Interferon: Clinical Application of Molecular Biology

Virus diseases have plagued mankind for thousands of years, but, until recently, no medical treatment for them has been available. Although the use of vaccines has almost eliminated several major viral diseases, such as polio and smallpox in the United States, medical research has provided no means for treating virus diseases already established in the body. No drugs have been made available for combating viruses even though drugs such as penicillin have been used so effectively against bacterial infections.

The interferon system has become recognized as a major natural defense mechanism in man and animals against the innumerable viral diseases. This system appears in the body within a few hours after virus invasion. It can limit the spread of infection through the blood stream and plays a major role in recovery from established viral infections.

Interferon was discovered in 1957 when Isaacs and Lindenmann incubated chick cells with inactivated virus and found in the culture fluid a substance that interfered with the growth of active virus in fresh chick tissue (*1*). Many workers in virology, biochemistry, and molecular biology studied interferon, and out of this research came the rationale for an entirely new approach to the treatment of viral disease—induction of the synthesis of interferon in the body in order to stimulate the cell's natural defense mechanism. When the virus chromosome was established as the probable natural inducer of interferon during virus infection, attempts were made to find a drug that would mimic the virus nucleic acid and specifically act as interferon inducers.

With successful results in animal experiments as a backup, a "mimicking synthetic polynucleotide—familiarly called poly(I • C), a name indicating that it is a copolymer of polyinosinic acid and polycytidylic acid (one strand of each)—is now being readied for trials as a therapeutic agent against viral disease.

Molecular Biology of Interferon

The interferons produced by animal cells are proteins, or at least contain essential polypeptide components. Although no interferon has been purified completely, a 20,000-fold purification of chick interferon has been accomplished. The resultant specific activity of more than 10^6 units (*2*) per milligram of protein places this protein among the most active of biological substances. Antiviral activity is associated with three classes of proteins which vary in molecular weight and order of appearance after virus infection. Researchers believe one class may exist in the cell in a precursor state and the synthesis of the others is induced by the presence of viruses. Despite differences in size, no differences in biological activity or in antigenicity have been found; many workers consider interferons to be a family of proteins, just as antibodies are a group of heterogeneous proteins with the general property of combining with antigens.

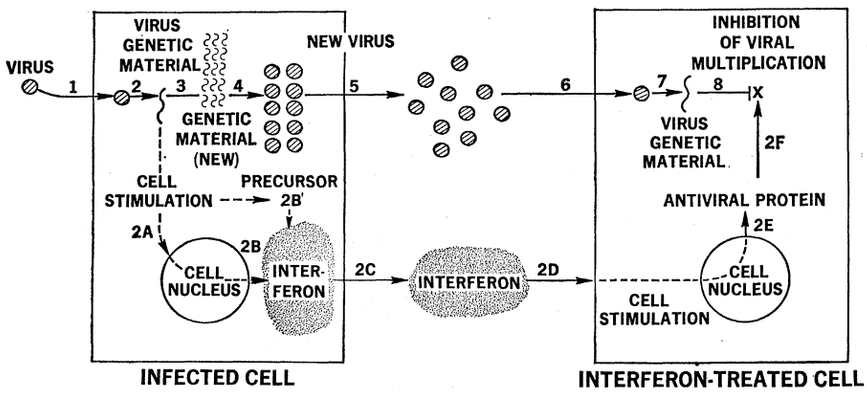
The interferons produced by a variety of cell types and species are not markedly different, and no conspicuously distinguishing features have been found among them.

Different viruses apparently induce the same interferon in an animal, and interferon induced by one virus will protect against many different viruses. In contrast to this lack of virus specificity, interferon is species-specific. Cells synthesize interferon that protects only cells of the same or closely related species. This means that chick interferon will not protect rabbits from viruses, but monkey interferon gives some protection to humans. Thus the information for interferon synthesis resides in the animal cell and not in the inducing virus. This information is believed to be chromosomal since, in cells infected with RNA viruses, actinomycin D—which blocks DNA-dependent RNA synthesis—prevents the production of interferon but does not prevent replication of the RNA virus. Presumably the gene for interferon is normally repressed and the infecting virus triggers derepression, although the mechanism of this action is not yet understood.

Mechanism of Antiviral Action

Interferon does not directly inactivate viruses, and therefore infected cells in an animal may not survive. However, the interferon which they produce as a result of infection is *excreted* and *reacts with other cells* to induce their resistance to virus. In this manner interferon can protect an animal from virus infection and promote recovery from established infections.

To investigate the molecular basis of interferon's antiviral action, researchers have used cells in tissue culture incubated with purified homologous interferon. Such cells bind only minute quantities of interferon, probably less than ten molecules per cell. It is not yet known whether interferon actually enters the cell. After the binding step, both DNA-dependent RNA synthesis and protein synthesis must occur for the development of antiviral activity. The need for these syntheses indicates that interferon itself is not the antiviral agent, but that it does induce the cell



Modification of interferon. [Courtesy of NIAID]

to produce new RNA and antiviral protein so that the cell is subsequently protected against virus. Molecular biologists have not yet determined how interferon induces these new syntheses, but there is a model system which may be comparable—namely, the repression-derepression of the lac operon, the genes governing the proteins involved in lactose metabolism in bacteria. If this model should apply to the interferon system, the cistrons that code for the messenger RNA of the antiviral protein would be controlled by an operator which interferon affects to allow transcription into RNA, which is then translated into antiviral protein.

The effects of these new syntheses of RNA and protein are observed when interferon-treated cells are challenged with virus. The virus penetrates the cell normally, but association of the virus chromosome with the cell ribosomes is greatly inhibited. As a result, the production of new viral nucleic acid and virus-specific proteins is reduced, resulting in an abortive infection. Presumably, inhibition of the formation of the RNA-ribosome complex is mediated by the antiviral protein whose synthesis interferon induces. Although ability of the ribosomes to associate with virus RNA is inhibited, the formation of the complex of ribosomes and host-cell messenger RNA is not inhibited and cell growth continues.

Clinical Application to Virus Infections

Interferon in man occurs during normal viral infections—it has been observed in the serum of patients with mumps, yellow fever, chicken pox, and influenza; and nonimmune children produced interferon after vaccination with an attenuated strain of measles virus (*3*). Hence it was thought that its administration to patients might reinforce a natural defense mechanism. Initial results of administration to humans were very promising. In a well-controlled study in England, 38 volunteers were each injected at two sites (on the arm) with monkey interferon and control material and they were subsequently vaccinated at the sites with vaccinia virus. The interferon treatment led to a statistically significant reduction in the incidence of infection (*4*). In Moscow, human interferon was administered to 200 people during an epidemic of influenza virus, with a definite prophylactic effect (*5*). In France, when large quantities of human interferon were injected into three infants with cytomegalic viral disease, which is normally fatal, the children survived (*6*).

Interferon has several advantages for clinical use. It is only weakly antigenic, and repeated doses of interferon can be administered and resistance to virus occurs each time. In addition, little or no toxicity to humans occurs when properly purified interferon preparations are used. A major disadvantage is that animal interferons are inactive or only slightly active in humans, and thus all interferon for injection into man will have to be made from human or simian cells. Although several systems for the production of interferon from human cells in tissue culture have been suggested, this goal has not been realized and is not immediately possible. Hilton Levy, one of the virologists who established the ribosomal basis for the inhibition of virus growth, believes that it will take at least 5 years before methods for the production of human interferon will yield enough for clinical use. According to George Galasso, head of the Antiviral Substances Program of the National Institute of Allergy and Infectious Diseases, research designed to produce large quantities of pure human interferon is now being supported.

Interferon Inducers

Since interferon itself was not available in sufficient quantities for administration to patients, a search was begun for substances that were obtainable in large quantity and that could induce interferon. A variety of chemically and biologically heterogeneous substances were found that would stimulate interferon formation in animals and protect against virus infection. These included killed viruses, microorganisms, bacterial endotoxins, rickettsiae, mold products such as helenine and statolen, and various large-molecular-weight polyanions. Because of their toxic effects or because only low amounts of interferon were induced, none of these were ideal for clinical use in man.

Isaacs had originally predicted that the nucleic acid of viruses was the stimulus for the synthesis of interferon and postulated that nucleic acid is not only the inducer of interferon, but also the target against which it acts. Experimental tests yielded conflicting results, and this hypothesis was considered unproved. Interest in this theory was revived when it was found that preferential inactivation of virus RNA and not virus protein led to inactivation of the capacity of the virus to induce interferon (*7*). Thus it appeared that the RNA of the virus was the stimulating moiety, and investigators, while testing a wide variety of RNA's, found that double-stranded RNA from several viruses and yeast, as well as synthetic double-stranded RNA's, could induce interferon. The active component of statolen turned out to be a contaminating fungal nucleic acid, and the large-molecular-weight polyanions were recognized as being very similar to polynucleotides in their anionic character.

The research group at the Merck Institute tested many chemically well-defined polynucleotides that might mimic the nucleic acid of the virus nucleic acid. They found that the RNA copolymer poly(I · C) is one of the most potent inducers of interferon in animals and also has high activity in the prevention of experimental virus infection (*8*).

A further clinical advance occurred when poly(I · C) was shown to be effective in treating viral infections that were already established in an animal. In this case, administration of poly(I · C) to rabbits with severe kerato conjunctivitis promoted recovery and there were no toxic effects to the animals (*9*).

Because of the potential clinical use of poly(I · C), a study of the toxicology of this agent was conducted at the National Cancer Institute (NCI) in 1969. Although poly(I · C) produced moderate toxic effects in monkeys and dogs, nevertheless safe dosages were established for carefully monitored studies in humans.

Poly(I · C) is now coming to controlled clinical trials at the National Institutes of Health (NIH). In one study, 18 volunteers were treated intranasally with either poly(I · C) or a saline placebo and then challenged with rhinovirus, which normally produces colds. Poly(I · C) had some protective effects and produced a reduction in the symptoms of upper respiratory illness (*10*).

Poly(I · C) has also been used in less controlled studies to treat patients with usually fatal viral diseases or those with severe viral diseases for which no other therapy is available (*10*). An infant with severe herpes simplex encephalitis was comatose and convulsive. Within 12 hours after treatment with poly(I · C) the convulsions stopped, his fever dropped, and his condition generally improved. Two children with subacute sclerosing panencephalitis were treated with poly(I · C). Deteri-

oration was slowed, and the patients' conditions remained stable for several months. However, the role of interferon was not established. At the University of Siena, Italy, herpes simplex eye infection in man is being treated with poly(I · C), and there is a significant therapeutic effect during the acute phase of the infection, with minimum toxicity (*11*).

Although poly(I · C) has not induced major antiviral effects in man, Samuel Baron, a colleague of Hilton Levy at the NIH, believes that this may be due to the low doses administered. Baron has participated in many of the clinical studies with interferon, including the important study in which poly(I · C) was shown to promote recovery from established conjunctivitis infection in rabbits. He says that the doses used up to now in humans "induce only a small fraction of the amount of interferon that can be produced during a naturally occurring viral infection." The amount that is produced normally "can be induced in mice and rabbits only by larger doses of poly(I · C) and some other inducers, and this amount is strongly protective. However, only low doses of poly(I · C) have been approved for use in man, and the low levels which they induce exert only mild protection against viruses."

Poly(I · C) and Cancer Therapy

The potential of poly(I · C) in the treatment of cancer is now being studied at the NCI. Research with laboratory animals indicated that the drug changes the RNA and protein metabolism of cells and thus might exert a preferentially inhibitory effect on tumors. In one study in mice, poly(I · C) was tested for its inhibitory power on 14 tumors of both viral and nonviral origin (*12*). The rate of growth of the tumors decreased, and the animals survived longer than controls. In two cases the tumors regressed, and in one case the tumor disappeared. A possible molecular basis for these findings is that poly(I · C) treatment causes marked inhibition of the syntheses of protein and RNA in the tumor, to the extent of 60 to 95 percent. Poly(I · C) also inhibits animal tumors caused by chemical carcinogens (*13*). Levy thinks that, since poly(I · C) is a potent inducer of interferon, at least part of its antitumor action is through the interferon system.

Because of this antitumor activity in animals, poly(I · C) has been administered to 14 patients at the NCI with advanced malignancies (*14*). The titers of interferon increased with increasing doses of the polymer, but a plateau was not reached. Although at these low doses antitumor activity has not yet been demonstrated, it is too early to assess the value of poly(I · C) in cancer therapy.

Future Prospects

The successes of poly(I · C) treatment in man have been limited thus far. However, only low dosages of the drug have been administered in clinical trials, and with increasing dosages increasing amounts of interferon have been induced, an indication that the maximum level of induced interferon in man has not been reached yet. In addition, scientists at the NIH presented evidence in February of this year that human serum contains an enzyme, probably a ribonuclease, which destroys poly(I · C), thus reducing the effective dose administered (*15*). This phenomenon was not encountered in any of the animal experiments, but subsequently both minks and ferrets were shown to possess the enzyme. The problem now is to prevent degradation of poly(I · C) and to find conditions for potentiation of its action. A search is now being made for synthetic polynucleotides that will be resistant to the degradative action of human serum.

Both Levy and Baron agree that, because of possible toxicity, poly(I · C) may not be the most effective antiviral agent for use in man. However, it is relatively inexpensive and easy to make and purify, particularly when compared to the inaccessibility of human interferon. The United States is conducting extensive clinical studies with poly(I · C), and the United Kingdom and France are studying the administration of human leukocyte interferon to man. In Russia and Italy there are also large groups studying the clinical applications of the interferon system. Several other inducers of interferon are being experimented with, including modified natural nucleic acids and drugs that are not chemically similar to nucleic acids.

Research in the interferon system thus seems to be leading to successful treatment of viral diseases and tumors for which there has previously been available only minimal treatment.

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