Interferon (II): Learning About How It Works

Recent research is clarifying how interferon inhibits viral reproduction but its other actions are less well understood

*The first installment in this series focused on the potential clinical uses of interferon and its production (*Science, *15 June). This article will deal with progress in understanding how interferon works.*

More than 20 years ago, researchers discovered that interferon prevents viruses from reproducing. Recently, they learned that it also inhibits cell division and regulates many reactions of the immune system, effects that are now receiving a lot of attention, partly because investigators think that they may be behind interferon's apparent ability to inhibit tumor growth.

Of all interferon's effects, however, its ability to prevent viral reproduction is the best understood. Researchers have known for some time that interferon plays a major role in combating viral infections in the living animal. They showed, for example, that its concentration in the blood increases markedly within 24 hours of infection, reaches a peak, and then declines about 4 days later. About this time, antibody production by the immune system is becoming maximal. Thus, interferon may help to contain the infection until the more slowly reacting immune system can take over.

Further support for this hypothesis comes from Ion Gresser and his colleagues at the Institut de Recherches Scientifiques sur le Cancer in Villejuif, France. They injected animals with antibodies against interferon in order to deprive them of the agent's antiviral effects in the early stages of infection. Animals injected with the antibody developed more severe infections than the controls.

Many diverse substances, in addition to viruses, can induce cells to make interferon. The inducers include a number of bacteria that reproduce intracellularly, some protozoal parasites, low molecular weight substances including the antibiotics cycloheximide and kanamycin, and high molecular weight materials including lipopolysaccharides and, especially, double-stranded RNA's. Double-stranded RNA's are so effective at provoking interferon synthesis that some investigators think that all the inducers may work by causing the production within cells of the RNA's, which would then serve as the ultimate trigger for interferon production. In any event, most investigators agree that the mechanism of interferon induction probably resembles the classic bacterial process in which the inducer turns on a gene—here the interferon gene—by removing from it a repressor that has been preventing gene expression.

According to Pravinkumar Sehgal and Igor Tamm of the Rockefeller University and Jan Vilček and his colleagues at the New York University School of Medicine, fibroblasts produce more interferon if inhibitors of protein or RNA synthesis are added to the cells at appropriate times during induction than if the inducer is added by itself. This process, which is called "superinduction," is of interest both because it may help to increase the production of interferon for clinical use and because it tells us something about the control of interferon synthesis.

Stimulation of interferon production by inhibitors of protein and RNA synthesis seems at first glance to be a paradoxical effect. Interferon, after all, is a protein, and a decrease in its synthesis might be expected. Sehgal, Tamm, and Vilček explain superinduction by noting that interferon synthesis begins very rapidly after induction is initiated by double-stranded RNA and then shuts down within a few hours. They postulate that the inhibitors prevent the shutdown by stabilizing the messenger RNA (mRNA) for interferon. If the messenger lasts longer, it can direct the synthesis of more interferon. In addition, the inhibitors appear to increase the synthesis of the mRNA by an as yet unknown mechanism.

Once the interferon molecules are synthesized, they move out of the cells where they originated and diffuse to neighboring cells, where they bind to receptors on the cell surface. This binding somehow triggers the synthesis of at least three cellular proteins that act to prevent viral reproduction.* The newly synthesized proteins are inactive, however, until the cells are infected by a virus or exposed to double-stranded RNA. This requirement for activation may help to protect the normal protein and nucleic acid synthesizing mechanisms of the cell from inhibition in the absence of a viral infection.

One of the inactive proteins is a protein kinase, an enzyme that transfers a phosphate group from adenosine triphosphate (ATP) to an acceptor protein. In the presence of double-stranded RNA and ATP, the protein kinase adds the phosphate group to an initiating factor needed for the synthesis of viral protein, thereby inactivating the initiating factor and inhibiting viral protein synthesis (Fig. 1).

The second protein, also an enzyme, catalyzes the formation from ATP of an unusual compound designated pppA2′p5′A2′p5′A. (The p's represent phosphate residues and the A's adenosines.) This compound is a nuclease activator that activates the third protein—an endonuclease that breaks down RNA molecules. Breaking down the viral mRNA's before they have a chance to direct synthesis of much viral protein should inhibit viral reproduction.

There are some indications that viral protein synthesis is more sensitive to inhibition by interferon than cellular protein synthesis. For example, Peter Lengyel and his associates have shown that the nuclease activator inhibits the translation of a viral messenger into protein more effectively than that of a messenger for a cellular protein. In earlier results, Charles Samuel, who is currently at the University of California at Santa Barbara, and Wolfgang Joklik of Duke University Medical Center had found that translation of viral messengers was greatly reduced in cells treated with interferon, whereas translation of cellular messengers was unaffected. Other investigators, working with a different kind of cell, found that translation of both types of messengers was reduced as a result of interferon, however, and the issue is still unresolved.

*Among the investigators who have made major contributions to the discovery of these proteins and the unraveling of how they work are Ian Kerr of the National Institute of Medical Research in London, Peter Lengyel of Yale University, and Michel Revel of the Weizmann Institute of Science in Rehovot.

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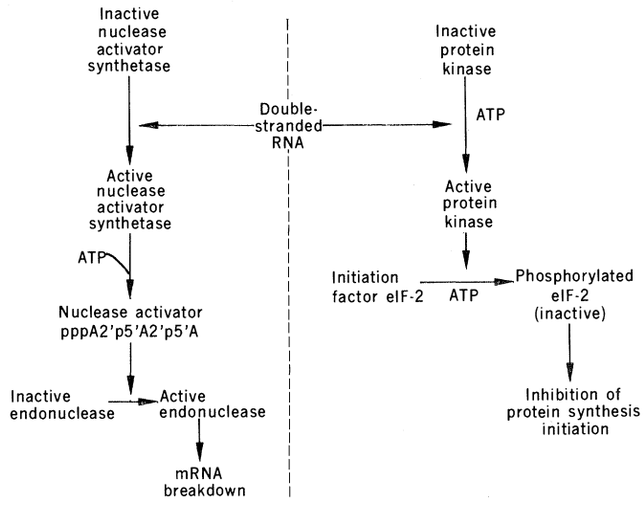

Fig. 1. Two pathways by which the enzymes produced in response to interferon inhibit viral protein synthesis. The enzymes must be activated, which can be accomplished by double-stranded RNA, in order to achieve their effects. The right portion of the diagram summarizes the steps leading to direct inhibition of protein synthesis; the left-hand side summarizes the reactions resulting in premature messenger RNA breakdown and consequent reduction in protein formation. [Adapted from a diagram by Peter Lengyel and his colleagues in *Proc. Natl. Acad. Sci. USA* **75**, 5893–5897 (1978)]

If all this were not complicated enough, there are additional mechanisms by which interferon may inhibit the reproduction of some viruses. Robert Friedman of the National Institute of Arthritis, Metabolism, and Digestive Diseases has been studying the effects of interferon on the replication of the murine leukemia viruses, which belong to the RNA tumor virus family. When these viruses chronically infect cells, their RNA genomes are copied into DNA, which is then incorporated into the genome of the cells.

According to Friedman, the production of the leukemia virus proteins in such infected cells is not inhibited by interferon. He says, "We can show that the virus is being made, but it is not released from the cell surface." In some cases, the virus particles are released but they are not capable of infecting other cells.

Release of the tumor viruses occurs through a budding process in which the virus first attaches itself to the underside of the cell membrane, which then pouches out and finally pinches off with the virus encapsulated in it. Friedman says that interferon produces several changes in cell membranes that might account for the loss of infectivity of the completed particle.

He speculates that incorporation of the viral genetic material into that of the cells allows the viral nucleic acids and proteins to be synthesized along with the cellular materials and thus escape interferon's inhibition of protein synthesis. The virus does not escape completely, however, because interferon disrupts later stages in its reproductive cycle.

Gresser and several other investigators have demonstrated that interferon inhibits the division of both normal and malignant cells in culture. Although this effect is not yet well understood, there are some clues to what is going on. According to Tamm, James Murphy, and Lawrence Pfeffer, who are also at Rockefeller, interferon prolongs the time interval between rounds of cell division. It produces slight decreases in the rates of synthesis of cellular DNA, RNA, and protein, but the effects are not sufficient to explain the slowed division of the cells, which grow to larger sizes than they normally would.

One of the first cell structures investigators examine these days when they are studying the control of cell division is the cytoskeleton, a network of filaments and tubules that undergoes distinct alterations when cells are transformed from the normal state to malignancy. Usually the cytoskeleton becomes disorganized and diffuse in transformed cells, a change that may account for the loss of growth control characteristic of malignancy.

The Rockefeller workers have observed the opposite effect on the organization of some of the filaments composing the cytoskeleton of cells treated with interferon. The filaments composed of the protein actin become thicker than they are in untreated cells. Tamm says this is consistent with the possibility that the effects of interferon on cell division are mediated through the cytoskeleton. Nevertheless, it is hard to eliminate the possibility that the filament changes are the effect—not the cause—of the slowed cell division.

Investigators have observed both stimulatory and inhibitory effects of interferon on the immune system, depending on the type of immune response under study and on the conditions of the experiment. For example, Howard Johnson and Samuel Baron of the University of Texas Medical Branch in Galveston find that, if cells in culture are exposed to interferon before they are exposed to an antigen, they produce less antibody. In contrast, if the cells are exposed to interferon a few days after exposure to antigen, antibody production is slightly enhanced.

Similar effects may occur in the living animal. Other investigators have shown that high doses of interferon, especially if given 2 days before antigen administration, greatly suppress antibody production, whereas low doses somewhat enhance it.

The production of antibodies is only one of many ways the immune system helps the body to ward off foreign invaders. Certain kinds of immune cells attack the invaders directly rather than by secreting antibodies. Some of these direct cellular attacks are also inhibited by interferon but others are stimulated. In particular, interferon enhances the cell-killing properties of a type of immune cell called the macrophage, according to Michael Chirigos of the National Cancer Institute.

This enhancement may be one way in which interferon restricts tumor growth. A great deal of evidence has indicated that activated macrophages can selectively kill tumor cells without affecting normal cells.

Moreover, Chirigos thinks there may be a connection between the antitumor activity of some agents, including BCG (bacillus Calmette-Guérin), and their ability to induce interferon. The agents in question all induce production of the agent and also stimulate cell-killing by macrophages. He suggests that their effects on macrophages may be mediated by their induction of interferon.

William Carter and Julius Horoszewicz of Roswell Park Memorial Institute have additional evidence that interferon mobilizes macrophages. They find many more of the cells in melanomas (a form of skin cancer) injected with interferon than in uninjected controls.

Finally, several investigators have now shown that interferon stimulates the activity of NK (natural killer) cells, another population of immune cells that may attack tumor cells.

Although there has been progress toward understanding the genetics of interferon production and action, there is still some confusion, especially regarding the chromosomal location of the gene for fibroblast interferon. Originally, Yin Hwee Tan, who is now at the University of Calgary, and Frank Ruddle of Yale University reported that chromosomes 2

and 5 both carry the gene. After further experimentation, Tan subsequently concluded that only chromosome 5 carries the structural gene; a genetic locus on chromosome 2 is involved in control of interferon production but is not the structural gene itself. Meanwhile, John Morser, working in Derrick Burke's laboratory at the University of Warwick in England, has obtained evidence that the gene for human fibroblast interferon is located on chromosome 9. Ruddle now says that the most recent results from his laboratory show that all three chromosomes carry a gene for fibroblast interferon. Geneticists consider it unusual for three separate chromosomes to carry exactly the same gene, although it would be less unusual if there were slight structural differences in the three genes.

Since there are three distinct types of interferon—leukocyte, fibroblast, and T or immune interferon—there is also the question of whether these are coded for by distinct structural genes. Alternatively, there may be only one interferon gene, with different cells having the capacity to produce different interferons by modifying either the mRNA or the protein whose sequences are specified by the common gene.

Sidney Pestka, of the Roche Institute of Molecular Biology, and Vilček have suggested that there are distinct structural genes, although they now concede that their experiment did not conclusively rule out the alternative possibility.

Pestka and Vilček prepared mRNA's from fibroblasts producing interferon and from an immature type of white blood cell that was producing leukocyte interferon. They injected the mRNA's separately into frog eggs. Eggs injected with the fibroblast messenger produced fibroblast interferon; eggs injected with the other messenger produced the leukocyte form.

The experiment rules out modification of the protein as the cause of the structural differences between the two types of interferon because the frog eggs would presumably produce the same alterations in the protein. But it does not rule out the possibility that the mRNA's underwent modification before they were isolated and injected into the eggs.

Modification of messengers is now thought to be an important feature of mammalian protein synthesis. But Pestka points out that they submitted their manuscript for publication in May 1977, just before the explosion of research on mammalian messenger processing documented the significance of the process. Thus, at the time, their conclusion that the mRNA's have different structures and are consequently the products of different structural genes for interferon seemed more clear-cut than it does now.

The original conclusion may still turn out to be correct, although Tamm points out that he and Sehgal have evidence suggesting that the mRNA for human fibroblast interferon is processed from a precursor roughly ten times larger than the final messenger, leaving a great deal of room for processing to produce alterations in the protein products.

The situation regarding the location of the genes needed for interferon's antiviral activity is somewhat clearer. At least one of them is located on chromosome 21, according to a number of investigators, including Tan and Ruddle. This chromosome may carry the gene for the interferon receptors known to be located on cell surfaces.

As interferon research now stands, the antiviral action of the agent is relatively well understood, but investigators are only beginning to tackle the mechanisms underlying its other effects. One important unanswered question concerns the relative roles of the three types of human interferons. They all act on viruses, cell reproduction, and the immune system, but their effects may vary in degree. Fibroblast interferon, for example, may not inhibit the division of a particular line of cells to the same extent that leukocyte interferon does.

Learning whether one interferon is more effective than another against a given tumor may help clinicians who are trying to determine whether the interferons will be useful agents for treating cancer. Interferon has not yet crossed the threshold to widespread clinical application, but the basic research now going on may give it a push in the right direction.—JEAN L. MARX

Burial Is Last Resort for Hazardous Wastes

Solidification may reduce leaching problem, but new rules, financial requirements may make disposal in landfills prohibitively expensive

Despite the many alternatives available for disposal of hazardous wastes, there are a great many materials that are too low in value to recycle, too difficult to degrade, too thick to inject into deep wells, and too contaminated with heavy metals and other nonflammable materials to incinerate. Some investigators consider these disposal methods to be volume reduction techniques because they leave a residue of hazardous materials. For most of these materials, the disposal option of last resort is burial in the ground. It's not the ideal solution, it's not necessarily even a good solution, but, realistically it's the only solution we now have. The problem then is to regulate landfills in such a manner that potential problems created by escape of toxic materials are minimized.

This is the last of four articles about the disposal of hazardous wastes.

The Environmental Protection Agency (EPA) has sponsored much research on the various facets of landfilling, such as engineering techniques, liners, covers, and gas generation. But if privately sponsored research is included, the greatest amount of effort has been devoted to the chemical solidification of wastes—the development of techniques to bind the wastes into a coherent mass before burial so that leaching of toxic materials by groundwater is minimized. Solidification is now used for only a very small percentage of hazardous wastes, but it promises to be one of the most important disposal techniques of the future. It also promises to be one of the most complex to evaluate. A recent survey by Robert B. Pojasek of Energy Resources Company Inc. of Cambridge found that at least 41 different companies and research

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