TEACHING EDITORIAL

The Interferons—Clinical Applications and New Technologies

"During a study of the interference produced by heat-inactivated influenza virus with the growth of live virus in fragments of chick chorio-allantoic membrane, it was found that following incubation of heated virus with membrane a new factor was released. This factor, recognized by its ability to induce interference in fresh pieces of chorio-allantoic membrane, was called interferon" (*1*).

This observation, made in 1957 by Isaacs and Lindenmann, culminated nearly three decades of study of interferences among viruses by many investigators. Isaacs and Lindenmann recognized that the interfering substance was produced by the infected host cells and named it interferon (*1*).

Perhaps the earliest recorded instance of interference by one virus with the replication of another was made by Jenner, who observed in 1804 that active herpetic infection may prevent the proper development of vaccinia lesions (*2*). Following observations that certain plants infected with one virus failed to develop manifestations of a second viral infection upon exposure to a second virus (*3,4*), the phenomenon of viral interference was reported for animal yellow fever viruses in *Macacus rhesus* (*5*). By the mid-1950s an extensive literature documented other instances of viral interference (*6*). Until the seminal observations of Isaacs and Lindenmann (*1*), however, the nature of this interference was not evident.

It has become clear, subsequently, that "interferon" is actually a family of proteins derived from different cell types in response to a variety of stimuli. Although originally associated with viral infections, interferons are produced in reaction to bacteria, foreign cells, macromolecules, and other chemical compounds. While active against viral replication, interferons also have potent effects upon the immune response, cell growth and replication, and numerous other cellular functions. These diverse functions have suggested that interferons may be useful therapeutically against viral, neoplastic, and immune disorders.

Although interferons appear to possess a wide range of biological and biochemical functions, the response to viral infection is best understood (*7*). Viral infections stimulate cellular DNA, which leads in turn to the production of messenger RNA coding for interferon. Subsequent translation leads to the production of interferon, which is released from the cell to interact with membrane receptors on adjacent cells. These cells are stimulated to produce proteins, which interfere with viral nucleic acid function.

Because the early studies of interferon function were done using impure protein preparations, it is uncertain which effects observed in these experiments were due to interferon and which may have been due to other substances in the preparations. Recently, using pure recombinant DNA interferons (*8*), some actions classically ascribed to interferon have been confirmed. Antiviral activity, antiproliferative activity, and stimulation of "natural killer" cell activity are intrinsic properties of pure interferons. Much more effort is required before the entire spectrum of actions of interferons is known with certainty. The exact number of interferon proteins that may be produced in specific cells, their structure, and their specific functions remain to be determined.

By the 1970s, methods of interferon induction had been devised that led to small quantities of interferons becoming available for study and characterization (*9*). Protein purification permitted elucidation of amino acid structure for some interferon sequences (*10,11*). Three classes of interferons were recognized, coded by different structural genes and possessing different amino acid sequences (*12*). The class of interferon produced depends upon the stimuli and the cell type stimulated. Leukocyte interferon (alpha) is represented by multiple molecular types, is generally not gly-

cosylated, and is monomeric (*13*). Fibroblast interferon (beta) may be molecularly heterogeneous, is glycosylated, and is dimeric (*13,14*). Immune, or antigen-induced interferon (gamma), is molecularly less diverse, is glycosylated, and is tri- or tetrameric (*13*). Interferons alpha and beta are acid stable and were termed type I in earlier literature; interferon gamma is acid labile and was termed type II. Since both leukocytes and fibroblasts can produce alpha and beta interferons and because gamma interferon can be chemically induced, the most unambiguous classification should be based upon amino acid sequence (or the corresponding structural DNA base sequence) (*12*).

Initial efforts directed toward the production of relatively large quantities of human interferon involved incubation of pooled donor buffy coat with an inducing virus (*9,15*). The resultant interferons were partially purified and then could be tested clinically. In the 1970s a large number of clinical trials were initiated in which crude interferon preparations were administered to patients with various viral illnesses (*16*), neoplasms (*17*), and arthritic conditions (*18*). Because the preparations used represented mixtures of proteins, only a small fraction of which was interferon, it was difficult to ascertain which of the observed effects were due to interferon itself. Furthermore, it was impossible to be certain which species of interferons were present in the mixtures. These preparations had been tested in vitro for antiviral activity and were assigned potency based on results in these bioassay systems.

Following the isolation of messenger RNA from appropriately stimulated cells, some of which coded for interferon, it became possible to prepare complementary DNA that could be spliced into plasmids. These plasmids, when introduced into bacterial cells and cloned, yielded colonies, some of which produced interferon (*19–21*). The use of partially purified interferons and the hybridoma technique developed by Köhler and Milstein (*22*) led to a family of monoclonal antibodies, which permitted effective purification of recombinant interferon (*23*). These monoclonal antibodies are used for epitope identification and characterization, and in addition, they form the basis for a number of immunoassays. With these new technologies, Hoffman-LaRoche, Inc.* in collaboration with Genentech†, produced human leukocyte interferon A on a commercial scale, making possible for the first time clinical trials using virtually pure, completely characterized interferon (*24–25*). These first human clinical trials using recombinant leukocyte interferon were begun in January, 1981, and the results suggest that recombinant interferons are biologically active and modify human viral and neoplastic diseases (*24*). Information regarding the biological effects of recombinant human leukocyte interferon A have been reported (*25–27*), but further extensive trials will be necessary to assess the relative antiviral and/or antitumor effects of other interferons or combinations of interferons. Additional alpha interferons will become available as will beta and gamma interferons.

Reliable assays are necessary for all aspects of interferon research, and because viral interference was the first biological property of interferons to be recognized, efforts to standardize interferon preparations involved assessment of their antiviral activity (*28*). Until recently, only bioassay systems were available for measurement of apparent interferon concentrations. Many assays have been described, all based on the ability of the interferon preparation to inhibit viral-induced cell lysis (cytopathic effect), to inhibit viral plaque formation in tissue culture, or to retard virus replication (*28*). These assays are generally imprecise, time-consuming, and subject to inaccuracies due to the presence (at least in crude preparations) of noninterferon substances, which may also possess antiviral activity.

Essential for the standardization of these systems is an internationally recognized standard reference preparation and two, Medical Research Council Research Standard B‡ and NIAID G-023-901-527§, are available for this purpose. These preparations of human leukocyte interferon are defined in terms of their activity in bioassay systems. They are not homogeneous preparations, and their activity differs in different bioassay systems (*8*). The relative immunoreactivity of these preparations in different interferon immunoassays is uncertain.

Despite these problems, a large body of information relative to the use of interferon bioassay systems has accumulated. The most widely accepted definition of an interferon requires that a candidate protein possess antiviral activity,** implying the ability to assess that activity (*12*). The relationship between the bioactivity and the immunoreactivity of the various interferons is currently unknown. Additionally, most immunoassays have not approached the sensitivity of antiviral bioassay systems (about 10 pg of interferon protein (*29*)). All of these considerations suggest that interferon bioassays will continue to be necessary for some time.

Investigators at the Roche Institute of Molecular Biology* have proposed that interferon action determined using molecularly pure interferons be reported in terms of the molecules/cell required to produce a certain effect (*8*). They call this estimate "specific molecular activity" and point out that other reference standards are not required to assess the activity of pure interferons. Specific molecular activity should be more meaningful than the current antiviral units derived using impure interferon reference preparations.

Following the purification of several interferons and the development of monoclonal antiinterferon antibodies, a number of immunoassay systems have been developed (*29–34*). These systems have utilized both monoclonal and polyclonal antiinterferon antisera, purified interferon for calibration and labeling, radio- and nonradiolabels, and immunometric as well as competitive-binding assay conditions. Elsewhere in this issue of the *Journal*, Bernier and colleagues describe a well-characterized, sequential, competitive radioimmunoassay for human leukocyte (alpha) interferon (*35*). These investigators have utilized partially purified leukocyte interferon obtained from Cantell (c.f. *9*) as their immunogen, and the resulting antiserum permits the detection of about 30 pg/ml interferon protein (3 pg in the assay tube). For radiolabeling they used pure recombinant human leukocyte alpha interferon obtained from Staehelin (whose group reported the development of immunoassays based on monoclonal antisera (*31*)), and calibrated their system using Medical Research Council Research Standard B (69/19). This system was found to be precise, recovered the 69/19 Standard (not surprising) and, of interest, was compared with a bioassay of the inhibition of cytopathic-effect type. This immunoassay did not recognize beta or gamma interferon but was not further characterized with respect to the alpha epitope(s) recognized. Reactivity with a preparation of lymphoblastoid interferon (presumed to be principally a mixture of alpha interferons) was the same as that observed for the 69/19 standard. Unlike monoclonal antibodies, the authors' antiserum would not be useful for affinity interferon purification nor as a structural probe. The potential clinical value of this assay, however, is well demonstrated by studies in patients with viral illnesses and in cancer patients receiving leukocyte interferon therapy.

Pure interferon preparations and interferon immunoassays will permit an accurate reassessment of many questions originally addressed using crude interferon preparations and bioassay systems. Under what circumstances does the body produce interferons? What are the normal physiologic effects of interferons? What is the fate of endogenous interferons? The effects of age, malignancies, inflammatory diseases, infections, drugs and immunosuppression as well as the effects of pathologic alterations in organ function (e.g., uremia, hepatic failure) on interferon response are of interest to investigators.

The therapeutic administration of pure interferon is now possible (*36–37*), and numerous other well-characterized interferon preparations are certain to become available for clinical trials soon. Hybridized interferons, possible with recombinant DNA technology, will add to the complexity of these trials (*25*). Alteration (e.g., glycosylation) of recombinant interferons after production has not yet been extensively studied (*37*). Immunoassays will be utilized for pharmacokinetic studies in patients receiving interferon. As the fate of both endogenous and exogenous interferons becomes known, the fact that at least some assays detect immunoreactive fragments will likely become evident.

Despite the very large body of knowledge that has accumulated regarding interferons, much remains to be learned. The production of recombinant interferons and epitope-specific monoclonal antibodies will permit assessment of structure/activity relationships for the first time. Initial observations suggest that some of the different biologic effects attributable to interferons may involve different molecular mechanisms, possibly indicating the presence of multiple cell membrane interferon receptors (*8*). There is need for the development of direct binding and cellular membrane receptor assays to clarify these points (*8*).

Advances in the technology of immunology and molecular biology have greatly enhanced our understanding of the interferon systems. Immunoassay will play an important role in future developments.

LYNN R. WITHERSPOON
Ochsner Clinic and Alton
Ochsner Medical Foundation
New Orleans, Louisiana

FOOTNOTES

* Roche Institute of Molecular Biology, Nutley, NJ 07110.

† Genentech, Inc., South San Francisco, CA 94080.

‡ The International Laboratory for Biological Standards, National Institute for Biological Standards and Control, Holly Hill, Hampstead, London NW3 6RB.

§ Research Resources Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20014 (G-023-901-527 has been calibrated against the MRC 69/19 leukocyte standard).

** "To qualify as an interferon a factor must be a protein which exerts virus nonspecific, antiviral activity at least in homologous cells through cellular metabolic processes involving synthesis of both RNA and protein" (*12*).

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