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Interferon Treatment of Human Neoplasia

By HANS STRANDER

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PREFACE

Information concerning the effects of interferons (IFNs) in the treatment of tumors—especially at the clinical level—has been compiled and presented in this volume. A rather complete survey is included of what has happened in just a few years of intensive international IFN research in this area. Since so many data have accumulated from both experimental and clinical oncology sources, references to information gathered before 1979 will be limited. Included are data presented at symposia and references that are difficult to obtain from general sources. The volume is almost entirely devoted to data on humans, but some mention is made of animal experimentation.

The book contains chapters dealing with experimental IFN effects, with special emphasis on the types of IFNs and their actions that cause regression of tumors. The volume starts with a survey of the various IFNs, how they are produced, and how they act. Their pharmacology and toxicity are discussed. A short chapter on animal tumor models used for possible application to human tumor disease follows. The book then deals with the treatment of benign tumor diseases. IFN treatment of malignant diseases is also discussed. IFN inducers and other forms of IFN therapy are mentioned. Concluding the volume is a chapter summarizing the present situation and suggestions for future research.

Readers most likely to find this book of particular interest will be investigators actively involved in IFN effects and the possible mechanisms underlying the effects achieved with human tumors. This book will also be of interest to oncologists and other specialists working with IFN at the clinical level. It should also fulfill the needs of investigators interested in a broad introduction to the area. It is clear that IFNs have become a permanent part of the armamentarium used in the treatment of tumor disease in man and thus should be of general interest to all engaged in clinical oncological research.

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CHAPTER 1

INTERFERONS (IFNs)

"The writing of an article helps to make the writer better informed on the subject he discusses."
Morris Fishbein (1938)

I. Introduction

Interferons (IFNs) are proteins or glycoproteins able to exert antiviral activity through their effects on the intracellular events of the viral cycle. They belong to the family of biological response modifiers and are constituents of the body's defense system. IFNs were first defined in 1957 (Isaacs and Lindenmann), although the phenomenon of viral interference had been reported much earlier (for a review, see Nagano, 1975). Three classes of IFNs have since been described, but it is quite possible that new types of IFNs will be discovered in connection with biological studies (see Van Damme *et al.*, 1981).

IFNs can be induced in an organism by (1) virus infection, (2) a variety of nonviral inducers, (3) mitogens, (4) antigens, and (5) tumor cells. Since IFNs are produced under such varied circumstances, the exact role played by these molecules in connection with various disease states must be deciphered. In addition, one would wish to understand their relevance to resistance to disease (Wilkinson and Morris, 1983b).

Isaacs is said to have been in 1962 the first to consider large-scale production of IFN. In the 1960s and early 1970s, the various factors associated with such large-scale production were examined, particularly in Canada, Finland, France, the Soviet Union, the United States, and Yugoslavia. In 1961, Gresser reported that IFNs could be produced in substantial amounts by human leukocytes.

This system was then studied in Finland, leading to the initial production of semipurified human leukocyte IFN-α (see Cantell *et al.*, 1981). Such IFNs were used during the 1970s on both viral and tumor diseases. Subsequently, this type of natural IFN-α has been used in other types of disease (cf. Merigan *et al.*, 1982; Strander, 1983a, 1984). It soon became evident that natural IFN-α could cause side effects in the form of headache, malaise, and fever (Strander *et al.*, 1973). Later

studies showed that even pure preparations caused similar side effects (Scott *et al.*, 1981).

The results of IFN-α treatment of a variety of tumors were summarized in a report by a World Health Organization (WHO) Scientific Group in 1982. Since that time, promising results have been obtained in renal cell carcinoma, chronic myelogenous leukemia (CML), hairy cell leukemia, Kaposi's sarcoma, and several other diseases. Among the most exciting effects were the ones on the various papillomavirus-associated diseases (juvenile laryngeal papillomatosis, common warts, and condyloma acuminata).

Natural IFN-β was first produced in large amounts in 1972–1973 and has since been used on a variety of tumor patients, especially in Western Europe and Japan. The large-scale production and use of IFN-γ has just begun.

An excellent review of the anti-tumor activities and pharmacokinetics of IFN, as well as a summary of the results of IFN treatment of tumors in humans, was written by Stewart (1979a). Several more recent reviews are listed in the Addendum to Chapter 13, before the bibliography. The aim of the present review is to provide summaries of the rationale for IFN use in the treatment of human neoplasia and of the results obtained in this area to date.

II. Types

Interferons (IFNs) have been divided into three classes: α, β, and γ (cf. Collins, 1983a; Pestka and Baron, 1981; Pestka, 1983b; Pestka *et al.*, 1984). A fourth class, IFN-ρ, has been suggested by Wilkinson and Morris (1983c). They found a substance with the essential characteristics of a classical IFN but with antiviral activity expressed only in trisomy 21 human fibroblasts.

The IFN-α family contains many types of molecules, and it has been suggested that up to 40 subtypes may ultimately be found (J. Collins, personal communication). Several IFN-α subtypes have also been described in the murine system (Shaw *et al.*, 1983). The reason for this heterogeneity is unknown. Whether there are multiple subtypes of IFN-β and IFN-γ remains a matter of controversy (Collins, 1983b). For a description of the old and new IFN nomenclatures, see Anonymous (1980). The main types of IFN used in clinical trials are listed in Table I.

It took quite some time before IFNs were purified to homogeneity (cf. Knight, 1978; Knight *et al.*, 1981; Rubinstein, 1982a). The use of monoclonal antibodies (see Milstein, 1982) has been extremely im-

TABLE I

IFN PREPARATIONS USED FOR CLINICAL TRIALS

Name	IFN class	Subtypes	Number of subtypes	Purity	Comment
Natural	α	Various	15–40	Impure, semipurified or purified	More impure in earlier trials
Recombinant	α	$\alpha 2$	1	Purified	Produced in *E. coli*; arginine at position 23; deletion at position 44
Recombinant	α	A	1	Purified	Produced in *E. coli*; lysine at position 23; deletion at position 44
Recombinant	α	D or $\alpha 1$	1	Purified	Produced in *E. coli*; 29 amino acid variations from αA
Lymphoblastoid	α	Several	5–8	Semipurified to purified	From cultured lymphoma cells *in vitro* or in hamsters
Natural	β	One (?)	1	Semipurified	Can be purified; made from fibroblasts or SV40-transformed cells
Recombinant	β	β_1	1	Purified	Cysteine at position 17
Recombinant	β	β-Ser	1	Purified	Serine at position 17
Natural	γ	1 (?)	1	Impure or semipurified	More impure in earlier trials
Recombinant	γ	γ_1	1	Purified	Probably different from natural γ

portant in this respect. Recombinant DNA technology has also had enormous impact on IFN research (Wetzel, 1980; Weissmann *et al.*, 1982a; Fiers *et al.*, 1982).

Goeddel *et al.* (1980a) reported that human leukocyte IFN-α produced by *Escherichia coli* was biologically active, since it could protect squirrel monkeys from lethal encephalomyocarditis (EMC) infection. By 1981, the structures of eight different cloned human leukocyte IFN-α cDNAs had been described (Goeddel *et al.*, 1981). Many distinct IFN-α sequences have since been determined, although this is just the beginning of an extensive research area (Weissmann *et al.*, 1982b). The properties of the genetically engineered IFN-α2 preparation have been reviewed (Nagabhushan *et al.*, 1984).

Analogues or hybrids of human IFN-α have also been prepared, but the clinical potential of such molecules remains to be seen (cf. Lee *et al.*, 1982a; Alton *et al.*, 1983). So far, it has not been possible to find active IFN fragments (Wetzel *et al.*, 1982). Human IFN-β was cloned in 1979 by Taniguchi and collaborators (Goeddel *et al.*, 1980b; Taniguchi *et al.*, 1982). Recombinant human IFN-γ followed in 1982 (cf. Gray *et al.*, 1982; Rinderknecht, 1984).

Human lymphoblastoid IFN may be produced by exposing lymphoma cells to a viral inducer. It seems to consist of several primary IFNs, the exact structures of which are unknown. There appears, however, to be little, if any, glycose present in these molecules (Allen and Fantes, 1980). IFN-β is produced at the same time.

The biochemical properties and structures of the various human IFNs have been reviewed (Hayes, 1981; Rubinstein, 1982b; Vilček, 1982b). For a discussion of the evolution of the IFN molecules in humans, see De Grado *et al.* (1982). These authors have proposed a common ancestor for both virus-induced IFNs and IFN-γ.

III. Production and Purification

An important contribution to IFN research was made by Gresser (1961) when he demonstrated that peripheral leukocytes are able to produce substantial amounts of IFN. The use of human leukocytes for this purpose is in keeping with the modern concept of multiple uses of donor blood (Högman, 1979). During the 1960s, a substantial amount of work was done in Cantell's laboratory on the production of large amounts of human IFN-α by suspended leukocytes (see Strander, 1971). This culminated in the production of stable, semipurified preparations useful for clinical trials in the early 1970s (Mogensen and Cantell, 1977; Cantell and Hirvonen, 1978). For a more recent discus-

sion of the preparation of human natural IFN-α, see Horowitz and Horowitz (1984). Monocytes seem to be the main producers of IFN-α in leukocyte preparations following Sendai virus induction (Saksela *et al.*, 1984).

Natural IFN-α preparations have limitations, however. Schoub *et al.* (1983) found differences among individual preparations and stressed the importance of doing comparative studies on the various batches before their use in clinical trials. Others have criticized the use of human leukocyte cultures for the production of IFN because of the possibility of slow virus contamination of semipurified preparations (Wadell, 1977). Such a problem is illustrated by the acquired immunodeficiency syndrome (AIDS). Of 2952 cases reported to date, 31 cases under investigation by the Centers for Disease Control (CDC) in the United States have no identified risk factors other than having received blood transfusions within the 5 years preceding the diagnosis (see Curran *et al.*, 1984). Observations made on infants with AIDS suggest transplacental, perinatal, or postnatal transmission of an as yet unidentified infectious agent (see Scott *et al.*, 1984). Taking into consideration the seriousness of the neoplastic diseases being treated by IFNs, the risks involved are, in my opinion, not strong enough to prevent the use of natural IFN preparations. Furthermore, human leukocyte IFN-α has been given to thousands of patients, and none of them has developed AIDS so far.

Many tumor cells, including human lymphoma cells, spontaneously produce IFN (Adams *et al.*, 1975b). Twenty-one different human lymphoblastoid cell lines were screened for ability to produce IFN following exposure to Sendai virus (Strander *et al.*, 1975). One cell line, which showed a good response, the Namalwa cell line, has since been used for the large-scale production of human lymphoblastoid IFN-α, especially in England, Japan, and Austria. Imanishi *et al.* (1982) have used human lymphoblastoid cells grown in hamsters for this purpose. For a discussion of the preparation of lymphoblastoid IFN, see Fantes and Finter (1984).

Horoszewicz *et al.* (1978c) found that the best IFN-β producing strain of human diploid foreskin fibroblasts had a translocation between chromosomes 5 and 15, although normal fibroblasts are also generally good IFN-β producers. For a discussion of the production and purification of natural human IFN-β, see Billiau *et al.* (1979c), Leong and Horoszewicz (1981), Van Damme and Billiau (1981), and O'Malley *et al.* (1984).

Human natural IFN-γ was developed for clinical use in several laboratories around 1980 (cf. Papermaster and Baron, 1981–1982;

Johnson *et al.*, 1981; DeLey *et al.*, 1981, 1982). Other groups have initiated such production (Braude, 1983b; K. Cantell and M. L. Kauppinen, personal communication). In some of these studies, diterpene esters have been used as inducers of IFN-γ (see Yip *et al.*, 1981). Purification of human natural IFN-γ has been described by Braude (1983a).

Le *et al.* (1982) found a cloned human cutaneous lymphoma cell line with a helper T cell phenotype which can be induced to produce approximately equal amounts of IFN-α and IFN-γ. Unfortunately, this preparation cannot be given to patients because of the use of a phorbol ester for the induction.

An important contribution to the area of production and purification of IFNs was the development of a monoclonal antibody to human leukocyte IFN-α (Secher and Burke, 1980). Originally described by Köhler and Milstein (1975), the establishment and screening of hybrids producing monoclonal antibodies have been developed to near perfection (Morser *et al.*, 1981; Staehelin *et al.*, 1981a,b). For a review of recent techniques for the production of monoclonal antibodies, see St. Groth and Scheidegger (1980) and Berd *et al.* (1982). Using these improved techniques, mouse hybrids secreting monoclonal antibodies to human IFN-β (Hochkeppel *et al.*, 1982) and IFN-γ (Hochkeppel and De Ley, 1982) were soon developed.

Lymphocytes also produce other substances with lymphotoxin activity (Granger *et al.*, 1978) which may play a role in the IFN system. Biotechnical laboratories are currently involved in the study of these and other lymphokines for their possible clinical application (see Fiers *et al.*, 1983). IFN can be produced on a large scale by bacteria (cf. Pestka, 1983a; Kingsman and Kingsman, 1983). It must be remembered, however, that it has not been determined whether the products obtained from the various recombinant systems are equal in potency to the natural products.

Several different recombinant IFN hybrids have been produced for clinical trials (see Stebbing, 1983a). Perhaps the most important aspect of these hybrids, however, is that they will extend our understanding of the structural importance of the various parts of the IFN molecules and will be helpful for the design of more effective compounds for clinical use. New IFNs can be formed by recombining the DNAs that code for the different IFN subtypes. The clinical significance of these substances is unknown, although they have been shown to be biologically active in some tissue culture systems (see De la Maza *et al.*, 1982).

There are three recombinant IFN-α preparations currently in clinical use: α2, which has an arginine residue substituted at position 23 and a deletion at position 44; αA, which has a lysine at position 23 and a deletion at position 44; and αD, which differs from αA at 29 sites.

IFN-β and IFN-γ present special problems because of the presence of glycosylation. For example, although glycosylation is not a prerequisite for the various biological activities exerted by IFN-γ *in vitro* (see Doyle *et al.*, 1982), it will be necessary to compare glycosylated and nonglycosylated IFN-γ preparations in clinical studies.

The common recombinant IFN-β has a cysteine residue at position 17. A variant, γ-Ser, modified by the substitution of a serine residue at this position, has increased stability (see Khosrovi, 1984). It has, in addition, been shown to have antiviral, antiproliferative, and natural killer (NK) cell activation properties similar to the parent molecule.

IFN-γ has also been produced using recombinant technology. For a review of the molecular cloning of human IFN-γ cDNA and its expression in eukaryotic cells, see Devos *et al.* (1982). There are no known differences among recombinant IFN-γ preparations (see Borden *et al.*, 1984d). Vilček's group recently demonstrated, however, that natural IFN-γ can be separated from the recombinant IFN-γ produced in *E. coli* by monoclonal antibodies. This may be due to a conformational difference at least *near* the active regions of these molecules (Le *et al.*, 1984). If this is the case, the current method of recombinant IFN-γ production will need to be reassessed and perhaps other host cells considered. In this regard, it is worth noting that human IFN-γ has been expressed in cultured monkey cells (Gray *et al.*, 1982).

In view of the multitude of methods of production and purification, the quantitation of IFN preparations used in clinical trials is extremely important. Hence, standardized biological assays have been developed (Myers, 1984). International units (IU), defined by these assays, are used to express the concentrations of different IFN preparations. Monoclonal antibodies have also proved useful in the rapid quantitation of IFNs (see Staehelin *et al.*, 1981c). A discussion of points to consider in the production and testing of IFN for human use may be found in Liu *et al.* (1984). The suggestions put forward on the basis of this discussion should be followed up.

IV. Induction and Production Control

Different types of IFNs can be produced both as single products and as mixtures in varying proportions. The production is dependent

on the cells used as well as the inducer. For a list of the various IFN inducers, see Torrence and De Clercq (1981). Interferon induction by viruses is an extremely complex process (see Marcus, 1982), the regulation of which is not yet well understood at present. Control systems are known to exist, however, at three levels: (1) at the level where the IFN genes are accessible for transcription, (2) at the transcriptional and posttranscriptional levels, and (3) at the translational level (see Burke, 1982, 1983). For a review of the posttranscriptional and translational control of gene expression in eukaryotes in general, see Revel and Groner (1978).

Over 20 years have passed since Wheelock first identified IFN-γ (1965). Since that time, production of IFN-α, -β, and -γ has been demonstrated in various cell types. Human bone marrow stromal cells can produce high levels of IFN-β (Shah *et al.*, 1983), although low levels of IFN-α are probably produced as well. T cell lines also preferentially produce IFN-β (Matsuyama *et al.*, 1982). Cyclosporine A inhibits the synthesis of IFN-γ (Reem *et al.*, 1982). Using a reverse hemolytic plaque assay, Palacios *et al.* (1983) showed that human IFN-γ is produced by OKT3$^+$, 4$^+$, 8$^-$, HLA-DR T lymphocytes. When human peripheral monocytes were exposed to killed bacteria, a subtype of IFN-α was initially induced. After 2–3 days, an IFN resembling IFN-γ was detected and, finally, an atypical IFN-α, sensitive to pH 2 treatment, appeared (Rönnblom *et al.*, 1983b). Some bacteria stimulated the T lymphocytes to produce IFN-γ-like molecules. The IFN-α was produced by nonadherent, predominantly Fc receptor-bearing, non-T, non-B cells. It would, on the basis of these results, be interesting to try to mimic some of the production sequences observed *in vitro* for the *in vivo* treatment of infections or neoplasms in experimental animals. For a discussion of the cellular modulation of IFN induction by polyribonucleotides, see Borden (1981–1982).

V. Genetics

The genetics of the IFN system have been reviewed by many authors (Stewart, 1979a; Slate and Ruddle, 1979; Seghal, 1982a,b; Epstein and Epstein, 1981–1982, 1983). In the mouse, all of the IFN genes are located on chromosome 4 (Lovett *et al.*, 1984). It will be interesting to see how the various IFN genes map in other mammalian cells (see Slate and Ruddle, 1981). Some data are already available (see D'Eustachio and Ruddle, 1983).

In 1982, C. J. Epstein *et al.* (1982) concluded that the gene product of the human chromosome 21 locus IFRC (a specific cell surface receptor for IFN-α) was the real IFN-α receptor. Chromosome 21 also controls the antiviral response to IFN-γ (Epstein *et al.*, 1981) and contains the gene coding for the IFN-γ receptor (Weil *et al.*, 1983b).

CHAPTER 2

GENERAL ACTION

I. Action on Cells in General

The biochemical effects of IFN on cells have been studied extensively over the past years (cf. Lengyel, 1982; Williams, 1983). IFN action is a complex process involving a multiplicity of substances and molecular mechanisms (cf. Hovanessian, 1979; Lengyel, 1981).

Heron and Berg (1978) studied the effects of temperature on IFN action. They found three effects of natural human IFN-α to be temperature dependent; namely, the development of the antiviral state, augmentation of the generation of NK cells, and growth inhibition. Cell-mediated lympholysis and the mixed lymphocyte reaction peaked at 38–39°C. The anti-growth effects increased with rising temperature. These findings challenge the use of antipyretics during IFN therapy.

The biochemistry of the IFN-induced antiviral state was reviewed by Revel (1979) and more recently by McMahon and Kerr (1983). The state seems to be controlled by several components. Clinically, the most important of these is (2′-5′)A synthetase (cf. Williams and Kerr, 1980; Dougherty *et al.*, 1981–1982), because it can be used as a marker of IFN action on heterologous cells; for example, on human tumors xenografted onto nude mice (Cayley *et al.*, 1982). It is not known how important this system is in comparison to an induced protein kinase and other affected pathways in the cell. The kinase is also likely to play a role, however, since the same conditions that activate the (2′-5′)A system trigger the kinase. Munoz *et al.* (1983) suggested that under some circumstances degradation of cellular RNA upon virus infection does not take place in IFN-treated cells. The important point at the moment, in my opinion, is that all of these pathways, starting with an interaction between IFN and the cell membrane and leading to the antiviral state, have begun to unravel.

IFNs often exert their most intense effects on homologous cells (see Gillespie and Carter, 1981–1982). Types of homologous cells, however, may respond differently to various IFNs. Several proteins are induced in IFN-exposed cells (see, for example, Sundström and Lundgren, 1983), and it will be interesting to follow the cloning of cDNA segments complementary to the corresponding mRNAs (see Lengyel *et al.*, 1982). Extremely small differences in polypeptide pat-