Interferons and Cancer

Although interferons were discovered in 1957 (1), we do not yet fully understand their production, mechanism of action, biologic role, or therapeutic potential. Originally described as antiviral agents, interferons have other activities which include regulation of cell growth (2) and regulation of the immune response (3). Several recent findings have made interferons of current special interest to oncologists. Through the persistent effort of Dr. Kari Cantell, Central Public Health Laboratory, Helsinki, Finland, an amount of human leukocyte interferon sufficient for meaningful clinical studies of the effects of interferon on a few diseases has finally become available. Employing this interferon, a group at Stanford University, Stanford, California, under the direction of Dr. Thomas Merigan, has obtained evidence suggesting that human interferon may be useful in the treatment of chronic hepatitis B virus infection (4) and has found that interferon treatment of herpes zoster complicating leukemias and lymphomas has led to more rapid resolution of lesions and prevention of systemic effects (5). In addition, preliminary results from the laboratory of Dr. Hans Strander (Karolinska Institutet, Stockholm, Sweden), who has been treating 28 osteogenic sarcoma patients with Cantell's human interferon, suggest that the group receiving interferon has a lower rate of metastasis than does a concurrent control group (6). Strander's studies, although not yet involving sufficient numbers of patients to be highly significant, appear to warrant serious consideration of human interferon as an antitumor agent; animal studies supply strong evidence that in some situations interferons are effective in inhibiting tumor growth (7).

Interferons are proteins. Their production by animal cells can be induced by various substances (1), and they in turn inhibit a wide spectrum of RNA and DNA viruses by inducing an antiviral state. However, interferons are generally animal species-specific in their range of antiviral activity; only human interferons have been shown to be effective in treatment of human cells, although it is certainly possible that some animal interferons will prove active.

INTERFERON PRODUCTION

Interferon production is an induced activity. Under normal conditions animals produce interferons as one of a series of responses to virus infections, and interferons seem to be important in recovery from some primary virus infections (7). Lymphocyte interferons are lymphokines, inasmuch as their production may be induced by exposing macrophages and T-lymphocytes to mitogens or antigens (8). Interferons may also be induced in tissue cultures by double-stranded RNA forms such as polyribinosinic-polyribocytidylic acid (poly I·poly C) and in animals by, for example, various intracellular parasites (bacteria, metazoa, protozoa), polymers, endotoxins, or double-stranded RNA forms (9).

When exposed to an interferon inducer, cells in culture make an interferon messenger RNA, which may be translated in cell-free systems or in frog oocytes (10, 11). The amount of interferon produced by cells is proportional to the amount of interferon messenger RNA induced (12). The amount of messenger RNA may be increased by a superinduction procedure which involves treatment of cells with inhibitors of RNA and protein synthesis, after exposure to an inducer but before collection of interferon begins (13). The superinduction procedure appears to prolong the half-life of the interferon messenger RNA (12). The production of interferon presents the cell biologist with an interesting induction system in which negative feedback controls operate and an easily characterized protein is made.

ANTIVIRAL ACTIVITY OF INTERFERONS

Although the mechanism of action of interferon is not yet known, it seems to be the result of a complex series of events (14). Interferons bind on the cell surface to a specific receptor which seems to contain both ganglioside and peptide components [(15); Kohn LD: ___

ABBREVIATION USED: MuLV = murine leukemia virus.

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Editor's note: Periodically, the Journal publishes solicited guest editorials as a means of transmitting to investigators in cancer research the essence of current work in a special field of study. The Board of Editors welcomes suggestions for future editorials that succinctly summarize current work toward a clearly defined hypothesis regarding the causes or cure of cancer.
Unpublished data]. Binding of interferon to a cell surface receptor does not necessarily result in the development of antiviral activity, but in sensitive cells, i.e., those with the proper effector apparatus, binding causes chemical, morphologic, physical, and immunologic alterations in the plasma membranes (16–18). These alterations, possibly through a mechanism involving cyclic AMP (19, 20), seem to result in the production of an enzyme precursor which when activated can produce an intracellular antiviral substance (21). This enzyme is a protein, the production of which probably requires the synthesis of a new species of messenger RNA (22).

After an interferon-treated cell is infected with a virus, the production of a viral product, most likely a double-stranded form of viral RNA, seems to trigger the activation of the enzyme precursor (23, 24). The activation may involve phosphorylation of the precursor by a kinase also activated by a viral double-stranded RNA. The antiviral substance formed in cell-free extracts is a recently described adenosine oligomer with an unusual 2',5' linkage (Kerr IM, Ball LA: Personal communication). This antiviral substance may inhibit virus replication by several mechanisms, which possibly include inhibition of virus-directed transcription, or translation, or ribonucleolytic cleavage of viral messenger RNA.

SITE OF ANTITUMOR ACTIVITY OF INTERFERONS

Several hypotheses have been advanced to attempt to explain a putative antitumor activity of interferon. These include inhibition of tumor virus replication and cell transformation by virus and inhibition of tumor growth, directly or indirectly, through primary effects on the immune system.

Interferon was thought to inhibit tumor viruses through the same mechanism as that involved in the inhibition of other viruses (14). For the inhibition of both the DNA and RNA tumor viruses studied most completely, however, the mechanism of interferon action seems to have unusual features. For simian virus 40, the accumulation of early viral messenger RNA is inhibited (25), whereas in the best studied of other systems, early viral messenger RNA is not reduced by interferon treatment (26). Even more surprising were the findings on interferon inhibition of MuLV replication, a system in which interferon treatment does not seem to inhibit MuLV-directed protein or RNA synthesis (27, 28). Instead, defective virus with markedly reduced infectivity is synthesized (29–31). In some systems, the MuLV produced in interferon-treated cells is so defective that it cannot bud from the cell surface (32, 33). At least two abnormalities have been found in the defective MuLV released from interferon-treated cells: a large glycoprotein, possibly a precursor to the viral gp69/71 in the viral membrane (34), and a defective p30 reverse transcriptase complex with decreased enzyme activity (Bandyopadhyay AK: Personal communication).

Whatever the mechanism, however, interferon treatment results in a marked decrease in the production of infectious virus and in cell transformation by virus (35). Interferon may thus prevent virus recruitment of new cells into the transformed state, which may explain the repeated observations in many systems that interferon treatment inhibits induction of tumors in mice by onogenic viruses (2). Interferon treatment also inhibits the growth of established virus-induced mouse neoplasms and has been successfully employed to inhibit transplantable tumors and chemically induced neoplasms in mice (2). Less success was usually attained in the treatment of solid transplantable tumors than in treatment of ascites tumors; however, interferon treatment inhibited the development of both the subcutaneous nodules of Lewis lung carcinoma at the site of transplantaion and the development of pulmonary metastases from the transplant (36). Interferon administration reduced the incidence of fibrosarcomas and lung adenomas induced in C3H mice by 3-methylcholanthrene (37). These transplantable or induced tumors are not obviously due to viruses. Their inhibition by interferon suggested that interferon treatment might have a direct effect on tumor growth.

The inhibition of growth of tumors which apparently are not virus induced gave rise to speculation that interferon might be a growth control regulator and that the effects on tumors are caused by rapid tumor growth. If interferon does regulate the growth of cells, interferon treatment should show some effects on normal (nontumor) cells. In some studies, this has proved to be so, and in vitro interferon inhibited cell DNA synthesis and replication (38). Interferon treatment inhibited the regeneration of the liver in partial hepatectomized mice (39). Newborn C3H or Swiss mice treated with high concentrations of partially purified interferon exhibited weight loss and diffuse hepatic cell degeneration (40). Similarly, in a newborn child treated with human interferon for congenital cytomegalovirus infection, significant weight loss was recorded before the therapy was halted (41).

A third possible site for interferon-induced inhibition of tumor growth is the immune system. In both in vivo and in vitro studies, interferon treatment inhibited the antibody response (42). This effect of interferon was dose-dependent and could not be separated from the antiviral activity of the interferon preparations. Interferon also inhibited cellular immune responses and delayed-type hypersensitivity (43). Similar studies also showed an inhibition by interferon treatment of in vitro mitogen-stimulated DNA synthesis by T-lymphocytes (44). In mice, interferon treatment inhibited the response to allografts (45), sensitization to picryl chloride (43), and the delayed-type hypersensitivity response to sheep red blood cells (46).

Possibly related to studies on the immune system was the finding that interferon treatment inhibited phagocytosis (47) in mice and caused several specific alterations in the surfaces of lymphocytes. Interferon-treated lymphocytes had an increased capacity to absorb alloantisera (17) and an enhanced expression of surface H-2, antigens (48).
Thus interferon treatment might inhibit tumor growth in several ways through primary effects on the immune system. Alterations induced by interferon in the plasma membranes of lymphocytes might be responsible for an increased cytotoxicity. Interferon treatment could also alter the surfaces of tumor cells (as it does in lymphocytes and L1210 cells) to increase the expression of tumor-specific transplantation antigens. The increased phagocytosis induced by interferon treatment could have a role in the inhibition of tumor growth by causing rapid destruction and elimination of tumor cells. Finally, the inhibitory effects on antibody production could cause a decreased level of tumor-protective (blocking) antibodies, thus permitting the immune system to deal more effectively with tumors.

ATTEMPT AT A UNITARY HYPOTHESIS TO EXPLAIN THE VARIOUS ANTITUMOR EFFECTS OF INTERFERON

Many diverse effects of interferon treatment have been discussed so far. These include inhibition of viral RNA and protein synthesis, formation of defective virus particles, inhibition of normal and tumor cell growth, and inhibition of various aspects of the immune response. Can all of these activities be explained by interferon action at a single site? This is a difficult synthesis, but it is possible that changes induced by interferon on the plasma membranes of cells might be common initial sites of action. Antiviral action seems to be started by the binding of interferon to plasma membrane receptor sites and then, in sensitive cells, its affecting activation sites. These steps appear to cause several alterations in the plasma membrane. For RNA tumor viruses, the interferon-induced alterations in the plasma membrane could be responsible, at least in part, for the structural defects in virus produced by these cells. For other viruses, the early events (binding and activation) may be responsible for the intracellular activities that later lead to the inhibition of virus growth.

Alterations in the plasma membrane could also help to explain interferon's inhibitory action on normal and tumor cell growth. The phenomenon of contact inhibition of growth would suggest that events at the cell surface are important in determining the replication rate of cells. Interferon-induced changes in the plasma membrane could signal a cutback in the growth rate of malignant cells.

Finally, many of the effects of interferon on the immune system could be related directly to effects on the plasma membrane. Certainly, as discussed above, most of the effects on the immune system which may be related to the antitumor activity of interferons are membrane-associated events. These include increased phagocytosis, an increase in expression of transplantation antigens, and an increase in cytotoxicity of interferon-treated lymphocytes. Therefore, although it is a very speculative hypothesis, the actions of interferons may be due to a primary effect on the plasma membranes.

WHAT CAN BE DONE?

In spite of the promise of the investigations outlined above, the number of researchers in the field of interferon studies is small, and support for basic research has been modest. There are several reasons for this situation. Because of its great specific activity (one unit has been recently estimated to be less than $10^8$ mg of protein), interferons have been difficult to obtain in large enough quantities for purification or for a significant number of clinical studies. In addition, assays for interferon are biologic, based on the ability of a preparation to inhibit virus replication; they are time-consuming and relatively inaccurate. Finally, in any one species several substances appear to have the general properties of interferons. For example, human white cell interferon preparations contain several molecular species of interferon. Interferon produced in human diploid cells has one component that antigenically resembles one of the interferons from white cells. Human T-lymphocytes, stimulated by mitogens or antigens, produce yet another distinct species of interferon. Although all of these interferons induce antiviral activity, it is not clear that they act in the same way.

It is no great wonder, then, that many editors and peer review groups are unenthusiastic about interferon research. Only because interferon had promise as an antiviral agent was it fortuitously included among the drugs to be studied by the Antiviral Substances Program of the National Institute of Allergy and Infectious Diseases, Bethesda, Maryland. For several years, under the excellent supervision of Dr. George Galasso, this program was probably the greatest source of funds for interferon research in this country. In many areas of interferon research, the Antiviral Substances Program was responsible for keeping up some momentum at a time when progress would otherwise have been stalled. At present, however, I believe that the exciting research leads on the molecular biology of interferon production and action, together with the preliminary data, discussed above, on the clinical uses of human interferon, should stimulate cancer researchers with a wide range of interests to consider doing studies in these areas. Also, large-scale bioengineering techniques should be used in the attempt to deal with the problems of interferon production and purification.

Such efforts may lead to solutions of basic problems in the understanding of biologic controls of animal cells. In addition, the leads that Gresser (2) and Strander (6) have put forth may be confirmed, and interferon could prove to be useful in the therapy of cancer.

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