are now known to produce at least three different forms of the material. In 1975, Jan Vilček of the New York University School of Medicine and Edward Havell, who is now at the Trudeau Institute in Saranac Lake, New York, discovered that the principal interferon made by leukocytes (white blood cells) is not the same as the major form of interferon produced by fibroblasts (connective tissue cells). The third human interferon, which was identified through the efforts of several investigators, is called T or immune interferon because it is produced by the T cells of the immune system. All the interferons, whether of human or other origin, are moderately large glycoproteins (protein plus carbohydrate) with molecular weights of about 15,000 to 20,000.

Most of the clinical experience has been with leukocyte interferon, largely as a result of the efforts of Kari Cantell of the Central Public Health Laboratories in Helsinki. He developed a method of preparing the interferon from "buffy coats," the white blood cells obtained during the separation of whole blood into its various components. Cantell's program, which was developed in cooperation with the Finnish Red Cross, has supplied much of the interferon that is used in research around the world, including the 40 billion units purchased by the ACS.

Since the mid-1960's, a number of investigators, including Ion Gresser and his colleagues at the Institut de Recherches Scientifiques sur le Cancer in Villejuif, France, have acquired evidence that interferon inhibits the development of several cancers in experimental animals. Among the human cancers that have recently been reported to show some response to interferon treatment are osteogenic sarcoma, multiple myeloma, melanoma, breast cancer, and certain types of leukemia and lymphoma.

The most extensive clinical trial conducted so far has been of patients with osteogenic sarcoma, a virulent bone cancer. The usual treatment of this cancer is surgery to remove the affected bone. Nevertheless, the cancer spreads rapidly, usually to the lungs. Only about 20 percent of the patients with no evidence of metastases at the time of diagnosis survive 5 years.

In 1971, Hans Strander and his colleagues at the Karolinska Hospital in Stockholm began a study to see whether interferon could prolong the survival of patients with osteogenic sarcoma. They found that it did. Of the 35 or so patients treated with the agent, almost 65 percent showed no evidence of metastases 2½ years after diagnosis. Only about 30 percent of the control patients, who were treated at other Swedish hospitals and did not receive interferon, remained free of the disease for that long. In addition, Strander and his associates have observed improvement in the condition of a few patients with multiple myeloma or Hodgkin's disease, which is a type of lymphoma, after therapy with interferon. (Lymphomas are cancers of the tissues that produce the white blood cells called lymphocytes.)

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Thomas Merigan of the Stanford University School of Medicine has evidence that interferon therapy is effective against at least one type of lymphoma in addition to Hodgkin's disease. He and his colleagues found that interferon reduced the size of the tumors of three patients who had a slowly progressing nodular lymphocytic lymphoma. However, they could not detect any improvement in the condition of three patients with a rapidly progressing, diffuse histiocytic lymphoma. When interferon therapy began, the patients with the rapidly progressing disease were all in the advanced stages of the cancer after more conventional therapies had failed.

Evidence that interferon can cause regression of melanoma, a highly malignant form of skin cancer, comes from Edward Holyoke, Julius Horoszewicz, and William Carter of Roswell Park Memorial Institute in Buffalo. The Roswell Park group is one of the few now using fibroblast, rather than leukocyte, interferon. When they injected the fibroblast interferon directly into the melanoma nodules of five patients with metastatic disease, the injected nodules regressed, or even disappeared, in all but one of the patients.

Horoszewicz and Carter, with Takuma Nemoto, also of Roswell Park, have used fibroblast interferon for the treatment of a few patients with metastatic breast cancer. When they injected the in-

Current and Future Sources of Interferon

Anyone who talks to the investigators doing research on interferon will surely learn one thing: they do not have as much of the agent as they want, especially for clinical studies. If the clinical trials they can conduct continue to give positive results, the demand for interferon will only increase dramatically.

Until recently, all the human interferon tested in the clinic has been leukocyte interferon obtained from "buffy coats," the layers of white blood cells produced during the separation of whole blood into plasma, red blood cells, and other medically useful materials. These white blood cells, for which there are few other uses, can be saved, cultured, and stimulated to produce interferon by exposing them to the appropriate viruses.

In this country, at least until the current interferon boom, most of the buffy coats were thrown out, however, a situation causing some dismay among investigators eager to test the clinical efficacy of interferon. In the words of Mathilde Krim, codirector of the Interferon Evaluation Program at the Memorial Sloan-Kettering Cancer Center, "most of the 4 million buffy coats produced every year in this country go down the drain." A few European programs, notably that of Kari Cantell in Helsinki, provided the bulk of the human leukocyte interferon used in the early clinical trials.

But even if all the buffy coats were devoted to interferon production, they could still provide only a limited supply of the agent because normal white blood cells do not divide in culture; they are used once and then discarded. Moreover, some investigators have expressed concern that leukocyte interferon, prepared as it is from cells obtained from many hundreds of donors and then pooled, might be contaminated with potentially harmful viruses. Thus, there is a major research effort aimed at finding additional interferon sources.

Another possible source of human leukocyte interferon is white blood cells that have been transformed, usually by viruses, so that they have lost their normal growth control. These cells do divide in culture and might provide unlimited quantities of the agent. For example, a group of investigators at the Burroughs-Wellcome laboratories in London has devised a fermentation facility, with a capacity of up to 1000 liters, for producing human leukocyte interferon from a line of transformed cells. The cells they use, however, are transformed by the Epstein-Barr virus, which is suspected of causing cancer in humans. These and other virus-transformed cells carry partial or complete segments of the viral genome. Any interferon produced by such cells would have to be rigorously purified to ensure that it is not contaminated with viral genes having the potential of causing cancer. Nevertheless, if the purification problem can be overcome, techniques such as the one being developed at Burroughs-Wellcome may eventually be scaled up to produce large quantities of human leukocyte interferon.

The discovery of fibroblast interferon has provided still another source of material for clinical and basic studies. Fibroblasts, which can be easily prepared from human foreskins obtained during circumcisions, do not have to undergo transformation in order to multiply in culture. Consequently, it should be easier to ensure that the fibroblast cell lines used for interferon production are free of undesirable viral contaminants. At least two commercial laboratories in the United States, HEM Research in Rockville, Maryland, and Calbiochem-Behring Corporation in La Jolla, California, are already producing human fibroblast interferon, although still not in the quantities everyone wants.

While some investigators are working to devise more efficient methods of producing human interferon from cultured cells, others are pursuing different, less conventional methods of manufacturing it. These include complete chemical synthesis and the use of recombinant DNA techniques to introduce the interferon gene into bacteria, which could then serve as "interferon factories."

In order to synthesize interferon, the sequence of the amino acids of which it is composed must be known. The first step in determining the sequence is complete purification of the material. Among the laboratories working to purify one or another of the interferons are those of Christian Anfinsen at the National Institute of Arthritis, Metabolism, and Digestive Diseases, Sidney Pestka at the Roche Institute of Molecular Biology, William E. Stewart II at the Memorial Sloan-Kettering Cancer Center, and Yin Hwee Tan at the University of Calgary. For example, Pestka and Menachem Rubinstein, also at Roche, have reported the purification of human leukocyte interferon. They determined the amino acid composition of the material, which has a molecular weight of about 18,000 and contains about 150 amino acids. The Roche workers are currently determining the sequence of those 150 amino acids.

terferon directly into the tumors, they observed varying degrees of tumor regression.

Jordan Gutterman of the M. D. Anderson Hospital and Tumor Institute in Houston has obtained partial regressions of breast tumors in patients he has treated with leukocyte interferon. Gutterman is reluctant to talk about the details of his clinical trial because the data have not yet been published. Nevertheless, it was the encouraging results from his preliminary studies that prompted him to suggest that the ACS provide interferon for more extensive clinical trials.

Leukemias may also respond to interferon treatment provided high enough doses are administered. Norwood Hill at the Wadley Institutes of Molecular Medicine in Dallas found that most of a small group of eight children with either of two types of acute leukemia responded favorably, if only temporarily, when they were given very high doses of leukocyte interferon. The children did not respond to lower doses, however.

Not having enough interferon to give higher doses when signs of response are noted is frustrating to the researchers. The Wadley group produces its own interferon by a modification of Cantell's method, but still feels handicapped by an inadequate supply. "We make all the interferon we use," says Hill, "but not all that we need."

Chemical synthesis of a polypeptide the size of interferon will be no mean feat, although the job would be somewhat simplified if it turns out that fragments of the molecule are biologically active. Some investigators think it may be easier to have bacteria do the synthetic work for them, provided recombinant DNA techniques can be used to insert the interferon genes into bacteria—and provided the genes are expressed there. The recombinant DNA methods require that the researchers have the genes in hand.

Several investigators, including Pestka and Jan Vilček of the New York University School of Medicine, are now trying to isolate the messenger RNA's for leukocyte and fibroblast interferon. The messengers could serve either as probes for isolating the genes or as templates to be copied into DNA by the appropriate enzymes.

Simply inserting the interferon genes into bacteria does not guarantee that they will be expressed in the form of active interferon proteins, however. Moreover, interferon is known to contain carbohydrate, and bacteria will probably not be able to attach the right sugars to the interferon protein, even if they can make it. Attachment of the carbohydrate residues to any chemically synthesized protein will also be a problem.

How a carbohydrate deficiency will affect the activity of the material is unclear, although there is evidence from Stewart and from Anfinsen that interferon without the carbohydrate retains its activity, at least in cultured cells. There is still the possibility, however, that in live animals it will be less effective. For example, it might be broken down more rapidly than the natural substance. "Then again," says Pestka, "there may be no problem."

The efforts to find new ways of producing human interferon are based on the assumption that the material is species-specific. The idea is very controversial, but William Carter of Roswell Park Memorial Institute suggests that it may be possible to use interferon from animal sources instead.

Recently, he and his colleagues showed that interferon prepared from pig leukocytes has antiviral activity in cultured human cells. Human leukocyte interferon has been known for some time to prevent virus reproduction in cultured cells from a variety of animals.

According to Carter, these two interferons lack carbohydrate and this deficiency may explain their ability to work on cells from other species. In other words, the carbohydrate might confer species-specificity on the interferons. Other investigators have challenged the idea that human leukocyte interferon lacks carbohydrate, however. This issue has not yet been resolved, partly because pure material was unavailable for study until recently, although Pestka now says that the purified leukocyte interferon he is studying appears to have little or no carbohydrate.

Another route around the species-specificity problem would be to use substances that induce interferon production by the body itself. Many such substances are known and have been under study for several years. Double-stranded RNA's in particular are good interferon inducers. One of the best is a synthetic RNA in which all the bases in one of the two strands are inosines and all the bases in the other are cytosines. [This synthetic RNA is sometimes called poly(I)·poly(C).] Tests with animals have indicated that poly(I)·poly(C) is toxic. In human subjects it was not especially toxic, but it was not a particularly effective interferon inducer either, according to Hilton Levy of the National Institute of Allergy and Infectious Diseases, who has helped to conduct clinical trials of the agent.

A number of attempts have been made to bolster the interferon-inducing potential of poly(I)·poly(C) while decreasing, or at least not increasing, its toxicity. Carter and Paul Ts'o of the Johns Hopkins School of Hygiene and Public Health have taken one tack. They produced a distortion in the poly(I)·poly(C) molecule that should make it more susceptible to attack by enzymes that break down RNA. They hypothesize that this molecule will last long enough to induce interferon synthesis, which occurs very quickly after exposure to an inducing agent, but not long enough to produce toxic effects or to trigger dangerous immune responses. According to Carter, in preliminary tests, the altered molecule is less toxic but just as effective as the original in inducing interferon production.

Meanwhile, Levy and Edward Stephen of the U.S. Army Medical Research Institute of Infectious Diseases have adopted the opposite approach. They have sought to prolong the lifetime of poly(I)·poly(C) by complexing it with the synthetic polypeptide called polylysine. The complex has proved to be a potent interferon inducer in both nonhuman primates and man. Moreover, it has protected monkeys from viral infections, including infection by rabies and yellow fever viruses, that are usually fatal to the animals. Levy and Arthur Levine of the National Cancer Institute have already conducted phase I clinical trials of the complex in human patients. High doses induce the production of as much as 15,000 units of interferon per milliliter in the patients' serum but produce an unacceptable degree of side effects. Lower doses that were better tolerated still induce the production of 2000 units of interferon per milliliter of serum. This is about ten times the serum concentration achieved by direct injection of interferon.—J.L.M.

At present, researchers do not know exactly how interferon restricts tumor growth, but they doubt that its inhibition of virus reproduction is the key. Although viruses are known to cause a number of cancers in experimental animals, and they are suspected of being involved in the development of some human cancers, their exact role in the latter is unclear and for the most part unproved.

Other, more recently discovered actions of interferon may underlie its antitumor effects. Several investigators have demonstrated that interferon inhibits the division of both normal and malignant cells in culture and alters a number of immune responses both in cultured cells and in the living animal. The immune system is generally thought to play an important role in preventing cancer development.

patients who may benefit from interferon are individuals receiving organ transplants. Martin Hirsch of Massachusetts General Hospital says that interferon injections given immediately before, and then for 6 weeks after, kidney transplantation decreased the incidence of infection by cytomegalovirus (CMV). About $1^1/_2$ weeks after the interferon injections were stopped, the patients became infected with the virus. Hirsch wants to expand this trial by giving higher interferon doses to the transplant patients and continuing the treatment for 10 weeks instead of 6. He points out that CMV infections are most troublesome during the first 3 months after transplantation, when immunosuppression is greatest. A 10-week regimen should be long enough to tide the patients over the most dangerous period.

Many, but not all, viral infections can

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Even when interferon does not produce regressions of cancer, it may still benefit the patients by warding off viral infections that would otherwise prove fatal to immunosuppressed individuals. Strander, for example, noted that the patients he treated with interferon were not plagued by viral infections.

The Merigan group has conducted two clinical trials to see if interferon can control viral infections in cancer patients. In one, they used it to treat a few children who developed chicken pox while they were on chemotherapy for leukemia. In otherwise healthy individuals, chicken pox is a relatively benign infection whose lesions are confined to the skin. But the infection often spreads to the visceral organs and may prove fatal in immunosuppressed patients. According to Merigan, only two of the nine children given interferon developed the visceral infection, but six of the nine control children did.

In a more extensive study, including a total of 90 cancer patients, the Stanford workers showed that high doses of interferon significantly reduce the pain and severity of shingles infections and also help prevent its spread to the visceral organs. Shingles, which afflicts mainly older adults, is a painful infection of the nerve endings caused by the same virus that causes chicken pox in children.

Another group of immunosuppressed be prevented by vaccination, as any cold sufferer knows all too well. Those that cannot be prevented just have to run their natural courses because there are few drugs for treating viral conditions.

One of the more serious viral diseases for which there is currently no drug therapy is hepatitis B infection. Although vaccines to protect against this virus are undergoing clinical trials, they are not yet generally available. Meanwhile, hepatitis B is a major health problem, especially in the less developed countries, where the infection is endemic.

There are hints that interferon therapy may help to control the chronic infections, although no one knows if things will pan out. Merigan, for example, has found that the agent reduced the concentration of Dane particles, which are thought to be the infectious form of hepatitis B virus, in the blood of three patients with chronic hepatitis B.

Despite the promise interferon has shown in its preliminary clinical trials, there are suggestions that it may not always be beneficial for viral disease and may even be harmful in some circumstances. Gresser has evidence that it can contribute to the development of the symptoms of the infection produced by lymphocytic choriomeningitis virus (LCM). When newborn mice are injected with this virus, many of them suffer impaired growth, liver degeneration, and death within a few weeks. Injection with interferon alone produces the same effects in the animals. But if the newborn mice are injected both with the virus and with antibody to interferon many more survive, even though the virus concentration in the blood of the animals is higher than it would be if they had been injected only with virus.

Mice that survive the acute phase of LCM infection often develop kidney disease later in life. Antibody to interferon also inhibits development of the kidney disease, according to Gresser. The mechanism by which interferon produces these deleterious effects is unknown. Although Gresser points out that no such effects have ever been observed in adults treated with the agent, he cautions that it may be potentially dangerous to very young infants.

In the children and adults treated thus far, the side effects of interferon have been relatively mild, especially compared to those of the potent chemicals often used in cancer chemotherapy. Patients treated with interferon often experience fever, chills, and loss of appetite. Perhaps more serious is the observation that it may suppress the bone marrow, leading to anemia, inadequate immune responses, and the possibility of hemorrhaging. In addition, interferon sometimes alters liver function. All these changes are reversible, however, when interferon administration is stopped.

Moreover, investigators are not even sure that interferon itself causes the untoward effects; they would not expect the human material used in the clinical trials to be toxic to human patients. But most of the preparations tested were very impure—only about 0.1 percent of the protein they contained was actually interferon. The possibility that the impurities cause the problems is one reason why investigators are investing so much effort in purifying interferon. Because interferon inhibits cell division, however, high doses of even the well-purified material may suppress division of bone marrow cells, although Carter says that the fibroblast interferon prepared at Roswell Park, which is about 100 times purer than most other preparations, does not appear to inhibit the division of normal cultured cells.

Constrained as they are by the limited supplies of interferon available for research, clinicians are still eager to find out whether its early clinical promise will be fulfilled. If it is—and this is by no means certain—medicine will have a new weapon for combating conditions as diverse as cancer and the common cold.—JEAN L. MARX