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Interferon Treatment of Human Neoplasia

Hans Strander

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Volume 46

Interferon Treatment of Human Neoplasia

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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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HANS STRANDER

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

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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). Vilćek'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-

terns were detected when the proteins induced by pure recombinant IFN-α and partially purified natural human IFN-γ were compared (Weil *et al.*, 1983a, 1983–1984). Furthermore, it has been shown in both normal and malignant cell lines and in freshly isolated human tumor cells that recombinant IFN-γ induces the same peptides as recombinant IFN-α, as well as several additional ones that vary among cell types (Epstein *et al.*, 1983). The implications for this in terms of IFN therapy is unknown.

The antiviral assay of IFNs has been well standardized (see Finter, 1981). Stebbing and May (1982) compared various natural and recombinant IFN-α, -β, and -γ in such an assay employing vesicular stomatitis virus (VSV). They could not detect any significant differences in pairwise comparisons using the various IFNs. The time schedule for optimal action of IFN-α and IFN-β *in vivo* might differ considerably from what would be optimal for IFN-γ (Dianzani *et al.*, 1978). It has been found by De Somer's group that purified IFN-γ is able both to inhibit the growth of lymphoblastoid cells and to potentiate NK cell activity of fresh donor lymphocytes, although in neither case was it more active than IFN-α or IFN-β of similar antiviral potency (De Ley *et al.*, 1980).

As previously mentioned, hybrids between different leukocyte IFN-α subtypes have already been produced, and some of them have been tested in the laboratory for various properties (see, for example, Pestka *et al.*, 1982a). It is not known which, if any, of these different hybrids will have clinical relevance. In addition, it is possible that many of them will prove to be antigenic when tested *in vivo*.

IFN sensitivity and inducibility are firmly connected with the differentiation process (Burke *et al.*, 1978). It has been suggested that IFNs may inhibit the differential gene expression involved in eukaryotic cell differentiation (Grossberg *et al.*, 1981). It has, in fact, been clearly established that IFNs can exert selective effects on the expression of some genes involved in differentiation (Lotem and Sachs, 1978). Work with IFN-resistant clones of Friend leukemia cells seems to indicate that the antiviral and differentiation effects of IFN act through different mechanisms (Affabris *et al.*, 1982). For an interesting general discussion of the differentiation problem and phenotypic reversal of myeloid leukemic cells, see Sachs (1978).

Tomida *et al.* (1980) found that IFN could enhance the differentiation of mouse myeloid leukemic cells. IFN did not itself induce the differentiation, but it did augment induction by several other substances. IFN could, however, induce lysozyme activity in these cells and behaved in a synergistic manner with other inducers in this re-

spect. It is interesting that the induction of differentiation in this system can also be achieved with hormone preparations. Tomida *et al.* further demonstrated in 1982 that IFN-α and IFN-β could enhance the induction of differentiation of promyelocytic leukemia cells in humans. The potential of such systems in tumor therapy is, as yet, unexplored (see Tomida *et al.*, 1983a), but will soon be investigated. The role of IFN in normal myelopoiesis has not been firmly established. It will be interesting to see how IFN affects the differentiation process in these cells that maintain an equilibrium between proliferation and differentiation (Dayton *et al.*, 1983).

In *in vitro* systems, where cells can be induced with various substances to produce hemoglobin, it can be shown that various human IFNs can increase production at low doses, whereas high doses are deleterious to hemoglobinization (Cioè *et al.*, 1983). This might be an important observation affecting the construction of optimal clinical schedules.

Verma *et al.* (1981) found that human leukocyte IFN-α can block granulocytic differentiation. In suspension cultures, an accumulation of granulocyte–macrophage progenitor cells, cluster-forming cells, and morphologically identifiable myeloid precursors was seen with IFN-α treatment. Human placental conditioned medium, used as a source of colony-stimulating factor, could effectively counteract this effect. Therefore, the authors suggested that natural human leukocyte IFN-α might play a regulatory role in the control of normal granulopoietic proliferation and differentiation. Trinchieri's group made the important discovery that IFN-γ, but not IFN-α or IFN-β, induces monocytic differentiation in myeloid cells (Perussia *et al.*, 1983a). Immature myeloid cells from normal bone marrow or from the blood of patients with CML can be made to differentiate into monocyte-like cells by IFN-γ. Even myeloid cells as mature as metamyelocytes can be induced to undergo monocytic differentiation. This could be an important function of human IFN-γ and has direct bearing on the treatment of various human tumors with IFN-γ preparations. Model systems have been developed *in vitro* to study IFN-α and IFN-γ together with inducers of differentiation in order to work out a strategy for IFN therapy directed at leukemic cell differentiation (Hamburger *et al.*, 1983).

It has been shown that human amniotic fluid contains IFN activity (cf. Chany *et al.*, 1983; Tan and Inoue, 1982). The role played by these IFNs, however, remains unclear. They could play a role during embryonic development, by protecting the cells from virus infections or contributing to the immune tolerance of the mother. It is of interest

that most pregnancies are also associated with elevated (2′-5′)A synthetase levels. This suggests that IFNs work actively in an immunoregulatory sense against viral invasion and the dissemination of disease (Williams *et al.*, 1982).

Hattori *et al.* (1983) found that a human histiocytic lymphoma-derived cell line could be made to differentiate by exposure to IFN-β or recombinant or natural IFN-α. In contrast, a promyelocytic leukemia-derived cell line that would differentiate toward cells of the monocyte lineage in response to certain inducers did not differentiate when cultured with IFN. Robert *et al.* (1984) studied the influence of semipurified natural human leukocyte IFN-α on differentiation of chronic lymphocytic leukemia cells *in vitro*. Both proliferation and differentiation were induced in leukemic cells in two of six tested patients. In two other patients, only differentiation was induced. Sonnenfeld *et al.* (1983) found an interesting correlation between carcinogenic potential and the ability to inhibit IFN-α or IFN-β production.

The diversity of IFN actions has been emphasized every time clinical application is discussed (cf., for example, Gresser, 1977b; Taylor-Papadimitriou and Balkwill, 1982). It has even been postulated that the IFN response may play a role in the aging process (Bocci, 1980a). It is known that IFNs cause a large increase in the amount of HLA mRNA in exposed cells (Fellous *et al.*, 1982). IFNs can also affect phosphorylation of fibrinogen and other plasma proteins by affecting platelet kinase activities (Hovanessian *et al.*, 1983).

We know that IFNs can enhance several particular cell functions. Exposure of cultured neurons to human natural IFN-α, for example, causes enhanced excitability of the neurons (Calvet and Gresser, 1979). Tunicamycin, an inhibitor of glycosylation, can potentiate the inhibitory effects of IFNs both on virus multiplication and on cell growth (Maheshwari *et al.*, 1983b). Renton and Mannering (1976a,b) made the discovery that IFN-inducing agents could cause a depression of the hepatic cytochrome *P*-450-linked monooxygenase system in rodents. They predicted that viral infections and treatment with agents that induce IFN would impair the metabolism of drugs in humans (see Mannering *et al.*, 1980). This is an important concept to consider with regard to combination treatments, as the metabolism of a variety of drugs might be changed when given simultaneously with IFN. Reiners *et al.* (1984) have since shown that the levels of depression of promutagen activation correlate with cytochrome *P*-450 content and the induction of IFN-γ. This suggests that some IFNs, for example, IFN-γ, may play an active role in the hepatic promutagen/procarcinogen activation.

It has been emphasized that the effects of IFNs not only have to deal with what might happen in the host, but also with the changing behavior of the tumor cell (Siegal *et al.*, 1982). In the system employed by these investigators, it was found that different IFNs caused increased type IV collagenase levels surrounding tumor cells leading to increased invasiveness of Ewing's sarcoma with IFN exposure.

It has been suggested that the anti-tumor effects of IFN may be related to their ability to modulate differentiation in tumor cells. Rivière and Hovanessian (1983) made the interesting observation that tumor cells in organisms may themselves not only produce IFN but may also respond to their own IFN. The practical implications of this finding remain to be determined.

Sister chromatid exchanges do not seem to be affected by human leukocyte IFN-α in peripheral blood lymphocytes from normal donors (Viiayalaxmi, 1982). It has been suggested that IFN has antimutagenic properties (Zasukhina, 1982) but that a fragile site on chromosome 16 can be induced by IFN or ethanol. This gap is considered to be a normal chromosome variant, however (Hecht *et al.*, 1981). Actually, in animal systems, it has already been shown that IFN treatment can prevent stable integration and expression of transfected plasmids containing cloned genes from hamster ovarian cells. In contrast, IFN does not prevent the transient expression of one of these genes in its unintegrated form (Dubois *et al.*, 1983b).

Chany-Fournier (1983) has reviewed the evidence for loss of malignancy in transformed cells exposed to IFNs and, in particular, the continuous treatment of Moloney sarcoma virus (MSV)-transformed cells with IFN. These cells recover normal phenotype and contact inhibition and lose the ability to form colonies in agar. This experimental model consisting of the polymerization of cytoskeleton and new production of collagen and fibronectin emphasizes the role played by this type of transformation in the anti-tumor spectrum of IFN. Pfeffer and Tamm (1982) found that volume increase was a sensitive indicator of IFN effects on cellular phenotype. The phenotype reversion of transformed cells that can be induced by IFNs has, in fact, been associated with changes in the cell cytoskeleton (Brouty-Boyé *et al.*, 1981). Clones of x-ray-transformed cells passaged in the continuous presence of IFNs progressively acquire characteristics of a nontransformed phenotype. This reversion induced *in vitro* by IFN preparations has been observed in clones of transformed cells containing C-type virus particles as well as in virus-free clones (Brouty-Boyé and Gresser, 1982).

IFN also causes a dose-dependent inhibition of ornithine decarbox-

ylase activity stimulation. It has been suggested that the anti-tumor activity of IFN can perhaps to some extent be attributed to this inhibition (Streevalsan *et al.*, 1979). In order to destroy cultured tumor cells, different IFNs sometimes have to be employed in addition to appropriate effector or mononuclear cells (see Baron *et al.*, 1983). Direct cytolysis which can be achieved with preparations of IFN-γ may also be an important mechanism of IFN anti-tumor action (Tyring *et al.*, 1983). The anti-growth effects exerted by IFNs are described in Chapter 3.

II. Action on the Cell Surface

Important alterations of the cell surface are induced by IFNs (see Friedman, 1981a). Following exposure to IFN, cell surface receptors for concanavalin A (Con A) are found to be redistributed (Pfeffer *et al.*, 1980a). Whether IFNs must penetrate the cell membrane to induce these changes is unknown (Friedman, 1978b, 1979). For a discussion of the interaction between membrane gangliosides and IFN, see Vengris *et al.* (1980).

During IFN treatment *in vitro*, marked structural changes can be detected in the plasma membrane, thus affecting motility, proliferation, and plasma membrane rigidity. The role that these changes play in the *in vivo* response of patients treated with IFN remains unknown (Tamm *et al.*, 1982). IFN effects on the cell membrane must also be considered in discussions of early virus–cell interactions (see Kohn, 1979). The mechanisms underlying IFN-induced resistance and the species specificity barrier seem to be located primarily at the cell surface.

It has been proposed that IFN-α molecules have either two binding sites or two regions constituting a single binding site, one in the —COOH and the other in the —NH_2 half of the molecule (Streuli *et al.*, 1981). For a review of the molecular characterization of IFN receptors, see Zoon and Arnheiter (1984). By 1981, it was evident that IFN-γ receptors are different from the receptors of the other IFNs (Branca and Baglioni, 1981; Aguet *et al.*, 1982). Consequently, the designations "Type I" for the IFN-α and IFN-β receptors and "Type II" for the IFN-γ receptors were proposed (Orchansky *et al.*, 1984). In addition, different affinities for the subtypes of IFN-α and IFN-β may exist (Gardner and Vilček, 1979).

Human lymphocyte cultures are known to simultaneously produce several types of substances with antiviral activity (see for example, Van Damme *et al.*, 1983). In view of the existence of multiple IFN

receptors, a combined treatment schedule seems logical (Aguet *et al.*, 1983).

Grollman *et al.* (1978) noted several similarities between the receptors for IFN and those for glycoprotein hormones. Both receptors consist of a glycoprotein as well as a ganglioside component. In addition, receptor–substrate interactions in both systems lead to changes in membrane structure, adenosine 3′,5′-monophosphate levels, and transmembrane flux of ions. How these similarities may be exploited in the IFN treatment of tumor patients remains to be determined.

Maxwell *et al.* (1984) have studied the binding of recombinant DNA-derived leukocyte IFN-α to peripheral blood cells of patients with CML. After five doses of IFN-α, a decrease in binding from 600 to 75 molecules per cell was observed. This was found to be the result of a loss of receptors. No correlation could be shown between clinical hematologic response and the extent of receptor down-regulation.

III. Tumor Viruses and Oncogenes

In 1981, Georg Klein predicted that chromosomal alterations involving rearrangements of cellular oncogenes might result in altered expression of these genes. Ryan *et al.* (1983) have shown that the family of human transforming genes maps to different human chromosomes. Cairns (1981) also suggested that genetic transpositions might cause human cancer, and a model for genetic transposition in carcinogenesis has already been published in this series (Klein and Lenoir, 1982). It has, in fact, been shown that high levels of a gene product coded by a normal human oncogene can induce tumorigenic transformation (E. H. Chang *et al.*, 1982). The role played by viral oncogenes in tumorigenesis is a fascinating subject (see Marshall and Rigby, 1984). Viruses have been implied in T cell malignancies in adults (see Gallo, 1984), and analogues of retrovirus transforming genes are frequently expressed in human malignant cells (Eva *et al.*, 1982). Insight into oncogene function will open the way to new forms of cancer therapy (Wylie and Weiss, 1984), thus cancer therapists will have to be familier with the concept of protooncogene, oncogenes, and the alteration of the genomes of cells (see Weinburg, 1983).

The role of IFNs in oncogenesis and, for example, transduction with cellular oncogenes (Swanström *et al.*, 1983), is largely unknown. Clearly, more research on the effects of IFN on DNA arrangements is required, as gene dosage effects and the increased expression of nor-

mal cellular genes seem to be important steps in carcinogenesis in at least some instances (Klein, 1981). In theory, phenotypic reversal from a transformed state to a nontransformed state could be achieved by a biological response modifier, such as IFN (Samid *et al.*, 1984). It has been shown that IFNs have an inhibitory effect on the transformation process and that this effect does not seem to be limited to viral transformation (Dubois *et al.*, 1983a). If this is true, IFNs might help in preserving the integrity of different cellular genes.

Epstein–Barr virus (EBV) has been discussed in connection with both oncogenes and certain malignancies (see Ernberg and Kallin, 1984). The success in determination of the DNA sequence of the EBV has been extremely important (Anonymous, 1984) in providing a "key for the unlocking of mechanisms of gene control." Different IFNs are able to reduce the frequency of cells positive for EBV-specific nuclear antigen induced by transformation but are unable to prolong the EBV transformation interval of non-T mononuclear leukocytes infected by EBV (Chang, 1984). EBV is thought to play a role in the development of nasopharyngeal carcinoma. Since IFN can be produced by EBV-infected cells, IFN studies on nasopharyngeal carcinoma patients should be undertaken to provide models for future work (Klein *et al.*, 1974).

In an interesting experimental system, NIH 3T3 cells were transfected with the human EJ bladder oncogene and with cloned Ha-MuSV DNA. Treatment with mouse cell IFN caused a dramatic reduction in transformation (Samid *et al.*, 1983). These investigators also examined the effect of IFN on RS 485, an established line of NIH 3T3 cells transformed by the human c-Ha-*ras* 1 gene activated by a Ha-MuSV long terminal repeat (LTR). After 30 generations in the presence of IFN, a reduced growth rate was observed, and after an additional 10 cell generations, flat revertant colonies were seen. The cells in these colonies had lost their malignant character. When IFN was removed, a transformed morphology reappeared after approximately 20 cell generations. These observations suggest a correlation between normal phenotype and IFN treatment and one possible mechanism of IFN action against malignant tumors.

We do not know, at present, if viruses other than the ones directly implied in the cause of some human cancers may play a helper role in carcinogenesis (see, for example, Desgranges *et al.*, 1983). If this is the case, treatments affecting the IFN system in a positive manner might be even more anticarcinogenic. See also Chapter 8 on the human papillomaviruses.

IV. Biological Response Modifiers

IFNs are felt by most investigators to belong to the family of "biological response modifiers," and some consider them more specifically to be lymphokines. For discussions of the concept of lymphokines, see Dumonde *et al.* (1969) and Bendtzen (1978). Still others regard IFNs as hormones. The hormonal concept of IFN has been amply discussed by Inglot (1983). She suggests that IFNs and growth factors are to be regarded as two families of nonclassical hormones with opposite actions. IFNs can, in fact, regulate the growth of many cells, including melanoma cells in culture (Creasey *et al.*, 1983). The clinical importance of target cell receptor down-regulation by circulating peptide hormones has been emphasized (see King and Cuatrecasas, 1981). It will be interesting to see if the IFN system follows the same principles. Relationships between IFNs and neuroendocrine hormones have already been suggested (Blaylock and Smith, 1981).

Combination therapy with IFNs and hormones would, therefore, seem a logical choice in many instances. It should be mentioned that human natural IFN-α has been shown to increase estrogen receptor activity in human breast cancer tissue, human uterus endometrium, and rabbit uterus (Dimitrov *et al.*, 1981, 1984). A response was seen at concentrations of 10–1000 IU/ml. Higher doses did not give rise to a further increase in activity. Cytosol fractions with low binding activity did not respond. Highly purified lymphoblastoid IFN-α or recombinant IFN-α produced the same effect. The mechanism behind this augmentation of receptor activity is unclear. It will be interesting to continue this work in patients.

The potentiation of IFN activity by mixing various IFN preparations is clearly a system that deserves extended studies for both theoretical and practical reasons (see Fleishmann *et al.*, 1979). The interplay between IFNs and cellular growth factors should also be interesting to follow (Holley *et al.*, 1977). In 1969, Chany *et al.* reported the presence of IFN antagonists in extracts of various human sarcomas. They have since demonstrated the enhancement of various biological effects of IFN by other substances (Chany *et al.*, 1980). Fleishmann *et al.* (1984a) have isolated an IFN inhibitor in their IFN-γ preparations. The importance of the sarcolectins—IFN antagonists that can be extracted, for example, from hamster sarcomas and normal muscle—is at present unknown, but they can affect the antiviral state preestablished by IFN and hence could be important for the antitumor effect, especially if the latter is caused by direct effects on the tumor cells (Jiang *et al*, 1983). Clearly, it would be interesting to see

whether many of the more common human tumors contain sarcolectins. Such studies are currently in progress.

Rhodes (1983) studied the effects of retinoids, retinoic acid, and β-carotene on human IFN-α and IFN-β. These substances inhibited IFN stimulation of monocyte membrane function. Interestingly, β-carotene inhibited the cytostatic action of IFN on lymphoblastoid cells, and this inhibition was reversed by retinoic acid. This suggests a regulatory mechanism whereby β-carotene could potentiate the stimulatory effects and inhibit the suppressive effect of IFN on host effector cells. To summarize, it is known that IFN activates cells of the immune system but is antiproliferative, while the net effect of β-carotene in the systems so far investigated is to potentiate both activation and proliferation. This may be of importance with respect to the anticancer role of dietary pro-vitamin A.

V. IFNs and Prostaglandins

The interactions between the IFN and prostaglandin systems are an intriguing subject, especially since elevated prostaglandin production seems to be a marker of high metastatic potential in the neoplastic cells of breast cancer (Rolland *et al.*, 1980). Hydrocortisone and dexamethasone, inhibitors of prostaglandin E synthesis, decreased the induction of both prostaglandin and IFN in IFN-pretreated cells, while various other hormones were devoid of this activity (Zor *et al.*, 1982). Other prostaglandin synthetase inhibitors, including indomethacin and aspirin, did not alter IFN production, although prostaglandin synthesis was abolished. These investigators concluded that while the induction of IFN and prostaglandin E may be the consequence of the same initial cellular event, prostaglandin E does not have a regulatory effect on IFN synthesis.

Fuse *et al.* (1982) studied the effects of human natural IFN-β on the synthesis of prostaglandins in IFN-sensitive and IFN-resistant cells. They found that IFN stimulated prostaglandin synthesis and that this enhanced synthesis could be inhibited by prednisolone or indomethacin. These results suggested that IFN stimulates prostaglandin synthesis by promoting the release of arachidonic acid from phospholipids. It is of interest that prednisolone and indomethacin partially inhibited the anti-cell growth activity of IFN. This should be taken into consideration in clinical trials with IFN.

CHAPTER 3

ANTI-GROWTH EFFECTS

I. The Anti-Growth Concept

After the discovery by Paucker *et al.* (1962) of the anti-growth properties of IFN preparations, it was debated whether or not this activity was due to IFN itself. By 1976, it was, however, quite clear that these effects were probably due to the presence of the IFN molecules in the preparations (Stewart *et al.*, 1976). This conclusion has been further substantiated in many laboratories (see, for example, Evinger *et al.*, 1980b). All of the known IFNs can affect the growth and function of both normal and malignant cells. The kinds of changes that can occur have been described in a review by Taylor-Papadimitriou (1983). She divides the cell functions that are inhibited by IFN into growth functions, inducible activities of proteins, and systems of cellular differentiation. She also lists the various cell functions that are enhanced by IFN and the changes in cell membranes reported to be induced by IFN. These lists are extensive, and the difficulty ahead of us is to sort out the observed changes and construct a comprehensive picture of the effects of IFN on patients.

The growth of normal cells can be inhibited by IFNs. For example, human natural IFN-β leads to a decrease in the proliferation rate of human fibroblasts (Pfeffer *et al.*, 1979). In the treated cells, one can see changes in the fibronectin pattern and a decrease in cell locomotion (Pfeffer *et al.*, 1980b). Human IFNs have also been shown to inhibit motility in other cultured cells (Broaty-Boyé and Zetter, 1980). The sensitivity of lymphocyte-derived tumor cells to the anti-growth effects of IFNs in experimental systems is affected by the stage of differentiation of the cells (Paraf *et al.*, 1983). It has been suggested that IFN participates in the process of cell growth arrest during cell differentiation. In the Friend leukemia cell system, addition of "physiological" concentrations of IFN to differentiating cells results in a pronounced inhibitory effect on cell growth, an increased number of cells in the resting phase of the cell cycle, and a decrease in the preferential rate of the cellular phosphoprotein P-53 (Kimchi *et al.*, 1983).

Various tumor cells are known to react differently to IFN treatment. In embryonal carcinoma cells, IFNs are able to induce a partial antivi-

ral state in which the induced antiviral proteins can interfere with the replication of only some viruses (Nilsen *et al.*, 1980). It is interesting that malignant embryonal carcinoma cells can neither produce nor respond to IFNs, whereas differentiated cells obtained from embryonal carcinoma cell lines behave "normally" in both respects. Here, the differentiation steps lead to different effects of IFN on the enzyme systems of the treated cells (see Wood and Hovanessian, 1979). Thus, the differentiation process clearly affects IFN action in tumor cells.

An important study presented in 1974 showed that concentrated human IFN-α injected intramuscularly (i.m.) into mice, guinea pigs, rabbits, sheep, and humans gave rise to long-lasting plateaus of circulating IFN in the blood (Cantell *et al.*, 1974). It was described in the same publication that human sarcoma cells could be inhibited by natural human IFN-α preparations at blood concentrations achieved *in vivo*. This had direct clinical application. Since that time, it has been found that different IFNs behave differently in terms of pharmacokinetics (see Chapter 6, Section III). It is also of considerable clinical interest that tumor cells in experimental animals resistant to IFN-α and IFN-β can be sensitive to the anti-growth effects of various preparations of IFN-γ (Besançon *et al.*, 1983).

A problem in direct anti-tumor cell therapy is that there are differences in drug response among cells of a parental tumor, between the parental tumor and its metastatic subpopulations, and among various spontaneous metastases (Tsurno and Fidler, 1981). Kirkwood and Marsh have developed a tumor cell drug sensitivity assay for melanoma cells employing agar diffusion chambers *in vivo* in mice (Marsh and Kirkwood, 1980; Kirkwood and Marsh, 1983). Agar colony techniques have been used primarily for evaluating IFN anti-growth effects, however (see Chapter 3, Section III).

II. Anti-Growth Effects in Tissue Culture

Different cell lines react differently to IFNs. When the antiproliferative effects of natural IFN-α and IFN-β on 25 different human cell lines or strains were compared, IFN-β was more effective in inhibiting growth of all but one, the Burkitt's lymphoma cell line Daudi (Borden *et al.*, 1982a). The effect of IFN-α was usually established by 72 hours after IFN exposure, and no further growth inhibition could be seen at 120 hours. Conversely, IFN-β had a greater antiproliferative effect at 120 than at 72 hours. The authors were careful in interpreting their results. Nevertheless, they clearly demonstrated that different IFNs have different biological and cell regulatory effects. Ito

and Buffert (1981) reported that human urinary bladder carcinoma cells could be destroyed by exposure to semipurified human IFN-β preparations. Adenocarcinoma and osteosarcoma cells also reacted, although the response was weaker. An interesting finding made by these authors was that diploid fibroblasts were completely resistant to this cytotoxic effect. Cook *et al.* (1983) found that natural human IFN-β had pronounced anti-growth effects on various human brain tumor cells but not on a nontransformed cell line. The effects were noted after only 2–6 days. Similar results could be achieved with freshly explanted tumor cells from human brain.

The response of various lymphoblastoid cell lines to human natural IFN-α ranges from extreme sensitivity to resistance (Adams *et al.*, 1975a). Various cell lines were tested for IFN sensitivity employing natural human semipurified IFN-α (see Einhorn and Strander, 1978a). Comparisons were also made to IFN-β. It was clear in these studies that there were great variations in IFN sensitivity among different tumor cell populations. Of nine osteosarcoma cell lines tested *in vitro*, all were found to be inhibited in their growth by human natural IFN-α in tissue culture (Strander and Einhorn, 1977). What was especially interesting in the tissue culture work was the fact that cells could be affected at concentrations that can be obtained in the serum of IFN-treated patients (see also Chapter 3, Section I).

Rubin and Gupta found that IFN-γ might have cytocidal effects on certain tumor cells. They suggested that these types of IFNs or, less likely, factors present in natural IFN-γ preparations, may be potent anti-tumor agents (Rubin and Gupta, 1980). An extremely IFN-sensitive cell line from a malignant pleural effusion of a patient with metastatic renal cell carcinoma was developed for use *in vitro* for pharmacologic studies with human IFN (Chang *et al.*, 1983). Such sensitive cell lines should prove valuable for a variety of purposes in the future.

Nagai *et al.* (1982) presented anti-growth effects of IFN preparations used on medulloblastoma and glioblastoma cells in tissue culture. Both of these types of tumor cells seemed to react to IFN treatment. Screening of human glioma cells for IFN sensitivity can probably now be undertaken, since these cells grow well in culture (Benediktsson *et al.*, 1983).

Intriguing results, with unknown clinical relevance, were obtained when IFN sensitivity was studied in Burkitt's lymphoma patients (Ernberg *et al.*, 1981). Short-term incubations of fresh biopsies from Burkitt's lymphoma patients were tested for natural IFN-α sensitivity. Different biopsies from the same patient did not differ in IFN sensitivity, while biopsies from different patients were alternatively resis-

tant or sensitive. Thus, some Burkitt's lymphoma cells are probably already resistant to IFN *in vivo*. Similar results were obtained with lymphoblastoid cell lines (Adams *et al.*, 1975a). The patients were all treated with cyclophosphamide, and an inverse relationship between patient survival on this treatment and IFN sensitivity of the tumor cells was observed. The reason for this can only be speculated. One possibility would be that the immune system is important in these patients, and, thus, target cell resistance to the IFN molecules would be advantageous.

Horoszewicz *et al.* (1979) showed that resting tumor cells were more sensitive targets for the antiproliferative activity of human IFN-β than rapidly multiplying cells. This could mean that in some tumor systems IFN should be used on stem cells to suppress the reemergence of tumors in patients heavily treated with chemotherapy.

Borden *et al.* (1983b) found that increasing temperature could augment the antiproliferative effects of IFN on transitional cell carcinoma cell lines, and, remarkably, those who were not otherwise sensitive to IFN could be made sensitive by the temperature change, which was also associated with increased levels of (2'-5')A synthetase activity. An osteosarcoma cell lines in rats has been found to be much more inhibited by IFN at an increased temperature. In that particular system, 20 IU of IFN appeared to be cytotoxic at 39°C while 2000 IU/ml had to be used at 35°C to achieve a cytostatic effect (Delbrück *et al.*, 1980). Again, this emphasizes the problem of using antipyretic substances in connection with IFN therapy.

Following exposure to increasing concentrations of lymphoblastoid IFN, the extremely IFN-sensitive Daudi cells (Adams *et al.*, 1975a) developed a cell population that multiplied in the presence of 10^4 IU/ml of the IFN (Dron and Tovey, 1983). Clones exhibiting both moderate and pronounced resistance were isolated from such populations. Prolonged cultivation in the absence of IFN led to a reversion to intermediate IFN sensitivity by the clones with pronounced resistance. These clones possess specific high-affinity IFN receptors similar to those of the parental cells (Tovey *et al.*, 1983).

The different anti-growth effects of IFNs depend on several factors. Five human bladder carcinoma cell lines were tested for antiproliferative effects of human IFN *in vitro* (Borden *et al.*, 1984d). It was found that the antiproliferative effect of the various IFNs employed could be seen on continuous exposures and that IFN-β was more inhibitory than IFN-α. Cloned IFN-α was as effective as naturally produced IFN-α. It was proposed that the antimitotic effects observed might underlie the activity of IFNs in bladder carcinoma. In 1983, Yamada

and Shimoyama reported results on the treatment of 17 human cultured cell lines with natural human IFN-β and lymphoblastoid IFN. Daudi lymphoma cells were the most sensitive and three B cell lines, one T cell line, and one non-T, non-B cell line were moderately sensitive to both IFNs. Eleven other cultured cell lines were not sensitive. Cell lines that were sensitive to one IFN were always sensitive to the other, although there were different sensitivity levels registered. Both IFNs had a time-dependent cytocidal action but not a concentration-dependent one. It was concluded from these studies that IFN exerted cytocidal actions similar to antimetabolites and vinca alkaloids. When natural IFN-α and IFN-β were compared on osteosarcoma and lymphoma cells in tissue culture, it was found that the IFN-β was more effective on the osteosarcoma cells and the IFN-α on the lymphoma cells. Whether this finding can be extended to all kinds of tumors belonging to these classes is not known at present (Einhorn and Strander, 1977).

Groveman *et al.* (1983) tested recombinant IFN-α and IFN-β on transitional cell carcinoma cell lines. Proliferation of three out of four cell lines were significantly inhibited by these IFNs. A pure IFN-β produced on recombinant DNA gave comparable results to the naturally produced IFN-β. Naturally produced impure IFN-α, containing a mixture of various IFN subtypes, was more effective in this respect than the two recombinant IFN-α preparations tested *in vitro*. Kataoka *et al.* (1982) compared natural IFN-β, IFN-α, and lymphoblastoid IFN in their ability to suppress tumor growth. The IFN-β was found to be least active on Daudi cell proliferation, while three other hematological cell lines were insensitive to all IFNs. The IFN-β was most active on eight tested epitheloid cell lines, however. The conclusion made by the authors was that in the treatment of patients with malignancies it is important to use the correct IFN for the particular tumor in question. Five human IFN subtypes were compared on cell lines from various species and could be shown to differ in their relative activities on these various cell lines (Weck *et al.*, 1981). Again, we can conclude that different tumor cells respond differently to IFNs from various sources (see also Mayer-Eichberger *et al.*, 1981). There are techniques available for measuring antiproliferative and antiviral activities of different types of IFNs (Eife *et al.*, 1981).

Morimoto *et al.* (1983) compared various activities of recombinant human IFN-β produced in *E. coli* and natural fibroblast IFN-β. In all of the various biological systems—immune systems and systems measuring anti-growth effects in tissue culture—these two preparations seemed to exert very similar actions. In preliminary experiments com-

paring IFN-β and IFN-γ on different tumor cell lines, there appeared to be no great differences in anti-growth effects (Aota *et al.*, 1983).

Tomita *et al.* (1982) studied the effects of IFN-α, -β, and -γ on various lymphoblastoid cell lines and K-562 cells and found that in the Daudi cells, sensitive to IFN-α and IFN-β, up to 1000 IU/ml of natural IFN-γ showed no anti-growth effect. Satu *et al.* (1983a) studied various antiviral and antiproliferative activities of recombinant IFN-γ and compared it to natural human IFN-γ, natural human IFN-α induced in BALL-1 cells, and natural IFN-β in various *in vitro* studies. In antiviral assays, the recombinant IFN-γ required a longer treatment period than the human IFN-α and IFN-β to induce a level of substantial resistance. The recombinant IFN-γ was more specific and had greater cell growth inhibitory activity against epithelial cells than the human IFN-α and IFN-β. There were no effects on lymphoblastoid cell lines. In epithelial cells, there was some indication that recombinant human IFN-γ might have a cytocidal effect. Leukemic mouse L-1210 S cells were sensitive both to IFN-β and IFN-γ, but IFN-β and IFN-γ differed in their mechanism of interaction with the target cells (Hovanessian *et al.*, 1980). Rubin *et al.* (1983b) found that Hela cells and U-amnion cells were more effectively inhibited in their growth by IFN-γ than by IFN-α. Lymphoid cell lines, and especially the Daudi cells, were, however, relatively insensitive to the anticellular effects exhibited by human IFN-γ. Some proteins that are synthesized in response to IFN-α in Daudi cells were not induced after their exposure to IFN-γ.

Sikora *et al.* (1980) used cell fusion techniques to produce stable hybrids from neoplastic lymphocytes and worked with such a set of stable mouse–human hybrids. The neoplastic lymphocytes were from patients with nodular lymphoma and chronic lymphocytic leukemia who had shown a clinical response to human natural leukocyte IFN-α. The IFN preparation inhibited the growth rate of 14 of 17 such established hybrid cell lines, thus showing that the leukocyte IFN in this system had an inhibitory effect on neoplastic B lymphocytes. It is important to show whether such correlations can be obtained in various systems in order to develop a variety of suitable models for IFN therapy of malignant disease.

Czarniecki and Fennie (1982) and Czarniecki *et al.* (1984) studied the antiviral and antiproliferative effects of highly purified, bacterially derived human IFN on human melanoma cells. Treatment of cells with IFN-γ in combination with IFN-αA or IFN-β usually resulted in potentiation of both antiproliferative and antiviral activities, although antagonism was observed with cells from some patients. As found

by other investigators, the cells under study (human melanoma cells) had receptors for IFN-α and IFN-β that were different from those for IFN-γ.

The anti-growth effects obtained by combining IFN-β and IFN-α on tumor cells are confusing (see for example, Mayer-Eichberger *et al.*, 1981b). Relatively low concentrations of naturally produced IFN-γ have been shown to have antiproliferative effects on many types of malignant tumors *in vitro* (Vastola *et al.*, 1983).

Fleischmann *et al.* (1982) used two paired sets of nonmalignant/malignant cells in the mouse system for anti-cell growth experiments and treated the cultures with either IFN-γ or IFN-α/β. Treatment of the malignant cells with IFN-γ or the IFN-α/β mixture separately had a small effect on cell growth. On the other hand, when these IFN preparations were combined, there was a marked anticellular effect that resulted in killing of malignant cells. The conclusion drawn from these studies was that the anticellular activity of the combined IFN treatment was more effective on the malignant cells. This is, of course, important in combination studies if the pertinent effects of IFN at the clinical level take place directly on the tumor cells. In a series of experiments, Oleszak and Stewart (1982) found that maximum potentiation of IFN effects on tissue cultured cells occurred when different IFNs were mixed in similar concentrations. The clinical relevance of this finding remains to be determined.

Normal and transformed fibroblasts were killed more easily by actinomycin D if the cells were treated with human IFN (Inoue and Tan, 1983). A similar enhancement by adding IFN was also obtained with *cis*-platinum. Cyclophosphamide has also been considered as a substance which might be used in combination with IFN therapy, since it can cause the development of delayed-type hypersensitivity reactions in otherwise unreactive patients. Such reversal of T cell energy could lead to augmentation of the immune response in advanced cancer patients (Beard *et al.*, 1982).

The antiproliferative effect of IFN on the very sensitive Daudi lymphoma cell line has been the subject of a recent thesis (Leandersson, 1982). The Burkitt's lymphoma cells were found to react to IFN by accumulating in a cell cycle phase with G_0 characteristics. The cells were then arrested after mitosis. The rate of escape was dose dependent. The author suggested that the mediator of the antiproliferative effect in this system may be different from the one responsible for effects in other systems. Van der Bosch and Zirvi (1982) studied primary cultures of human colon tumors and exposed them to crude human leukocyte IFN-α as well as 4′-deoxydoxorubicin, an intercalca-

tor of DNA. The IFN caused a growth state-specific effect in the sense that stationary populations were killed, while fast-growing cultures were irreversibly growth inhibited by the same doses of IFN. The chemotherapeutic agents instead killed growing populations, whereas stationary cultures were barely affected by the same drug concentration. The interesting finding was that the IFN preparation antagonized the cytotoxic effect of 4′-deoxydoxorubicin when applied directly after chemotherapeutic exposure. Therefore, although the mechanisms are different, a combination therapy using IFN treatment and chemotherapy may not always be beneficial.

Namba *et al.* (1982) combined 5-fluorouracil and human IFN-β on various neoplastic cell lines and normal human fibroblasts. A combination of 5-fluorouracil and IFN was synergistic on some cell lines but neither synergistic nor growth inhibitory in an additive fashion on other cell lines. Of 5 high-grade astrocytoma tumor cell populations treated with natural human leukocyte IFN-α, moderate sensitivity was seen in one tumor tested *in vitro* (Bradley *et al.*, 1983). The combination of 1,3-bis-2-chloroethyl-nitrosourea (BCNU) and IFN did not seem to be advantageous. These authors concluded that this type of IFN was probably not useful in the treatment of malignant brain tumors if it acts directly on the tumor cells. A true synergistic, anti-growth activity was observed after 72 hours of exposure of Daudi cells to both α-difluoro-methyl-ornithine (α-DFMO), an enzyme-activated irreversible inhibitor of ornithine decarboxylase, and human natural leukocyte IFN-α. Such synergism could be observed regardless of the IFN/α-DFMO ratios (M. Rosenblum and J. Gutterman, personal communication). This should provide an interesting combination for clinical trials. Potentiation of the effects of these two drugs was also observed using an animal system. Total or near-total suppression of tumor growth was seen in malignant melanoma cells growing in mice (Sunkara *et al.*, 1983).

Gould *et al.* studied the effects of IFN-α and IFN-β on the response of human carcinoma cells to ionizing irradiation (Kakria *et al.*, 1981; Gould *et al.*, 1984). Several IFN preparations were used in these studies. They found that IFN-β sensitized bronchogenic carcinoma cells to irradiation toxicity in cases in which the IFN-α did not. Different IFN-β preparations gave similar results. Mouse IFN-α/β did not affect the radiosensitivity of the human cells. It was concluded that the human IFN-β preparations potentiated irradiation effects *in vitro*. Nederman and Benediktsson (1982) found that IFNs could not affect the sensitivity of glioma cells to irradiation and that an additive effect of these two treatments could be observed *in vitro*.

III. Colony Techniques

Techniques available for assaying multilineage hematopoietic progenitors that form mixed hematopoietic colonies have demonstrated that IFNs reduce the growth of such precursors in a dose-related fashion (Neumann and Fauser, 1982). In *in vitro* studies using bone marrow, IFN-α seems to show greater inhibition of myeloid colony formation than IFN-β. It should be noted, however, that only very high doses of the IFNs resulted in marked inhibition of colony-forming units (Van't Hull *et al.*, 1978). Verma *et al.* (1979) reported in studies on the effects of semipurified human leukocyte IFN preparations on myelopoiesis *in vitro* that the continued presence of the IFN preparation caused a decline in colony formation and a rise in cluster incidence with increasing IFN concentrations. Morphological examination of the clusters showed a progressively increasing percentage of a major granulocytic precursor. This suggested to the authors that human leukocyte IFN causes leukopenia by blocking differentiation of marrow myeloid precursors. They postulated that as the myeloid progenitor cell proliferates and differentiates, successive generations become more sensitive to the effects of human leukocyte IFN. An alternative explanation, however, is that IFN affects dividing cells, leading to an increasing percentage of immature, nondividing cells.

The *in vitro* testing of myeloma cells for sensitivity to IFNs and the possible use of such results for selecting patients for treatments look promising, especially if combined treatments with IFNs and cytotoxic drugs are to be advocated (Durie *et al.*, 1982). The human tumor cloning system is now being compared to other systems as an alternative first-line screen for components that have anti-tumor effects on a specific patient (Von Hoff *et al.*, 1984). The cloning of human solid tumors in soft agar is being used in many laboratories.

Some tumors show an especially high rate of colony formation suitable for *in vitro* IFN tests. These include colon carcinoma, melanoma, lung carcinoma, breast cancer, ovarian carcinoma, and sarcoma (Kern *et al.*, 1982). The human tumor cloning system for selecting chemotherapeutic agents was used in a prospective clinical trial by Van Hoff *et al.* (1983). Six hundred four single-agent trials were performed in 407 patients whose tumors were submitted for testing. There was a cloning efficiency of 41%. Of these, there was a 60% true-positive and an 80% true-negative rate for prediction of a response of a tumor to a single agent.

Ludwig *et al.* (1983) made the important observation that highly purified recombinant leukocyte IFN-αA and -αD and semipurified

natural IFN-α could stimulate clonogenic tumor grafts *in vitro*. They tested 225 human tumor samples and found that 30 of these (13%) showed growth stimulation. These observations were most frequent with cells from patients with acute myeloid leukemia (27%), renal cell carcinoma (20%), and breast carcinoma (19%), but less frequent in melanoma (6%). The data were confirmed by tritiated thymidine experiments in 21 patients with multiple myeloma, in which 90% of the samples showed a significant increase in isotope incorporation. This was also true in cell lines not containing any stromal cells. Therefore, the authors concluded that tumor growth can actually be stimulated *in vitro* by IFN exposure in some cases. Implications of this for the clinical use of IFN on tumor patients are obvious. Strayer *et al.* (1984) used natural leukocyte IFN-α for evaluation of antiproliferative activity on a panel of eight histological types of freshly prepared human tumor cells. Single-cell suspensions were treated. Colony formation was obtained in 40 cases. The most sensitive tumor cell type, of the ones tested, was renal cell carcinoma (69% response). Other tumor types showing sensitivity were carcinoids (50%), breast carcinoma (50%), melanoma (50%), and ovarian carcinoma (25%). It was possible to see a clinical correlation between *in vitro* sensitivity and *in vivo* response to leukocyte IFN-α in seven patients with renal cell carcinoma.

In an unpublished study by P. Ling *et al.* (personal communication), the sensitivity of primary human ovarian cancer cells to IFNs was studied *in vitro* using the tumor cloning system in semisolid agar. In 79% of the experiments with cells from ascitic fluid, the cells were sensitive to 100 IFN IU/ml. Sensitivity was found only in one test out of three with solid tumors. Different sensitivities seemed to occur with different IFNs, in line with results obtained by other investigators and on other tumors. Bradley and Ruscetti (1981) tested the effect of human IFN-β and IFN-α on colony formation in short-term soft-agar culture systems. All kinds of effects were seen from complete inhibition of growth to stimulation of growth. Eighteen of 40 evaluable tumor specimens showed at least a 70% inhibition. There were, however, four specimens showing at least a 3-fold stimulation of colony formation. It will be interesting to see how the effects in this system correlate with *in vivo* findings. It may be necessary to take possible tumor growth stimulation into account clinically. Welander *et al.* (1983) used the human stem cell assay to study effects of recombinant IFN-α2 on the growth of various cell lines from ovarian carcinomas. The IFN was also tested in combination with different chemotherapeutic agents. These authors found a possible synergistic effect

with doxorubicin and recombinant IFN-α2 on these cell lines, but combinations of IFN with other drugs showed only additive effects.

Fleischmann (1982) used IFN-γ and a mixed preparation of IFN-α/β separately and in combination in cloning studies with B16 melanoma cells. The IFN-γ seemed to be the most potent anticellular IFN in this system. Potentiation was achieved by mixing the IFN-γ and the other IFNs. The extent of the potentiation was dependent on the IFN concentrations of all IFN types, and there was a continued increase as the IFN concentrations were raised. These studies suggest that potentiation consists of a mutual, synergistic interaction between IFN-γ and the virus-induced IFN-α/β.

Aapro *et al.* (1983) combined human recombinant leukocyte IFN-αA with various chemotherapeutic drugs and tested the effects of combinations of these substances on various human tumor cell lines with a modified soft-agar clonogenic assay. Three human tumor cell lines (one each of myeloma, breast carcinoma, and colon carcinoma) showed sensitivity at clinically significant drug concentrations (in the serum). Synergistic activity against *in vitro* colony formation was found with vinblastine and the recombinant IFN-αA on a myeloma cell line (B226). Evidence of potentiation between the IFN preparation and *cis*-platinum was also noted. It remains to be seen how generalized such findings are, but in this series of investigations only subadditive, additive, or potentiation effects were seen, so the combinations strengthened the anti-growth effect in all instances.

IV. Theory behind the Anti-Growth Concept

It has been demonstrated that the growth-inhibitory and antiviral activities of purified natural leukocyte IFN preparations migrate together in chromatographic procedures (Evinger *et al.*, 1980a). Thus, it was concluded that the growth inhibitory activity was an intrinsic property of the human leukocyte IFN molecules. It should be noted, however, that Ware and Granger (1979) found that lymphotoxins and IFNs have overlapping biological activities, especially in their ability to inhibit cell proliferation. It has been debated whether there are substances other than IFNs exerting the anti-growth effects observed with natural IFN-γ preparations (see Yip *et al.*, 1982), although it now seems that the growth-inhibitory effects caused by such preparations are due to the IFN-γ molecules themselves (Rubin *et al.*, 1983a). The comparative effectiveness of IFN-γ in various anti-tumor functions, such as growth inhibition, remains to be established (Yip *et al.*, 1982).

Using various hybrid IFN DNA recombinants, Rehberg *et al.* (1982) showed that the antiviral and antiproliferative activities of various IFN molecules *in vitro* were promulgated through different mechanisms. Kimchi *et al.* (1981) determined that (2′-5′)A synthetase is involved in the antimitogenic effect of IFNs. The anti-growth effects of five cloned human leukocyte IFN-α subtypes, and molecular hybrids from some of them, were tested on six human cell lines (Fish *et al.*, 1983). These IFNs could be divided into two distinct groups on the basis of antiproliferative activity. Some assignment of groups on the basis of antiviral activity was also possible. In these studies, the relative antiviral efficacy of the cloned subtypes was inversely related to their antiproliferative activities. It has been suggested that purified human IFN-γ is a more potent anticellular substance than the other IFNs (Blaylock *et al.*, 1980). Chapekar and Glazer (1984) have presented data suggesting that IFN-γ-dependent toxicity against a human colon carcinoma cell line was accompanied by protein phosphorylation, which in turn is stimulated by exogeneous polyamines.

The fact that tumor cells exposed to IFN usually show a progressive increase in their intermitotic times has been known for some time (see d'Hooghe *et al.*, 1977). In studies by Balkwill *et al.* (1978), it was found that there was a lengthening of all phases of the cell cycle when various types of cells were exposed to human lymphoblastoid IFN *in vitro*. IFNs have been shown to inhibit the proliferation of cell lines growing on glass by hampering the transition from G_0 to the growth state (Sokawa *et al.*, 1977; Creasey *et al.*, 1980). Using different cells, Balkwill and Taylor-Papadimitrieou (1978) found that quiescent cells treated with IFNs in G_1/G_0 were delayed in their subsequent passage through the cell cycle. Serum was used to stimulate the cells in these experiments. There was an extension of both the G_1 and S + G_2 periods in the cell lines and the human cell strain tested. Daudi cells exposed to human lymphoblastoid IFN showed impaired cell growth, although the rate of DNA synthesis was not greatly inhibited even 2 days after the initiation of IFN treatment (Gewert *et al.*, 1983). When melanoma cells were exposed to IFNs, they seemed to undergo a decreased transitional rate out of G_0 to G_1 into the S phase and a prolongation of the S phase (Creasey *et al.*, 1980). In other studies, IFNs seemed to exert their effects in the early G_1 phase of the cell cycle, at which point the cells are not yet irreversibly committed to DNA synthesis (Lundgren *et al.*, 1979). Clearly, a multitude of anti-growth effects have been reported with IFNs.

V. Nude Mouse Experiments

In the nude mouse–human xenograft system, it is presumed that human IFNs can exert direct effects on human tumor cells, while mouse IFNs can only modulate the host systems. For example, Balkwill *et al.* presented data on their nude system in 1982, showing that human IFN therapy stimulated the (2′-5′)A synthetase levels in the tumor but not in the mouse spleen cells. In addition, human IFN-α did not influence the NK cell activity of the nude mouse (Balkwill *et al.*, 1982a). Kohno *et al.* (1982) studied the anti-tumor effects of human natural IFN-β using this system. They studied tumors transplanted subcutaneously (s.c.) from patients with ovarian carcinoma, laryngeal carcinoma, nasopharyngeal carcinoma, hepatoma, lung carcinoma, and melanoma. They injected the IFN-β at a dose of 10^5 IU per mouse. The injections were made s.c. around the tumor or intratumorally (i.t.). They saw a stronger anti-tumor effect with the i.t. injections. Several of the tumors tested were sensitive to IFN. It is interesting that both tested melanomas were sensitive to the IFN injections. The authors concluded that a dose of 10^5 IU per mouse is sufficient for the evaluation of anti-tumor effects and that larger doses are unnecessary. Actually, measurements of human tumors in the nude mice can be very reliable. Interobserver and intraobserver variations have been determined (Euhus *et al.*, 1984). Systems are also being developed to study human tumor metastases implanted in the subcutaneous tail tissue of nude mice (Murthy *et al.*, 1984), and a subrenal capsule assay has already been used for human ovarian cancer (Grönroos *et al.*, 1984).

Shimoyama *et al.* (1982) did model experiments in athymic nude mice and suggested that i.t. injections should be effective at the injection site in patients. However, many tumor cells have a low sensitivity to human IFN-β (see also Carter and Horoszewicz, 1980). The blood IFN level obtained even after intravenous (i.v.) injections in patients should be insufficient based on these studies, where the IFN was injected i.v. at a dose of $3–6 \times 10^6$ IU. It was assumed that the main effect of IFN therapy on tumor cells is a direct one. The cases that they treated with i.t. injections were a cutaneous T cell lymphoma and a rhabdomyosarcoma. If these studies were relevant to the clinical situation, then the nude mouse model would be excellent for studying the anti-tumor effects of IFNs. Clutterbuck *et al.* (1983) used IFN-α2 to treat human tumors inoculated into irradiated mice. Even high doses of the IFN failed to affect the growth of human carcinomas, melanomas, and myeloid leukemia xenografts.

The nude mouse model for studying the effects of human lymphoblastoid IFN on breast cancer xenografts has been extremely important and led to the development of fundamental model systems (Balkwill *et al.*, 1980, 1982b, 1983a; Taylor-Papadimitriou *et al.*, 1982). In these studies, it was found that human lymphoblastoid IFN prevented the establishment of transplanted breast cancer xenografts if given at the time of tumor implantation. Also, established breast cancer xenografts could be inhibited in their growth in a dose-dependent fashion. It will, of course, be interesting to correlate the effects seen on different types of tumors in the animals with clinical effects. Furthermore, the interesting observation has been made that not only human IFN can inhibit the growth of the human tumors in the mice, but that similar effects can also be obtained by using mouse IFN which cannot act directly on the human tumor cells. These findings stress the lack of understanding of the anti-tumor effect of IFN. Actually, not much has been gained with regard to the understanding of anti-tumor mechanisms involved over the last 5 years (see Gresser and Tovey, 1978).

The nude mouse system can be employed also to show the importance of IFN production. It was found by Reid *et al.* (1981) that both IFN production and NK cell activity in spleen cells were reduced in nude mice treated with anti-IFN globulin. Both parental and virus-infected (persistently infected with RNA viruses) tumor cells grew and formed in nude mice treated with the anti-IFN globulin, larger tumors than those growing in control nude mice. Primary human prostatic carcinomas have been difficult to grow in nude mice, but it was possible when the mice were treated with either anti-IFN globulin or antilymphocyte serum, giving rise to data consistent with the view that IFN might be an important part of the host's anti-tumor system. Perhaps this could occur through components of the immune system. It would be interesting if this system could be used more intensively as a model for IFN therapy of tumor patients. Shouval *et al.* (1983) showed that a human hepatoma cell line grew in a large number of nude mice if the latter were treated with sheep anti-mouse IFN globulin. Controls injected with the same number of tumor cells but not receiving anti-IFN treatment failed to develop tumors. These authors also found an inverse correlation between sensitivity of the hepatoma cells to NK cell activity *in vitro* and resistance to tumor growth *in vivo*. Other experimental evidence suggested that the IFN/NK cell system probably plays a role in limiting the tumorigenicity and invasiveness of the hepatitis B virus-infected human hepatocellular carcinoma that was used in some of these studies, and this mechanism is

probably similar to that which has been seen by employing other cells persistently infected with viruses. These authors also presented evidence that the tumorigenicity was limited by the host's immune response, since immunosuppression reduced the latency time for tumor development and also led to increased mean tumor weight.

IFN-β has been used in some experiments with the nude mouse–xenograft system. For example, Tanaka *et al.* (1983) showed that human natural IFN-β caused dose-dependent growth inhibition of human gliomas in the nude mouse. Ida *et al.* (1982) used human natural IFN-β for *in vitro* and *in vivo* experiments with human melanoma cells. In the *in vitro* experiments, the cells tested were highly sensitive to IFN-β when compared to other human cells. Accordingly, in the nude mice, tumor growth was suppressed by daily administration of the IFN-β. It is interesting that the i.t. injections gave the best results and that much less therapeutic potential was achieved with s.c. injections around the inoculated tumor. Intraperitoneal (i.p.) injections were completely unsuccessful. Interestingly enough, the three administration routes gave similar blood plasma levels and decline curves. This could indicate that the plasma IFN titer has no relative importance when anti-tumor effects are studied in the IFN-injected patient. Hashizume *et al.* (1983) transplanted human malignant melanoma cells into nude mice and found no difference in sensitivity to human IFN-β with respect to melanin productivity of the lines. The anti-tumor activity depended on the route of administration, and the i.t. route was most effective. Actually, i.t. and s.c. administration of high IFN doses sometimes led to complete disappearance of tumor cells.

Masuda *et al.* (1983) treated human osteosarcomas in athymic mice with human natural leukocyte IFN-α. There was an anti-tumor effect which was dose dependent. Daily administration led to a very significant anti-tumor effect. Recent experiments have revealed that human osteosarcomas transplanted into nude mice grow in a high percentage and can be treated and inhibited in the mice by IFNs (Bauer, Broström, Nilsson, Reinhold, Strander, and Nilsonne, Tribukait, unpublished observation). In the future, it will be interesting to compare the effects and clinical efficacy of various IFNs on these tumors in the mouse. Preliminary experiments have revealed that some tumors are sensitive and some are more resistant to human IFN in this particular system (Bauer *et al.*, 1984). Complete growth inhibition has so far been achieved in 50%, and it is interesting that the inhibition is most extensive in tumors with the lowest percentage of cells in S phase.

Combination studies with IFNs in the nude mouse model are war-

ranted. It will be interesting to see, for example, if monoclonal antibody therapy (Oldham *et al.*, 1984) should be combined with IFN treatment, especially since monoclonal antibodies can specifically inhibit growth of human tumors in these mice (Herlyn and Koprowski, 1982). Kitahara *et al.* (1981) have injected natural human IFN-β and human lymphoblastoid IFN into nude mice bearing transplanted human tumors. The conclusion drawn from these studies is that both IFN types can affect the tumors in the animals and that the effects are dependent on dose and concentration of the IFNs. The combination of human lymphoblastoid IFN and doxorubicin was synergistic against human acute lymphocytic leukemia cells transplanted into nude mice. Balkwill and Moodie (1984) reported that human lymphoblastoid IFN can potentiate the anti-tumor activity of suboptimal doses of cyclophosphamide and doxorubicin tested on human breast cancer xenografted in nude mice. The anti-tumor activity was greatest when simultaneous rather than sequential treatment with IFN and chemotherapy was given. Preliminary data also indicated that equal amounts of mouse IFN had no significant effects on the combination of cyclophosphamide or doxorubicin together with human lymphoblastoid IFN. This was measured either by looking at the anti-tumor effects or by looking at the toxicity of the therapy. This study might have a direct bearing on the clinical application of human IFN.

CHAPTER 4

EFFECTS ON THE IMMUNE SYSTEM

I. General Effects

IFNs exert extensive effects on the immune system (De Maeyer, 1981; De Maeyer and De Maeyer-Guignard, 1982; Vyakarnam, 1983) and especially on NK cells (see Wigzell, 1981). We know that IFN-α, IFN-β, and IFN-γ can suppress or enhance the antibody response in mice and humans. It is interesting in this regard that interleukin 2 seems to constitute a requirement for IFN-γ production (see Johnson, 1981–1982). IFNs can also affect cell-mediated immunity (see De Maeyer and De Maeyer-Guignard, 1981–1982) and several other immunological parameters (see Lucero *et al.*, 1981–1982), including other lymphokines (see Neta and Salvin, 1981–1982). It is important that one be aware of the difficulty in selecting *in vitro* systems that faithfully represent the immunological effects of IFN *in vivo* (see Bloom, 1980a).

IFN effects on NK cell activity have been the subject of numerous studies (see Masucci *et al.*, 1982). The specificity of the reaction must, however, be described in greater detail. It has already been shown in one system that allogeneic but not autologous tumor biopsy cells are sensitive to IFN-induced enhancement of cytotoxicity (Vanky *et al.*, 1980). Argov and Klein (1983) showed that short-term pretreatment of mixed lymphocyte culture (MLC) derived effectors with IFN enhanced the nonspecific component of the cytotoxic reactions, while the specific component was increased only by the continuous presence of IFN. Therefore, it has been proposed that IFN induces modifications favoring the proliferation of specific clones.

Fischer *et al.* (1983) reported that human peripheral blood monocytes showed augmented ability to lyse a variety of tumor cells in the presence of IFN and that this killing efficiency seemed to be due to an increase in the rate of killing and the recycling ability of the cytotoxic cells. The same authors also showed that excess tumor cells could impair the lytic machinery of freshly isolated monocytes, whereas monocytes treated with IFNs were less sensitive to this inhibitory effect. The role played by this system *in vivo* is presently unknown.

Patarroyo *et al.* (1983) assayed the lytic ability of human blood lymphocytes against autologous and allogeneic EBV-transformed B cell

lines. The effects, if present, were weak. When targets were superinfected with EBV, the strongest lytic reactions were obtained with IFN-treated lymphocytes.

In 1980, Masucci *et al.* (1980a) showed that IFN-α1 can enhance the NK cell activity of human lymphocyte populations. Additional effects included augmentation of antibody-dependent cellular cytotoxicity (ADCC), suppression of antigen- and mitogen-induced leukocyte migration inhibition, and growth inhibition of IFN-sensitive Burkitt's lymphoma cells. Hence, these authors concluded that IFN-α1 has comparable effects on immune functions to previously tested semipurified natural IFN-α preparations. Therefore, by these criteria, this particular recombinant IFN could substitute for the complex α-mixture. On the other hand, it has been clearly shown that IFN preparations can vary tremendously in their capacity to induce augmentation of immune functions, including cytolysis by monocytes and NK cells (see Ortaldo *et al.*, 1983b). Furthermore, the correct use of lymphocytotoxicity assays to monitor drug therapy *in vivo* is not an easy task (see E. Klein, 1981).

It has been established that natural human leukocyte IFN-α therapy induces changes in several immunological functions (cf. Einhorn, 1980; Einhorn *et al.*, 1981b, 1983b). Human leukocyte IFN-α in doses of $5–10 \times 10^6$ IU was given i.m. to patients with nodular, lymphocytic, poorly differentiated non-Hodgkin's lymphoma for 30 days (Rasmussen and Merigan, 1980). The number of B lymphocytes in the peripheral blood was reduced during the treatment, while there was no effect on the number of T lymphocytes. B lymphocyte responses in the patients were restored after termination of therapy. During a second course of IFN treatment in two of the patients, the restored responses were inhibited. Various cytotoxic functions of peripheral blood lymphocytes from 101 patients undergoing daily treatment with human natural IFN-α were examined by S. Einhorn *et al.* (1978b, 1980a, 1983a). All patients, except five who received 6×10^6 IU/day, were injected with 3×10^6 UI. ADCC and lectin-dependent cellular cytotoxicity (LDCC) were not altered within a week of continuous IFN therapy. After 3 and 6 months, a decrease in these functions was observed in the peripheral blood of almost all patients. On the other hand, it was found that NK cell activity measured against Chang cells increased following the first injection of IFNs and remained elevated during 1 year of IFN therapy. In myeloma patients, however, there were no correlations between NK cell activation and clinical parameters (S. Einhorn *et al.*, 1982b).

Other effects of natural human IFN-α on the immune system of

patients receiving i.m. injections have also been examined (S. Einhorn *et al.*, 1983a). The phagocytic activity of monocytes/macrophages increased following treatment with IFN-α *in vitro* (Einhorn and Jarstrand, 1982). There was, however, no change in the capacity of monocytes to ingest yeast particles immediately after initiating daily *in vivo* treatment with 3×10^6 IU of IFN-α. Instead, after 1 week to 6 months of treatment, monocyte phagocytosis had decreased in the majority of patients. The phagocytic activity of neutrophilic granulocytes was also investigated and found not to be affected by the treatment, and in most patients, the oxidative metabolism in the granulocytes increased 24 hours after the first injection of IFN (Einhorn and Jarstrand, 1984).

In 1981, Oettgen and Krown (published in 1982) summarized the results of clinical trials with human natural IFN-α performed at the Memorial Sloan-Kettering Cancer Center. Doses of up to 15×10^6 IU i.m. were used, and IFN was detected in the serum of all patients. Significant increases in NK cell activity against K-562 target cells were noted in almost all tested patients. The release of interleukin 1 by peripheral blood monocytes in response to endotoxin was found to be decreased during IFN therapy, which suggested that monocytes are stimulated by IFN to release interleukin 1 *in vivo*.

Kita *et al.* (1983) found that spontaneous tumor cell growth-inhibiting activity of human peripheral blood lymphocytes was increased following exposure to human natural IFN-α. Both NK and spontaneous tumor cell growth-inhibiting activity were increased after systemic administration of 3×10^6 IU of IFN, although differences in kinetics were noted. These activities are currently being studied, as tumor growth inhibition may be important for the anti-tumor activity of IFNs.

Ernstoff *et al.* (1983a) studied the effects of semipurified natural human leukocyte IFN-α, given i.m. to renal cell carcinoma patients, on NK cell activity, T cell subsets, and endocrine parameters. They found that NK cell activity rose during the first 8 days in patients who initially had low levels of target cell lysis. NK cell activity rose repeatedly during intermittent IFN schedules. A decreasing trend was noted in the ratio of peripheral helper/suppressor phenotypes. Of the various endocrine parameters studied, an increase in serum cortisol level was found to follow IFN-induced adrenocorticotrophic hormone (ACTH) stimulation. Tachyphylaxis of NK cell activity seen in these trials was attributed to localization in extravascular compartments, such as within the tumor. No catechol-like hormonal effect of the IFN injections was found. Serum IFN levels, which were rather low in

these trials, could not be correlated to NK cell activity. The clinical implications of these findings are unknown, but their future elucidation will be interesting. Bash *et al.* (1983) concluded, after injecting 10 patients with 10–60 × 10^6 IU of semipurified human natural leukocyte IFN-α in a single injection i.m., that the most common response is a decrease in NK cytotoxicity and ADCC at Day 1, followed by an increase on Day 3 with a return to the baseline at around Day 7. This pattern seems to occur in patients receiving rather high doses of IFN.

IFN-β can also affect immune functions. Ezaki *et al.* (1982a), for example, injected natural human IFN-β into 26 patients with various malignancies. Almost all of the patients had previously received heavy chemotherapy. The IFN was injected at a dose of 3–6 × 10^6 IU i.v. daily. Twenty-four patients were evaluable at the time of the report. Of these, two had partial remissions (8%) and seven had stable disease during the study period. Side effects were identical to those seen with other IFN preparations. NK cell activity increased in the treated patients. In some cases, it remained high, and in others, the level attenuated with time. Lymphoblastogenic responses to nonspecific mitogens and the premixed lymphocyte–tumor cell reaction remained unchanged during the course of treatment. Maluish *et al.* (1983c) studied immune parameters in 40 patients given human lymphoblastoid IFN by 6 hours of i.v. infusion or i.m. injections (doses of 0.1–50 × 10^6 IU for up to 5 weeks). There was no sustained elevation of NK cell activity. Depression of lymphoproliferative responses to mitogens and mixed leukocyte culture responses was noted, and there was an elevated monocyte-mediated anti-tumor cytostatic activity in one third of the patients.

A group at Duke University Medical Center (Koren *et al.*, 1983) injected purified lymphoblastoid IFN into seven cancer patients. In the first half of the study a single dose (0.1–4.0 × $10^6/m^2$) was injected i.m. In the second half, after 2 weeks of rest, chronic administration began with 15 injections given over a 5-week period. Half a million IU/m^2 was given to the first series of patients, and a maximum of 15 × 10^6 IU/m^2 was given to the last patient included in the trials during the final week of the 5-week schedule. Selected patients were given a rest for 4 weeks or longer and were treated afterward on a steeper escalation schedule with three injections per week. Initially, a decline in NK cell activity and ADCC activity was noted after the injections, with a nadir at 12 hours. This decline was partly related to the presence of nonadherent suppressor cells. The NK cell activity then returned to baseline, exceeded this, and stayed elevated for up to 1 week after a single injection. During chronic treatment, the same de-

cline in NK cell activity was seen repeatedly. The maximum stimulation was achieved during the first week and was greater for patients given higher doses of IFN-α. This stimulation was, however, more sustained in patients injected at lower doses, and in this sense very low doses (0.5×10^6 IU/m^2) appeared to be most efficient. Similar effects were seen on the peripheral ADCC system. Also, as seen in our own studies, there was a prominent and consistent drop in the lymphocyte count 8–12 hours after IFN injection. The possibility was raised that a redistribution of NK cells might explain the depressed NK cell levels (see also Kariniemi *et al.*, 1980). The conclusion of Koren *et al.* was that *in vivo* administration of lymphoblastoid IFN results in dose-dependent augmentation of NK and ADCC activity in the peripheral blood of cancer patients.

Neefe *et al.* (1983b) looked at immunomodulatory responses in patients with metastatic malignant melanoma treated with lymphoblastoid IFN-α. One group of patients was treated on an intense schedule approaching the maximum tolerated dose. They received 15×10^6 IU/m^2 every other day for 2 weeks in a cycle separated by rest periods of at least 1 week. Another group of patients received only 5×10^6 IU/m^2 weekly. The second schedule was intended to provide an opportunity for augmented anti-tumor immunity. The modulation of NK cell activity as well as ADCC reactions, and monocyte-mediated tumor growth inhibition in the peripheral blood were studied. Data from the first nine patients showed increased activities in all of these assays 2–3 days after IFN administration, but no sustained increases were observed. On the weekly schedule augmentation was more frequent. No tumor responses were registered, and it was therefore impossible to do correlation studies between immunological and anti-tumor effects.

Silver *et al.* (1983b) reported data on their series of patients treated with human lymphoblastoid IFN-α either by low-dose treatment with i.m. injections or high-dose treatment with continuous i.v. infusion. The T4 and T8 cells and NK cell activity in injected patients were measured against K-562 cells. Forty-six patients were evaluable at the time of the report, and it was seen that high-dose patients showed no significant changes during IFN infusion. By the end of the study period, there were significant increases in their NK cell activity and T4/T8 ratio and a concurrent decrease in their T8. By contrast, the low-dose patients showed a trend of increased NK cell activity in the peripheral blood during IFN administration, with no change over the study period. They did show a trend of decreasing T4/T8 ratios and a significant increase in T8. A favorable response was associated with an overall increase in NK cell activity, a decrease in T8, and an in-

crease in the T4/T8 ratio. An inverse relationship between NK cell activity and the concentration of existing immune complexes was also suggested by these authors. The same group (Silver *et al.*, 1984) injected lymphoblastoid IFN randomly into patients according to one of two protocols: a low-dose treatment consisting of 2×10^6 IU/m^2 i.m. daily for 28 days and then daily on alternate weeks, or a high-dose treatment with 5×10^6 IU/m^2 daily as tolerated for 10 days, repeated every 28 days. So far, PHA stimulation data, mixed lymphocyte reaction data, and NK cell activity data have been assembled. There were no significant trends noted over tht total time of the study for either the high-dose or low-dose patients. There was a slight association between the mixed lymphocyte reaction data and the PHA response. This was not true in a comparison with NK cell activity.

Purified human recombinant IFNs can also affect the immune system. Hengst *et al.* (1982, 1983) noticed that, in cancer patients treated with pure recombinant IFN-α2, lower doses (down to 3×10^6 IU s.c. daily) were more effective in increasing the studied immunological parameters than higher doses. In these studies, cells were tested for NK cell activity, ADCC, monocyte-mediated ADCC, and spontaneous monocyte-mediated cytotoxicity. In general, the antibody-dependent cellular cytotoxicity was usually decreased at least a few days after beginning treatment. The spontaneous monocyte-mediated cytotoxicity was generally increased in injected patients. The NK cell activity was increased in patients given low doses but was unchanged or decreased in patients given high doses. Breast cancer patients given 2×10^6 IU of the IFN-α2 per m^2 in Phase II studies also showed some increase in NK cell activity in the peripheral blood. There was some correlation between the increase of the spontaneous monocyte-mediated cytotoxicity and the NK cell activity. The authors concluded that it could be important to determine the optimal immunostimulatory doses of IFN for use in individual patients. Twenty-nine patients with various malignancies were treated with recombinant IFN-α2, and changes in NK cell activity against K-562 cells and the T cell subsets were studied using the Leu series of monoclonal antibodies (Ernstoff *et al.*, 1983b). Seventeen cancer patients received i.m. injections of $3–100 \times 10^6$ IU/day for 28 consecutive days or until tolerance levels were reached. Twelve patients were studied during a Phase I trial using the i.v. route with the same recombinant IFN-α2. NK cell activity rose during the first week of i.m. therapy at both high (i.e., $>30 \times 10^6$ IU) and low ($<10 \times 10^6$ IU) daily doses. With i.v. administration of comparable doses, NK cell activity tended to decrease in patients receiving the high doses. Changes in the T cell subsets were observed

in both trials, and the Leu 3a/2a (helper phenotype/suppressor phenotype) ratio rose in patients receiving i.m. IFN-α2 at higher doses. The rise in the Leu $3a^+$ and the fall in Leu $2a^+$ T cells were responsible for this observation. In contrast, the ratio 3a/2a fell in patients who received high doses of the IFN-α2 by the i.v. route. This, then, reflected a decrease in $3a^+$ cells and an increase in $2a^+$ cells. No changes were observed in T cells when low i.m. doses were used. In summary, there were several changes in immune parameters, and completely opposite results were obtained, depending on the route of administration and the dose. If immunological monitoring is important for the treatment of cancer patients with recombinant IFN-α, these observations are of the utmost importance.

NK cell and monocyte activity in advanced cancer patients receiving recombinant IFN-αA has been the subject of extensive studies at the National Institutes of Health (Maluish *et al.*, 1982). The relationship seems to be extremely complex, as both increases and decreases in various activities could be obtained, depending on the method of IFN administration. A suppressor factor was also found in the sera which could interfere with *in vitro* boosting of the NK cell activity by IFN. One hundred thirty-four patients with a variety of malignancies were treated in Phase I clinical trials with recombinant leukocyte IFN-αA, twice daily or three times weekly for 28 days. The doses varied. No appreciable increase in NK cell activity was observed with any of the regimens (Maluish *et al.*, 1983b). Monocyte function, measured as growth inhibition of human target cells, was elevated in 70% of the patients, while lymphoproliferative responses were depressed in most patients, as determined by response to Con A and by mixed leukocyte cultures. There was an increase in $OKT10^+$ cells in most patients and a transient increase in cells that could react with MO 2 antibodies. It is interesting that the depression of NK cell activity was most marked at the highest doses in the patients who received more frequent administration of IFNs. Ten patients with advanced colon cancer (Duke's Stage D) were treated with recombinant leukocyte IFN-αA (Tank *et al.*, 1983). The patients received 2×10^7 IU of IFN either chronically (twice a week) or cyclically (in three periods of 8 consecutive days). In the chronically treated patients, the NK cell activity increased on the second day after initiation of IFN therapy and then leveled off. The NK cell activity could only be increased during the first day in the cyclically treated patients. The Con A-induced γ-IFN production capacity (GIPCA) and phytohemagglutinin (PHA) responses were impaired in the patients before treatment. It was interesting to see that when GIPCA was augmented, the response to PHA was decreased and vice versa. Anti-tumor effects were not

reported on these patients. Einhorn *et al.* (1984) studied various immune reactions in 18 patients with disseminated colorectal cancer treated with recombinant human IFN-α2. The patients received either s.c. injections of 20×10^6 IU/m^2 three times weekly or pulse treatments with 50×10^6 IU/m^2 daily. The IFN was then given i.v. over 30 minutes for 5 consecutive days every fourth week. NK cell activity increased during continuous treatment, and in the patients in whom repeated cycles were given, all of the cycles were associated with elevation of the NK cell activity as measured on Chang cells. During treatment, IFN did not cause any enhancement of NK cell activity when it was added *in vitro* to lymphocytes from the patients. This did occur prior to treatment, however. Total T cells, suppressor cells, helper cells, and a number of cells detected by monoclonal antibodies against NK cells were not affected. The phagocytic activity of granulocytes was also unchanged, whereas the ability to reduce nitroblue tetrasodium by these cells increased after the first injection of IFN. On the basis of these results, it seems that the effects of recombinant IFN-α on the parameters tested are similar to those seen after *in vivo* injection of natural human leukocyte IFN-α preparations.

IFN-γ can also have an effect on immune functions. Harada and Matsumoto (1983) studied the effects of recombinant human IFN-γ on the immunological activities of human peripheral blood lymphocytes. There was augmentation of NK cell activity and the ADCC reaction. The recombinant IFN-γ preparation was comparable to α2 preparations and IFN-β in its effects on the NK cell system and more potent in augmentation of ADCC. This group also performed preclinical studies of recombinant human IFN-γ effects on cynomolgus monkeys. Induction of (2′-5′)A synthetase was lower with IFN-γ than with the IFN-α or IFN-β, as were serum levels following i.m. injections. The immune effects of IFN-γ will be discussed in greater detail in Chapter 4, Sections II and IV.

Some investigators have several IFNs in their studies on immune functions. Ng *et al.* (1983) used homogeneous preparations of bacterially cloned recombinant human leukocyte IFN-α and recombinant human IFN-β to study the shedding of melanoma-associated antigens in a human melanoma cell line. If these cells were incubated for 5 days with various IFN preparations, there was an increase in the surface expression and shedding of a cytoplasmic melanoma-associated antigen accompanied by a decrease in the susceptibility to NK cell lysis. On the other hand, if human peripheral blood lymphocytes were pretreated with IFN, there was an enhancement of NK cell activity against the melanoma target cells and of the ADCC reaction, using the

same system of melanoma cells coated with anti-melanoma antigen-associated monoclonal antibodies.

Maluish *et al.* reported in 1983 (1983a) on 34 patients who had received recombinant leukocyte IFN-αA by i.m. injections either twice daily or three times weekly for 28 days in doses of $0.1–36 \times 10^6$ IU. Twenty patients also received highly purified lymphoblastoid IFN by i.m. or i.v. injections. Sixteen patients were given IFN-γ three times weekly for 28 days and 15 patients received the IFN-inducer poly(IC·LC) (1 or 4 mg/m^2). It was found that NK cytotoxicity was depressed in many patients, particularly by higher doses and recombinant leukocyte IFN-αA. Also, lymphoproliferative responses to mitogens and Con A were depressed. Monocyte function, on the other hand, was elevated in most patients after the recombinant IFN-αA was given. These were rather unexpected findings, but they have since been confirmed in several laboratories, especially with high doses of the recombinant IFNs.

In summary, all of the IFNs can affect the immune system, and it is therefore not surprising that these substances given to patients cause both enhancement and inhibition of various immune functions. What is not very well understood, however, is the role played by the IFNs in normally occurring immune responses (see Moore, 1983). When this has been elucidated, we can start more relevant clinical experiments in order to evaluate the role of the immune system in malignant diseases. Herberman and Thurman (1983) have extensively discussed the monitoring of immunological parameters in patients with malignancies who were receiving IFN treatment. They conclude that it is not possible to draw any conclusions about the optimal biological response-modifying dose or schedule for IFN administration. More insight is needed concerning the most relevant immunological effects of IFN, including the observed hyporesponsiveness to NK cell activity augmentation following repeated exposure to IFN. It will be especially important to determine the therapeutic limitations of IFN as a biological response modifier. I should add that the most potent immunomodulatory effects, on the basis of the data presented in this chapter, are observed with low-dose IFN therapy.

II. IFNs and NK Cells

IFNs can exert potent effects on the NK system (see Chapter 3 and Wigzell, 1981). Natural killer (NK) cells are part of the immune defense against virus infections and tumors (see Herberman and Holden, 1979; Herberman *et al.*, 1979; Herberman, 1981a; Serrou *et al.*, 1982;

E. Klein, 1983). It is clear that the expression of NK cell activity is subject to regulations and that the NK cell activity at any one time represents a balance between various regulatory signals (Herberman and Oldham, 1983). For reviews on immunoregulation and NK cells, see Herberman (1982) and Hansson (1983). A model of NK cell-mediated cytotoxicity has been proposed by Wright and Bonavida (1982). Here the NK cytotoxic factors play an integral role in the lytic step. The effector cell must recognize the NK-sensitive target and then release the cytotoxic factors. In order to be lysed, the NK target cell must be able to activate the NK effector cell and absorb the cytotoxic factors. Warner and Dennert (1982) cloned NK cells *in vitro* and transferred them into NK-deficient hosts. Using this system, they obtained evidence suggesting that the NK cells play an important role in immune surveillance. It has been suggested by Blazar *et al.* (1979) that one of the important functions of NK cells is to kill virus-producing cells at an early stage of the virus cycle, preferably before the virus particles are assembled.

Small tumor load might be advantageous for the NK system. For example, in patients with stage I melanomas, NK cell activity against the tumor cells was maximal 2–4 weeks after tumor removal and was followed by a decrease to normal levels (Hersey *et al.*, 1980). The NK cell activity after surgery could be directly correlated to tumor thickness. On the other hand, in Stage II melanoma, NK cell activity did not increase, but instead fell to low levels after removal of tumors. This may reflect different immunological status of Stage I and Stage II patients, which could be important in IFN therapy. Therefore, analysis has to be made *in extenso* in treated patients.

Trinchieri *et al.* (1978) and Einhorn *et al.* (1978a) reported that IFN increases the cytotoxicity of human cells several-fold. The effect of IFN on NK cells has since been well documented (cf. Perussia *et al.*, 1980a; Trinchieri and Perussia, 1981–1982; Einhorn *et al.*, 1982; Welsh, 1981; Herberman *et al.*, 1981–1982). As early as 1978, Gidlund *et al.* showed that IFNs are able to enhance NK cell activity *in vivo* in mice. Einhorn *et al.* (1978b) showed that natural killer cell activity in the peripheral blood can be enhanced by injecting IFN into patients. Evidence presented by Herberman's laboratory suggests that a non-thymus-dependent consequence of tumor cell recognition is production of an acid labile IFN, which is followed by activation of NK cells (Djeu *et al.*, 1980). The IFNs act on human NK cells both by effector cell recruitment and increased effector cell cycling (Ullberg *et al.*, 1981). Saksela *et al.* (1979, 1980) showed that augmentation of NK cells in the peripheral blood was largely due to the recruitment of

"pre-NK" cells. Others have reported that the enhancement of cytotoxicity seen upon exposure of lymphocyte cultures to IFN is due to an increase in the number of lymphocytes passing the threshold of lytic function (Berthold *et al.*, 1981). Salata *et al.* (1983) suggested that enhancement of the cytotoxicity of the NK system by IFN may result in part from a conversion of $OKT3^+$ to $OKM1^+$ cells, which are more efficient killers.

The effector cell of NK activity, which can be boosted with IFN-β or IFN-α, is a nonphagocytic cell with a receptor for the Fc portion of IgG. The boosting has a rapid effect on the cytolytic process (Ortaldo *et al.*, 1981). Pattengale *et al.* (1982) found that lysis of fresh, noncultured, neoplastic B cells was accomplished by IFN-augmentable, Fc receptor-positive, nonadherent lymphoid cells. There was a correlation between NK susceptibility and disease activity in 11 patients whic chronic lymphocytic leukemia (CLL) and in one patient with lymphosarcoma. Neoplastic B cells from untreated patients with nonprogressive CLL had unchanged NK susceptibility profiles during the time of observation. On the other hand, there were four untreated patients with progressive chronic lymphocytic leukemia who had NK-susceptible neoplastic targets that disappeared during cytoreductive therapy and reappeared when progressive disease was found. In general, the peripheral blood of the patients with chronic B cell leukemias was found to have lower NK effector cell activities against standard targets than normal donor peripheral blood lymphocytes.

IFN-γ is known to be produced in the supernatant of mixed lymphocyte cultures when lymphocytes are sensitized to alloantigens. It appears in the culture fluid after about 2 days, and reaches a maximum concentration on the fifth day (Perussia *et al.*, 1980b). The producer cells are T lymphocytes and supernatants that can be isolated from such cultures enhance NK cell activity and protect NK target cell from lysis. Weigent *et al.* (1983b) showed conclusively that human IFN-γ preparations can enhance human NK cell activity. Interleukin 2 can also enhance NK cell activity (Weigent *et al.*, 1983a), and it has been shown that at least part of the NK boosting induced by preparations containing interleukin 2 is mediated through IFN-γ (Kawase *et al.*, 1983). So, under natural conditions, IFN-γ seems to be a key substance for optimal NK cell activity.

IFN can also act on target cells in NK cell reactions and in this sense can be antagonistic to NK killing (Trinchieri *et al.*, 1981a). This protective effect is specific for NK cell cytotoxicity (Trinchieri *et al.*, 1981b). Einhorn *et al.* (1979a,b) also showed that natural human leukocyte IFN-α augments the cytotoxicity of lymphocytes, while pre-

treatment of the target cells may decrease their sensitivity to the spontaneous cytotoxicity of the lymphocytes. It is not known whether this has clinical implication, but it has to be taken into consideration in discussions of the immune effects of IFN against tumor *in vivo*.

In an extensive series of experiments, Grönberg *et al.* (1983) suggested that tumor cells are able to escape NK cell elimination by IFN production by the effector cell-containing population. This statement was based on selection-dependent variations in NK sensitivity, properties of the kinetics of IFN production, and the sequence of the induction of resistance. The active component inducing resistance was characterized as a mixture of IFN-α and IFN-γ. Rönnblom *et al.* (1983a) demonstrated a dissociation between NK cells acting against tumor cells and IFN producing cells that are stimulated by the same types of targets. It has been suggested that the natural killing of tumor cells carrying EBV consists of a rapid IFN-independent NK response to glycoproteins on the surface of the induced cells and a slower IFN-dependent natural killing (Blazar *et al.*, 1983). Some of the involved systems in IFN–NK interactions are probably complex and indirect. It has to be emphasized that some of the results obtained when testing induced human killer lymphocytes may depend on the target cell type used (M. Masucci *et al.*, 1980a). That IFN is involved in the cytolytic process of NK cells on certain target cells is suggested by experiments employing two transformed fibroblast cell lines from the same original cell line (Ohmori *et al.*, 1979). A subclass of thymocytes from young mice was sensitive to lysis of mouse NK cells, and the modulation of the NK cell sensitivity of this subclass could be affected by IFN (Hansson *et al.*, 1980).

Human ocular melanoma cells showed weak susceptibility to spontaneous human peripheral blood leukocyte natural cytotoxicity, but increased killing was observed when IFN-augmented cytotoxic effector cells were employed (Rees *et al.*, 1983). CLLs are also NK susceptible (Zarling *et al.*, 1979). Moore *et al.* (1982) tested 22 human leukemias (13 acute myeloid and nine lymphoid) for susceptibility to spontaneous cell-mediated cytotoxicity by untreated lymphocytes and by lymphocytes pretreated with lymphoblastoid IFN-α. Some degree of IFN-amplified killing was found in five acute myeloid and five lymphoid leukemias. The other 12 leukemias were resistant. These results show that there is variability in the capacity of IFN-treated lymphocytes to lyse leukemic cells that have not been adapted to tissue culture. Sibbitt *et al.* (1984) studied NK cell activity of peripheral blood mononuclear cells (PBMCs) from 25 patients with lung carcinoma, malignant melanoma, or epithelioid cancers of the gastro-

intestinal tract. They found that the NK cell activities of patients with lung cancer and malignant melanoma were generally decreased compared to those of normal individuals. In patients with advanced disease, the response to IFN was impaired. There was indirect evidence to suggest that the cells that bind tumor targets were present in patients with the advanced cancer, but that these cells were inactive. A significant decrease in cytotoxicity was found in 70 women with severe dysplasia and cervical carcinoma. When the peripheral blood leukocytes of these patients and control patients were treated with IFN, there was enhancement of the mean cytotoxicity, except in those leukocytes isolated from patients with advanced cervical carcinoma (Seltzer *et al.*, 1983).

Synergistic effects of IFN-αA and IFN-αD on NK cells could not be detected (Lotzová and Savary, 1984). Other substances can, however, affect the actions of IFN. Retinoic acid can inhibit the spontaneous activity of human NK cells as well as the activation seen after treatment with partially purified human leukocyte natural IFN-α (Abb *et al.*, 1982a). Glucocorticoids in physiologic concentrations are able to decrease NK cell activity. Purified leukocyte IFN-αA and inducers of IFN are able to enhance NK cell activity in the presence of steroids, although to a lesser degree than in their absence (Holbrook *et al.*, 1983). It was suggested by these authors that glucocorticoid therapy be supplemented with IFN in order to avoid some of the immunosuppressive side effects caused by the steroids. One factor that has been reported to be of importance in NK modulation is the binding of monomeric IgG to human peripheral blood leukocytes as it seems to reversibly inhibit their NK cell activity (Sulica *et al.*, 1982). In experiments with prostaglandin E_2 and ethanol, Kendall and Targan (1980) demonstrated that the same NK modulator had the potential to activate as well as inhibit NK cell cytotoxicity depending on the order of exposure of NK cells, target cells, and NK-target conjugates to the modulator.

Kadish *et al.* (1981) studied natural cytotoxicity in 51 adult tumor patients and 27 normal subjects. Peripheral blood leukocytes from 31% of the patients and 7% of the controls failed to kill the target K-562 *in vitro*. Of patients with advanced cancer, only 50% were able to respond with cytotoxicity in the normal range. Pretreatment of peripheral leukocytes with IFN-α resulted in enhanced cytotoxicity of all normal subjects' leukocytes. In the patients without spontaneous cytotoxicity, there was a rise to a normal level in half of the patients after IFN exposure. Almost all of the patients whose peripheral blood leukocytes were unable to kill despite IFN treatment had disseminated

malignancies. IFN production was normal in all groups. Borden *et al.* (1982d) serially measured NK cell activity in the blood of 11 normal individuals over a period of 18 months. They found that each subject had a characteristic basal level of activity. It was evident that after i.v., i.m., or intraarterial (i.a.) injection of 3–9 $\times$ 10^6 IU of natural IFN-α there was augmentation of NK cell activity in patients with neoplastic disease. In these patients, *in vitro* and *in vivo* results could be correlated.

Thirty-nine patients with multiple myeloma were studied for NK cell activity in their peripheral lymphocytes prior to and during IFN therapy (Einhorn *et al.*, 1982b). The activity increased in the majority of patients and remained at an increased level during at least 1 year of therapy. The lower doses (3×10^6) of IFN-α seemed to induce a greater increase in NK cell activity than the higher doses (6×10^6). No correlation whatsoever could be seen between the response of the patients to IFN therapy and pretreatment levels of NK cell activity. Neither was any correlation revealed between the response of patients to IFN therapy and the enhancement of NK cell activity seen either *in vitro* or *in vivo* after exposure of cells or patients to IFN. NK cell cytotoxicity of peripheral blood was assessed using K-562 target cells in 14 melanoma patients who received 1, 3, or 9 $\times$ 10^6 IU of semipurified human leukocyte IFN-α for 42 consecutive days (Golub *et al.*, 1982a). The NK cell activity fell to below pretreatment levels during the first day. This was followed by an increase in cytotoxicity, with a peak at Day 7 and then a gradual decline to pretreatment levels. At the lowest dose, patients tended to skip the initial decline and maintain elevated NK cell activity over the entire period. There was also no correlation in this study between clinical response and rise of NK cell activity. Golub *et al.* (1982b) also showed, in their patients with metastatic malignant melanoma treated with semipurified human leukocyte IFN-α, that the increased NK cell activity found during the first week of *in vivo* treatment was due to an augmentation of the development of NK cells from precursors as well as direct effects on the NK cells themselves. The decline in NK cell activity seen after the first week of treatment appeared, on the other hand, to be primarily caused by a direct, negative effect on the NK cells. An important point in their study was that they did not find any evidence supporting the development of suppressor cells as a cause for the decline.

Lotzová *et al.* (1982) grouped normal individuals and solid cancer patients into high, medium, and low NK cell responder categories with regard to their NK cell activities in peripheral blood. It was found that predominantly higher responders were in the normal donor

population and low responders were among the cancer patients. Leukemic patients always had a low-response status. The difference between the high responders and the medium responders seemed to be due to a decrease in the number of active NK cells. It was more difficult to boost the high responders with human natural IFN-α *in vivo*. Lotzová *et al.* (1983b) also treated 24 cancer patients with human leukocyte recombinant IFN-α and studied their peripheral blood NK cell cytotoxicity in detail. They found that the cytotoxicity declined consistently 4–8 hours after single injections of IFN. Twenty-four hours after the injections, the cytotoxicity of patients with a low NK cell phenotype was significantly augmented, whereas that of patients with medium or high NK phenotypes was depressed. Depression was also observed in a number of medium and high NK response patients after receiving multiple injections of IFN, while some patients with the low-response phenotype responded with further potentiation. There was no correlation between the NK cell augmentation and the serum IFN levels. When NK cells were studied *in vitro* after patients had received IFN, they became refractory to further recombinant IFN-αA treatment.

Sugiyama *et al.* (1983) studied the NK system in patients with head and neck cancers. Natural human leukocyte IFN-α was given i.v. in escalating doses from 5×10^5 IU to 2×10^7 IU. Ten patients received in excess of 3×10^7 IU. Other treatments were not employed. Analysis was made of T cell subsets and NK cell activity before and after IFN-α treatment. An attempt was made to correlate the changes observed with the clinical course in order to develop a more effective way of giving the IFN therapy. Decreased numbers of helper T cells were seen, and the ratio of Leu $3a^+$ to Leu $2a^+$ cells decreased in the patients. IFN-α did not help in these cases, and the disease condition was aggravated during treatment. In some of the milder cases, IFN administration brought about improvement in these parameters. Peripheral NK cell activity was stronger after treatment than before treatment, and evidence indicated enhancement of the NK cell activity of each NK cell by the IFN-α. No distinct correlations between the clinical outcome and the parameters studied could be established.

IFNs other than natural IFN-α have also been able to affect NK cell activity *in vivo*. Spina *et al.* (1983) administered human lymphoblastoid IFN to patients with various malignancies in Phase I drug toxicity trials. The IFN was given i.m. twice daily at 12-hour intervals over 7-day courses in doses of $1.5–100 \times 10^6$ IU/day. Twenty-eight patients were studied with respect to various immunological parameters. Leukopenia was evident after 1–2 days of IFN administration. NK cell

activity in the peripheral blood was increased significantly ~2 hours after the initial injection, especially in patients receiving the higher doses. Most of the patients then experienced a decrease with a marked depression in NK cell function by Day 7 of therapy. The ADCC reaction paralleled the NK cell function test. There was no change in the percentage of circulating Fc receptor-bearing cells, which supports the theory that the cytotoxic cells, although present, were unable to express lytic functions. In studies of lymphoblastoid IFN treatment of cancer patients, Laszlo *et al.* (1983) found that enhancement of NK cells showed a complex dose–response relationship. Low IFN doses were less stimulatory than high doses in the short term, but gave more sustained stimulation over a 5-week course. The IFN in these studies was given i.m. three times per week. No effect was documented on various measures of monocyte function, hypersensitivity, immunoglobulin levels, and complement. The authors considered it important to emphasize that the very high doses used in most clinical trials were less stimulatory than the lower doses. Edwards *et al.* presented their data at the American Association for Cancer Research (AACR) meeting in 1984 from experiments with lymphoblastoid human IFN given in weekly i.m. injections at six doses ranging from 10^5 up to 3×10^7 IU. When doses were increased above 3×10^6, there was a negative correlation, so that NK cell activation became less pronounced. The authors suggested that low-dose IFN should be used in randomized clinical trials for determination of the functional significance of the human NK cell *in vivo*. Obviously, it would be of interest to use low-dose IFN together with pulses of heavy anti-tumor therapy, such as irradiation or chemotherapy. In studies of lymphoblastoid IFN treatment, Spina *et al.* (1983b) came to the conclusion that high-dose exposure of human lymphocytes to IFN may induce an NK cell refractory state, which would explain the decreased effectiveness.

The effects of various recombinant and hybrid recombinant human IFN-α on NK cell activity vary extensively (see Ortaldo *et al.*, 1983a, and Chapter 4, Section I). Edwards *et al.* (1982) made 23 separate determinations of NK cell activity in two of their clinical trials and found that NK cell modulation, as a result of treatment of the patients' PBMCs *in vitro* with 100–500 IU of recombinant IFN-α per milliliter, correlated significantly with the NK cell modulation that was seen upon administration of the IFNs *in vivo*. Similar results had previously been reported for natural IFN-α (Einhorn *et al.*, 1980a).

In all of the clinical trials at the University of Wisconsin Clinical Cancer Center, a positive change in NK cell activity within 24–28 hours of initial IFN administration was noted. In 15 cancer patients in

two clinical trials, Edwards *et al.* (1983) examined the relationship between IFN-induced stimulation of NK cell cytotoxicity *in vitro* and changes seen in NK cell cytotoxicity resulting from systemic IFN administration. Three IFN-α preparations were used in these studies; namely, a natural buffy coat IFN-α and two recombinant species, IFN-αA and IFN-αD. Enhancement occurred 24 hours after i.m. injection of the IFN. Patients exhibited individual differences in their responsiveness to NK cell activity enhancement by IFN *in vitro*, and these differences predicted rises in NK cell activity *in vivo* after IFN administration. Ortaldo *et al.* (1983c) tested two recombinant human leukocyte IFN-α, five hybrid IFNs containing varying portions of these two recombinant IFNs, and one recombinant IFN-γ over a wide range of concentrations for the ability to modulate the activity of NK cells. There were significant quantitative differences between the IFNs.

The studies of Lotsová *et al.* (1983b) have been mentioned previously in this chapter. They also measured NK cell cytotoxicity in the peripheral blood in 32 cancer patients receiving single and multiple injections of 3–86 $\times$ 10^6 IU of human leukocyte recombinant IFN-αA i.m. (Lotsová *et al.*, 1983a). Twenty-four hours after injection, there was a significant augmentation of the NK cell cytotoxicity in patients with a low NK cell phenotype. This was preceded by a decline 4–8 hours after the first injection. Most of the patients with medium or high NK phenotypes showed a depression with IFN. After multiple injections, there was a depression of the NK cell cytotoxicity in a number of high- and medium-response phenotype patients, while some patients with a low-response NK phenotype showed elevation of the NK effect. There was no correlation between NK cell augmentation and serum IFN levels. The IFN preparation used was active *in vitro* and able to cause a significant rise in the NK cell cytotoxicity of lymphocytes isolated from these patients before injections. In the data published by Maluish *et al.* (1983d), there was no clear relationship between response to recombinant IFN-αA in malignant patients and their changes in NK function, and, eventually, all of the patients who showed clinical benefit from the IFN therapy had a depressed NK response.

IFN-β has also been shown to augment NK cell activity in the peripheral blood of patients receiving IFN therapy (Pape *et al.*, 1982; E. Falcoff, personal communication). Therefore, such a system can also be included in monitoring tests before treating cancer patients with IFN-β. In the Chicago study of sarcoma patients treated with IFN-β, the effect of the therapy on NK cell function was studied in eight

patients (Braun *et al.*, 1984). The patients were given 10 daily injections of 10×10^6 IU of IFN-β followed by a 10-day interval. Test cells were of the K-562 line. Glass-adherent cell depletion augmented the depressed NK function that was found in five of eight sarcoma patients prior to therapy. Three other patients also had depressed levels of NK function, but there was no augmentation by glass-adherent cell depletion. Following therapy, the five patients with significant pretreatment suppression demonstrated augmented NK functions. In the other three patients, depressed NK function was found in whole PBMC preparations, but this was augmented by glass-adherent cell depletion. Therefore, this study clearly showed that treatment with natural IFN-β can change the level of NK function, depending on the activity of glass-adherent supproessor cells at the pretreatment stage.

How important is NK cell augmentation by IFN? The role played by NK cells in the inhibition of tumor growth by IFN has been debated (Ratliff *et al.*, 1982). On the other hand, evidence supporting involvement of the NK–IFN system in preventing metastases has accumulated from animal models (Sugino *et al.*, 1983). The role of the NK cells in the therapeutic results obtained with IFN therapy remains unknown (see Marx, 1980). In systems employing moderate doses of human natural IFN-α *in vitro* and *in vivo*, enhancement of NK cell activities is evident (see Einhorn *et al.*, 1981a). There are, however, no correlations demonstrable to clinical effects (Einhorn *et al.*, 1982b). Some investigators have found that repeated IFN administration suppresses the stimulation of NK cell activity seen initially (Goutner *et al.*, 1981). To summarize, then, we can say that NK cell activity in the peripheral blood increases following administration at moderate doses of human natural IFN (Einhorn *et al.*, 1980a), but it is not known whether this is of relevance for the anti-tumor effect exerted by the IFN in some patients (Einhorn *et al.*, 1982a). It seems, at least in some systems, that all types of IFN-α, -β, or -γ can induce resistance to NK cell killing of targets as well as stimulation of killing activity of NK cells against the target (Wallach, 1983). Whether selectivity can be achieved on this basis in any system in *in vivo* situations in humans is unknown. No evidence of a correlation between tumor response and NK cell activity has been seen in chemotherapy-treated patients either (Bhoopalam *et al.*, 1984).

I think it is important that different diseases be considered individually with respect to studies on NK cell activities and IFN treatment, since the NK cell system may be in a more activated or exhausted state in some diseases than in others. It would, under such circumstances,

be much more difficult to demonstrate the effects of IFN treatment on this system, especially if the effects are exerted at the local level (see, for example, Hawrylowicz *et al.*, 1982).

III. Other Effector Systems

Lymphocyte subpopulations separated on the basis of surface markers all contain, with the exception of the B subset cell population, cells with the ability to kill in short-term assays (see Masucci, 1984). The role played by IFN in these systems is at the moment incompletely understood. Actually, it is difficult to say if the use of *in vitro* cytotoxicity tests in general is relevant to *in vivo* situations, since they depend on the role of the directly cytotoxic T cells. The possibility exists that noncytotoxic T cells may be pivotal in the initiation of reaction responses (Robins and Baldwin, 1983). If the latter is true, noncytotoxic T cells should be measured and their response to tumor-associated antigens determined.

Mittelman *et al.* (1983) studied peripheral T cells in 33 patients with advanced cancer who were treated with either semipurified natural human leukocyte IFN-α or recombinant IFN-βA. The $OKT4^+$/$OKT8^+$ ratio defines the balance between helper/inducer and suppressor/cytotoxic T cell subsets. Both IFN preparations caused an immediate decrease in the ratio, but the T cell subsets that were responsible for the decrease varied with the source of the IFN. The decrease was due to an increase in $OKT8^+$ cells in the group treated with natural IFN, which was accompanied by a small decrease in the proportion of $OKT4^+$ cells in the recombinant A group. It seems that differential inhibition of various subsets of T cells may be an important way in which IFN can influence immune actions (De Maeyer-Guignard *et al.*, 1983). Adult T cell suppression of the transformation of B cells after EBV infection is mediated by IFN (Thorley-Lawson, 1981). PHA-induced transformation of adult B cells can also be suppressed by IFN, and it has been suggested that EBV and PHA-induced transformation share a common IFN-sensitive step. That the resistance of newborn lymphocytes to IFN may be an important principle to consider when IFN is discussed as an antiviral or anti-tumor agent has been proposed (Thorley-Lawson, 1981).

It has been suggested that circulating monocytes may serve as a target cell population for the *in vivo* action of IFN (Hovi *et al.*, 1981a). Human monocyte-mediated cytolysis can be augmented by IFN-β (Jett *et al.*, 1980), but significant effects of human leukocyte IFN-α2 on monocyte Fc-dependent phagocytosis, given to melanoma patients,

could not be demonstrated (Coleman *et al.*, 1984). IFNs are also known to inhibit suppressor T cell responses (Fradelizi and Gresser, 1982; Knop *et al.*, 1982; Tarkkanen and Saksela, 1982).

In a study in 1974, it was shown that partially purified human leukocyte IFN-α could suppress the mixed lymphocyte reaction as well as the *in vitro* response of human lymphocytes to PHA, Con A, and purified protein derivative (PPD) (Blomgren *et al.*, 1974). In older studies, it had been shown that when IFN was added to an assay *in vitro*, it inhibited proliferative responses of lymphocytes to mitogens (see Stewart, 1979a). Long-term treatment with human natural leukocyte IFN *in vivo* had no major effects on the mitogen responsiveness of the lymphocytes in the peripheral blood of treated tumor patients. It could be shown that this lack of lymphocyte-proliferative response following treatment *in vivo* was probably due to the fact that the lymphocytes were only treated with IFN prior to their use in *in vitro* assays (Einhorn *et al.*, 1983c). When human leukocyte IFN-α was given to 20 patients with osteosarcoma and their peripheral lymphocytes were tested *in vitro* for response to various mitogens, no alterations of response were noted (Einhorn *et al.*, 1979a). Harris *et al.* (1983) studied the influence of IFN-α therapy on PHA-induced lymphocyte DNA synthesis. Fifteen renal cell carcinoma patients received either 10^7 IU of natural IFN-α daily for 28 days (nine patients) or 10^6 IU of the same IFN (six patients). All patients were monitored at weekly intervals. By the end of the first IFN treatment cycle, 14 of 15 patients had significantly depressed PHA responsiveness. This immunodepression was reversed by indomethacin and was therefore probably prostaglandin mediated. The authors suggested that, due to the immunodepressive effects of the IFN-α therapy, IFNs should be given on an intermittent basis or together with indomethacin.

IV. Immunoregulatory Circuits

It has been found that recombinant IFN-γ is a potent macrophage activator and a potent inducer of HLA-DR, and is functionally related to interleukins 1 and 2. It differs in all of these respects from IFN-α and IFN-β (see Sherwin, 1984). IFN-γ probably exerts more potent immunoregulatory activities than the other IFNs (see Sonnenfeld and Merigan, 1979a). Purified human IFN-γ can induce interleukin 2 receptor expression on human peripheral T cells (Johnson and Farrar, 1983), as demonstrated by proliferation of the IFN-γ-treated cells in the presence of interleukin 2 and by the absorption of interleukin 2 by treated cells. The similarities between this type of induction and en-

hancement of the expression of antigens of the major histocompatibility complex are obvious. There is a close correlation between the magnitude of interleukin 2 and IFN-γ production under some experimental conditions (see Pearlstein *et al.*, 1983), and interleukin 2 seems to play a significant role in IFN-γ production following immune cell stimulation (Kermani-Arab *et al.*, 1983; see also Chapter 4, Section II).

Matsubara *et al.* suggested in 1979 that induction of IFN-γ may depend on the action of T lymphocytes and macrophages. For a discussion concerning the regulatory circuits involving IFN-γ and interleukins see Palladino *et al.* (1984). The present contention is that the production of IFN-γ by activated T cells may serve as a positive feedback signal for recruiting additional antigen-presenting, interleukin 1-producing, and then interleukin 2-synthesizing cells. This would allow production of effector cell sets during an ongoing immune response. The role played by IFN-γ in the activation of macrophages has been more and more firmly established (Männel and Ralk, 1983). The importance of tumor resistance for the macrophage-mediated host defense, however, is still an unsettled issue (see Rhodes *et al.*, 1979).

It will be extremely important to elucidate the role played by lymphokines in the production of macrophages capable of functioning as potent effector cells (see Cohn, 1978). The effect of IFN-α on peripheral blood mononuclear cells seems to be due to an increased responsiveness of the B cells to helper factors, which are in turn produced by radioresistant T cells (Rodriguez *et al.*, 1983). It is known that OKT3 monoclonal antibodies, which react with all human peripheral T cells and stimulate their proliferation at even minute concentrations, can induce IFN production by cultured mononuclear cells (T.-W. Chang *et al.*, 1982).

IFN can enhance production of some human lymphokines (Blomgren and Einhorn, 1981). Therefore, the clinical use of IFN-γ will be extremely interesting but also very complex, due to the many interactions of these molecules with other lymphokine systems (see Vilček *et al.*, 1983). In autologous mixed cultures, Argov *et al.* (1983) found that supernatants contained IFN-γ but that the concentration of IFN showed no correlation with either the proliferative response or the strength of the cytotoxicity. Conta *et al.* (1983) have shown that a Lyt 1^+ clone can produce IFN-α, IFN-β, IFN-γ, and lymphotoxins after Con A exposure. The same authors used a cell line to show that lymphotoxins and IFN molecules are regulated in a noncoordinate fashion. The interplays between lymphotoxins (see Rosenau, 1981) and IFN will be an important topic in future studies (see Wallach *et al.*, 1983).

Livingston *et al.* (1984) have tried to inhibit the suppressor cell activity in melanoma patients with cyclophosphamide at a rather low dose. These low doses of cyclophosphamide (100–500 mg/m^2) had profound effects on immune regulation and *in vitro* antibody production. This will be an interesting system to follow with respect to combination treatments with cyclophosphamide and IFN.

It seems that IFNs play immunoregulatory roles in both NK and T cell proliferation. They can also induce interleukin 2 receptors and activate suppressive T cells. Such interactions may constitute an important immunoregulatory circuit (Vose *et al.*, 1983) and will be extensively studied in the coming years.

CHAPTER 5

EFFECTS ON OTHER PARAMETERS

I. Receptors and Somatic Antigens

Vignaux and Gresser showed in 1978 that histocompatibility antigens could be more easily expressed on mouse embryo fibroblasts when these were treated with IFN. All IFNs can affect expressions of antigens (see, for example, Sonnenfeld *et al.*, 1981). Heron *et al.* (1978) demonstrated in 1978 that mononuclear cells from the blood experienced enhanced expression of HLA antigens and β_2-macroglobulins after exposure to IFNs of different origin and purities. Membrane immunoglobulins and antigens recognized by antiserum raised against human brain and T cells were not affected by the IFN. The same effects were observed on EBV-negative Burkitt's lymphoma cell lines. The IFN-induced enhancement required active protein synthesis. Fellous *et al.* showed that natural human leukocyte IFN-α could increase the expression of HLA-A and -B antigens and β_2-microglobulins on lymphoblastoid cells and peripheral blood lymphocytes, while it was incapable of affecting the expression of HLA-DR antigen (Fellous *et al.*, 1979). Attallah and Strong (1979) made a study on HLA antigens after IFN exposure, since they are targets for antibody-mediated and cell-mediated cytolysis. Quantitative absorption techniques were used together with anti-HLA sera. It was found that human lymphoblastoid IFN enhanced the expression of HLA antigens on human peripheral blood lymphocytes by about 8-fold. This was specific for both the HLA-A and HLA-B regions of the histocompatibility complex. No increase in the expression of the Ia region could be detected. Therefore, it is quite clear that HLA antigens can be increased by exposing cells to purified human IFN-α (cf. Heron *et al.*, 1978; Fellous *et al.*, 1979; Burrone and Milstein, 1982). In the latter study, it was established that the increased HLA synthesis was due to an increase in the amount of HLA mRNA present in the cells after exposure to IFN.

In the English studies on IFN treatment of small-cell carcinoma of the bronchus, expression of HLA-DR and Fc_γ receptors by peripheral blood monocytes from untreated patients with the same disease was investigated. When lymphoblastoid IFN was given (a total of 4×10^8 IU/m^2 over a 5-day period), there was a marked increase in both the

monocyte HLA-DR antigen expression and the Fc_γ receptor expression in comparison with untreated patients and normal donors. These increases declined somewhat, but they could still be detected at 3 weeks if the lymphoblastoid IFN was given intermittently by the i.m. route (Rhodes *et al.*, 1983).

It was shown by Basham and Merigan (1982a,b) that recombinant IFN-γ increased the HLA-DR (Ia-like) antigen synthesis and expression in human cells. Furthermore, in an interesting paper, Basham and Merigen reported in 1983 that recombinant IFN-γ increased the synthesis and expression of the HLA-DR (Ia-like) antigens as well as the low-molecular-weight subunit of HLA, β_2-microglobulin, on human melanoma cells. Human leukocyte IFN-α did not cause such an increase even at concentrations 400 times higher than what was used in the case of the IFN-γ. This was also true for recombinant IFN-α, which did not cause increased expression of the surface antigen either. This suggests a more specialized role for IFN-γ in the immune regulation in humans, and it also emphasizes the possibility of combining IFN-γ with IFN-α in the treatment of patients with various malignancies.

Augmentation of delayed-type hypersensitivity responses can be achieved by using H2 receptor antagonists like cimetidine (Avella *et al.*, 1978). This histamine H2 receptor antagonist stimulates lymphoblastogenesis and lymphokine production and can be expected to affect the immune system (Watson *et al.*, 1983). It should be mentioned that IFN-induced binding antigens are sensitive to anti-H2 but not to anti-Ia sera (Lonai and Steinman, 1977).

Masucci *et al.* related target selectivity of IFN-induced cytotoxicity to the Fc receptor content (1980b). Aguet *et al.* (1981a) suggested that enhancement of the Fc_γ receptor could be one of the mechanisms by which IFN exerts its immunostimulating effect. Wallach *et al.* (1982b) found that IFN-γ could induce HLA-ABC and β_2-microglobulin, mRNAs, or proteins at concentrations that were over 100 times lower than those needed to induce the (2′-5′)oligo-A synthetase and the antiviral state. Such a tremendous difference was not found for the other IFNs. It is clear that the *in vitro* expression of Ia antigens on macrophages in murine systems is regulated by substances with the characteristics of IFN-γ (Steeg *et al.*, 1982). Dolei *et al.* (1981) found that IFN-β was also able to accumulate Ia antigens in culture fluid from lines exposed to this IFN. A similar increase in the expression and shedding of HLA-ABC and β_2-microglobulin was observed. IFN-α was also able to cause such enhancement, with the exception of the increase of Ia expression. Pober *et al.* (1983) measured Ia antigens on

cultured human umbilical vein endothelial cells. These cells usually do not express these antigens. If the cultures were treated with PHA, there was induction of the expression of Ia antigens and every endothelial cell present became Ia^+ and HLA-AB increased concomitantly. It is interesting that human IFN-γ, produced by Chinese hamster ovarian cells transfected with the human IFN-γ gene, also induced endothelial Ia expression. The authors hypothesized that this inducible expression of the Ia antigen may be important for allograft reactions and for the recruitment of T cells.

It is therefore possible that IFNs can influence effects and functions of the immune system by increasing the Fc receptor expression on cells. It has also been shown that human natural leukocyte IFN-α can increase the expression of Fc_γ receptor on human Burkitt's lymphoma cells (Fridman *et al.*, 1980). Ralph *et al.* (1983) found that the Fc receptor-inducing activity of lymphokine preparations was inhibited by a neutralizing monoclonal antibody to IFN-γ, and the differentiation modulator for monoblast cells also seemed to be IFN-γ. Perussia *et al.* (1983b) reported that the Fc receptor for human monomeric IgGL was induced on myeloid cells cultured together with natural IFN-γ for 8 hours. This was repeated with recombinant IFN-γ, while other types of IFN did not specifically induce the appearance of the Fc receptor in this system. The effect was also evident on nature polymorphonuclear cells. These authors presented evidence to suggest that the receptor, which is present on human monocytes or immature myeloid cells and which can be selectively inducible by IFN-γ, has a specificity that is similar to the Fc receptor 1 described on mouse macrophages. Furthermore, Guyre *et al.* (1983) showed that natural and recombinant human IFN-γ can lead to a nearly 10-fold increase in the number of Fc_γ receptors of normal human monocytes and human cell lines. IFN-α and IFN-β also caused an increase in these receptors, but this rise was more modest. Rhodes and Stokes (1982), finally, demonstrated that natural human IFN-β was able to increase the expression of both Fc_γ receptors and HLA-DR antigens on human peripheral blood monocytes. Retinol and retinoic acid were found to be antagonistic to these IFN effects. It can be concluded that the receptors and antigens studied can play a crucial part in IFN action, especially in the case of IFN-γ.

II. Tumor Cell Antigens and Other Markers

It was shown in 1976 that IFN treatment of mouse L-1210 cells was accompanied by increased expression of histocompatibility surface

antigens (Killander *et al.*, 1976). In humans, it would be interesting to study the expression of Thy 1 antigen on human melanoma cells exposed to IFN, since it could be used as a marker on the melanocytes at certain stages of differentiation (Hersey *et al.*, 1983). Therefore, it could also be expected to change after IFN exposure if these cells contain receptors for IFN. Human natural IFN-γ seems to be more effective than IFN-α or IFN-β in stimulating the production of immunoassociated antigen HLA-ABC and β_2-microglobulin on human melanoma and lymphoma cells (Dolei *et al.*, 1983). Unfortunately, most markers of various malignant neoplasma are not very reliable, so the follow-up of specific markers in order to evaluate IFN effects is questionable for most neoplasms (see Klavins, 1983).

It would also be interesting to learn from experimental animal work how IFN could affect tumor angiogenesis, which is probably an important clinical concept (for a review, see Shubik, 1982).

III. Various Laboratory Parameters

Monitoring for IFN therapy in individual patients is desirable as in the use of results obtained in studies on various therapeutic variables (see Strander, 1982c). For example, the (2′-5′)A system has been successfully employed to see whether IFN-β, given s.c., can affect PBMCs (Revel *et al.*, 1982). It has already been emphasized that the (2′-5′)A synthetase assay in peripheral white blood cells can be advocated for regular use on IFN-treated patients (Wallach *et al.*, 1982a). Arai and Nagai (1983) studied immunological parameters and (2′-5′)A synthetase activity in peripheral blood lymphocytes of IFN-treated patients with malignant brain tumors and could not see any correlations between the studied parameters and clinical responses. After injections of 3×10^6 IU of IFN-β, the (2′-5′)A synthetase activity was increased at 4 hours and then gradually decreased after 11 hours to the pretreatment value at around 24 hours. This intracellular enzyme, induced by IFN, was also measured in 44 patients studied by Merritt *et al.* (1984b). Twenty-eight of these patients were untreated individuals, while 16 of the patients were receiving natural semipurified leukocyte IFN-α. PBMCs were concentrated and enzyme levels were determined. The measurements were reproducible on various occasions in every individual, but the levels varied between individuals. Enzyme activity was increased within 8 hours after the IFN had been administered, and the elevated levels could be maintained for at least 24 hours. By giving daily IFN treatments, the enzyme level could be kept high. In two of the IFN-treated patients, the levels did not

change after IFN injections, but in these two patients, higher pretreatment levels were found than in the others. Determination of the (2'-5')A synthetase can be done quickly and reliably, and this should be an interesting parameter to follow in IFN-treated patients.

A comparison was made of the biological effects of two recombinant human IFNs (R-αA and R-αD) (Hawkins *et al.*, 1984b). Eight patients with various malignant tumors were given weekly injections of either αA or αD. The frequency of side effects was much lower with the αD preparations. This was revealed both by measuring maximum temperature and by investigating the incidence of side effects. Mean titers in the serum were similar with the two recombinant preparations. The αD preparation had less antiviral activity on human cells, while its effects on total granulocyte counts, NK cell cytotoxicity, and (2'-5')A synthetase activity were comparable to those of αA. This shows that the measured species-specific antiviral activity of IFN preparations does not predict other biological properties when comparisons are made to other preparations of IFN.

Kirkwood *et al.* (1984) performed an important study in which they looked for induction of (2'-5')A synthetase in patients receiving natural human leukocyte IFN or recombinant IFN-α. They studied the enzyme in PBMCs. Two doses of IFN were used, 1 or 10×10^6 IU/day for 28 days i.m. There was no relationship between anti-tumor response with human leukocyte IFN in three patients with metastatic renal cell carcinoma and induction of the (2'-5')A synthetase in the peripheral blood. The studies now undertaken by this group include trials with IFN-α2, in which melanoma patients are treated and (2'-5')A synthetase present in the tumors is being measured. It will certainly be interesting to see if there are going to be any correlations between the induction of the enzyme in the tumors and the clinical response. Merritt *et al.* (1984a) demonstrated that the IFN response of (2'-5')A synthetase in PBMCs is determined by the IFN dose given—in this case, lymphoblastoid IFN—and not by the pattern of the circulating IFN activity. It might be important to follow up the relationship of the induced synthetase with the presence of clinical responses in patients injected with IFNs. If the (2'-5')A system and the dependent nuclease will be used for monitoring of therapy, it has to be remembered that these systems are complex. Not only IFN treatments but also cell growth and cell differentiation regulate the levels of the nuclease (Silverman *et al.*, 1983). The (2'-5')A system certainly seems intricate (Kerr *et al.*, 1983). It is probably of importance for the control of both cell growth and specific cell functions, and the IFN effects are really intriguing.

Kauppila *et al.* (1982) injected five healthy women with daily s.c. injections of semipurified human leukocyte IFN at a dose of 3×10^6 IU/day from the Days 3–23 of the menstrual cycle. Serum steroid and peptide hormone concentrations were monitored at 3-day intervals. Values had also been obtained from the preceding cycle. It was found that serum estradiol and progesterone concentrations were significantly decreased during the treatment cycle, suggesting that IFN might interact *in vivo* with the function of both follicle-stimulating hormone (FSH) and luteinizing hormone (LH). There were no significant changes in the serum peptide hormone concentrations [FSH, LH, prolactin, insulin, growth hormone, and thyroid-stimulating hormone (TSH)]. Neither were the levels of endometrial nuclear and cytoplasmic estrogen and progesterone receptors (ER_n, ER_c, PR_n, and PR_c) affected by the IFN treatment. There were also increased activities of serum alkaline phosphatase and γ-glutamyltransferase present during the IFN therapy, and this, as mentioned in Chapter 2, Section I, indicated that IFN treatment can interfere with liver function. There was also a decrease in the peripheral leukocyte count. It was suggested that IFN treatment may effect the growth of hormone-dependent neoplasms, since effects were obtained on the estradiol and progesterone concentrations.

IFN has also been shown to interact with thyroid cells, and in rats, iodide uptake by functioning rat thyroid cells is increased by adding mouse IFN to the cells (Friedman *et al.*, 1982). There is a difference in IFN sensitivity by functioning as opposed to nonfunctioning thyroid cells, and therefore it would be important to know how IFN can affect thyroid function in general *in vivo* in humans. Orava *et al.* (1983) treated five volunteer women by daily s.c. injections of 3×10^6 IU of semipurified human natural IFN-α. The daily administration caused significant decreases in circulating concentrations of T3, T4, and free T4, whereas serum TSH was not affected. This could be interpreted to mean that IFN acts at the thyroid cell membrane level. The possible clinical implications are unknown.

Treatment of patients with daily s.c. injections of 3×10^6 IU of partly purified human leukocyte IFN-α has been able to cause changes in serum lipids. The high-density lipoprotein cholesterol was decreased after 1 week, with a minimal value at 2 weeks, and the original blood level was reached then about 2 weeks after the last IFN injection (Cantell *et al.*, 1980b). Dixon *et al.* (1984) noticed that human natural leukocyte IFN-α can decrease high-density lipoprotein cholesterol (HDLC) and total cholesterol levels in patients with breast carcinoma. They also found that recombinant IFN-α caused a similar

decrease in high-density lipoprotein cholesterol and total cholesterol. The authors concluded that a definite relationship existed between the administration of IFN-α and the decreased plasma levels of these cholesterols. Ehnholm *et al.* (1982) showed that daily injections of semipurified human leukocyte IFN-α to healthy subjects lowered the plasma levels of cholesterol, very-low-density lipoprotein + low-density lipoprotein cholesterol (LDLC), HDLC, and apolipoprotein A-I. Massaro *et al.* (1984) studied the mechanism by which exogenous IFN therapy alters plasma lipid levels. Ten malignant melanoma patients received 30×10^6 IU/m^2 of recombinant human IFN-α2 i.v. daily for 5 days every third week. Plasma proteins were isolated from fasting blood samples. It was found that plasma cholesterol levels were significantly decreased on Day 4 of IFN administration, and there were significant decreases in LDLC and HDLC. There were similar decreases in lipoprotein–protein concentrations. The results suggested that the decreases in lipids caused by injections of recombinant IFN-α2 are specific for the plasma cholesterol which is transported in the HDL and LDL subfractions.

An inhibitor of complement fixation reactions was associated with IFN treatment and isolated from the serum of IFN-injected patients (Aho *et al.*, 1976). The nature of this inhibitor has never been established. It would be important to see whether it also appears in patients injected with more purified IFNs and IFNs of different types and whether it has clinical implications. It would also be interesting to follow IFN effects on the hypercalcemia associated with certain types of cancer (Seyberth *et al.*, 1975).

Mirro *et al.* (1984) treated seven patients with myeloblastic leukemia in relapse with high-dose and continuous infusion of human lymphoblastoid IFN for 10 days. Doses given were $15–30 \times 10^6$ IU/m^2 per day. It was found that the patients developed bleeding and prolonged activated partial thromboplastin times. The study demonstrated that high-dose continuous i.v. infusion of human lymphoblastoid IFN reversibly depressed the activity of Factor 12 and vitamin K-dependent factors. Hovi *et al.* (1981b) showed that natural IFN-α and recombinant IFN-α1 specifically enhanced the secretion of plasminogen activator, a specific serin protease.

Horn *et al.* (1983) studied 83 breast cancer patients for carcinoembryonic antigen (CEA) plasma levels, IFN plasma levels, liver function tests, and clinical status. A 73–83% positivity rate was obtained when the results of these four tests were compared. This possibly provides a basis for tests in future breast cancer trials employing IFN treatment.

Patients with metastatic malignancies were given single i.m. injections of 10^7 IU of partially purified preparations of either IFN-α or IFN-β by Lucero *et al.* (1982). The levels of circulating IFN were lower after injection of the IFN-β than after the injection of IFN-α. Despite this difference in pharmacokinetics, the NK cell activity of the peripheral blood was enhanced with both IFNs. CEA levels were not influenced by the IFN injections, but, on the other hand, the natural IFN-α caused an increase in the serum levels of β_2-microglobulin in the circulation while this was not achieved with the IFN-β. This could be confirmed in experiments on cell cultures, and only the IFN-α was able to cause a release of β_2-microglobulin by either leukocytes or fibroblasts.

It is difficult at the moment to monitor IFN therapy. Levin and Hahn (1981) showed that healthy persons have little or no IFN in their blood. In 94% of their tested persons, the PBMCs were not in an antiviral state. In contrast, patients with acute viral diseases had increased IFN levels in their blood, and also in 70%, their PBMCs were found to be in an antiviral state. Studies on the intracellular antiviral state are important in order to optimize IFN treatment of various patients.

CHAPTER 6

PHARMACOLOGY AND TOXICITY

I. IFN Effects in Animals—General Implications

The various animal models available for IFN studies have been reviewed by Schellekens and Meide (1983). These authors stated that the total number of papers on IFN seems to increase at a rapid rate (Lindenmann, 1982), while papers on animal studies with IFN are declining in number. The main reason for this has been the lack of large amounts of mouse IFN, but this has now changed and some of the animal IFNs have been produced on a large scale and are available in sufficient amounts to make animal work more attainable. It is important that such studies continue in the tumor area. A most interesting finding is the one made by Werenne *et al.* (1983) that young calves constitute a possible model for *in vivo* studies on human IFN action. The *in vivo* effects of IFN against bovine papillomavirus would be of considerable interest in this context, since papillomaviruses are probably involved not only in benign tumors on which IFN studies have been performed in humans, but also to some extent in malignant tumors (Gissman *et al.*, 1983).

Enhancement of NK cell activity and a rise in (2′-5′)A synthetase in mice go hand in hand when animals are treated with polyA·polyU (Youn *et al.*, 1983). In animals, many systems are affected by IFN injections or inducers. Extensive work was done by Lindahl in Gresser's laboratory on the immunological significance of the IFN system (Lindahl, 1974). A new emphasis was made "that the role of the IFN system is not limited to inhibition of viral replication, but may be of far greater importance to the host than previously recognized." This has certainly turned out to be the case.

What can we then learn from recent animal data? It is well known that the host genotype has an influence on the effects of IFN therapy in mouse systems (De Maeyer and De Maeyer-Guignard *et al.*, 1982). It is amazing, actually, that this aspect has not been studied extensively in humans. It has also been shown that IFN and IFN inducers may be effective or ineffective antiviral substances, depending on the presence or absence of certain host genes (see, for example, Haller *et al.*, 1981), and the antiviral state toward influenza viruses in the mu-

rine system clearly is dependent on dominant resistance genes (Horisberger *et al.*, 1983).

In experimental systems, Schultz *et al.* (1978) demonstrated that resting macrophages were transformed into activated cells by exposing them to IFN preparations. It is clear that macrophages are probably important for IFN action, and this has been shown especially in viral infections in experimental animals (Stebbing *et al.*, 1978). Work by Kirchner *et al.* (1983) also suggests that cells that are important *in vivo* for the functioning of the IFN system probably are of the macrophage lineage. During viral infections, both IFN-α and IFN-β are produced. In the herpesvirus infections in murine systems, detectable IFN does not have to be induced in order to boost NK cells in the peritoneal cavity after injection of viruses.

Of direct clinical importance is the use of anti-IFN antibodies on animals having various types of diseases in order to see which of them will worsen in the absence of a fully available IFN system (Fauconnier, 1981–1982). One important such finding is that IFN may be a part of the primary host defense against malaria. In a virus carrier state established by a congenital infection of mice with lymphocytic choriomeningitis virus, there was evidence of IFN production and increased levels of (2′-5′)A synthetase in the liver and spleen of the adult mice (Saron *et al.*, 1982). It was suggested that IFN may play a role in the continued pathogenesis of the virus carrier. Studies on rhesus monkeys infected intradermally with vaccinia virus and treated with semipurified human natural leukocyte IFN-α revealed that IFN therapy in this virus system can be effective *in vivo* against a virus that is insensitive to the antiviral action of IFN in many different cell types *in vitro* (Schellekens *et al.*, 1979). This indicates that IFNs may exert antiviral actions by activating defense systems of the host.

II. Animal Toxicity

IFNs are extremely potent substances in many respects. This has to be remembered in clinical trials. Furthermore, they can even induce disease (see Gresser, 1983b). An animal that could be used for studying various types of problems concerning human IFN preparations is the cynomolgus monkey (Yamasaki *et al.*, 1982; Yamada *et al.*, 1983). It is interesting that chimpanzees react with fever upon injections with natural and recombinant IFNs, but no side effects were observed either with natural human IFN-α preparations or with the recombinant $\alpha 2$ preparation when these were given to rhesus monkeys (Schellekens, 1982). This certainly limits the availability of experimental

animals for studies on various aspects of IFN administration with human implications. Dawson *et al.* (1983b) made studies on recombinant IFN-γ in chimpanzees. The purity of the material was more than 95%. Dose-dependent rises in temperature were registered, and there were also dose-dependent effects on white cells and platelet counts and an increase in the level of aspartate aminotransferase. However, all values were within the normal range.

For clinical work, it is important to remember that Gresser's group was able to show that glomerulonephritis was inducible in newborn Swiss mice with mouse IFN preparations and that there were glomerular basement membrane changes that were followed by deposition of immunoglobulins in these organs (see Morel-Maroger *et al.*, 1978). IFN can give rise to tubuloreticular structures lying in the cisternae of the endoplasmic reticulum, resembling the structures found in endociliar cells of patients with systemic lupus erythematosis (SLE) (for a summary, see Moss *et al.*, 1983). The role played by these structures is unknown, but the evidence that they are really due to IFNs seems substantial. Gresser *et al.* (1975) also showed that if newborn mice are injected with large doses of virus-induced IFN, they can die with extensive liver damage. This has to be taken into consideration when treatment of children is undertaken with IFNs. Heremans *et al.* (1978) found that IFN could accelerate autoimmune hemolytic anemia in mice. Furthermore, in 1981, Gresser *et al.* reported conclusively that pure mouse IFN could inhibit growth, induce liver and kidney lesions, and kill suckling mice. They concluded that the IFN itself in the IFN preparations was responsible for all of these effects. This is important for all future *in vivo* work in humans. IFNs are potent substances requiring careful handling clinically.

III. IFN Titrations and Pharmacokinetics

IFN concentrations *in vitro* and *in vivo* are usually determined by biological antiviral assays employing an international IFN preparation as a comparative standard. Immunoassay systems for detecting IFNs clinically are being developed, but it has to be emphasized that such systems contain many pitfalls (see Meager, 1984). Different assay systems for *in vivo* treatments have been developed. Hahn and Levin (1980) have concentrated on this problem in particular.

Using the rhesus monkey model, it was shown by Dawson *et al.* (1983a) that human recombinant IFN-γ could be detected in the serum of the animals after i.m. injection. These findings suggest that IFN-γ does not have to be injected i.v. in order to achieve detectable

serum titers in humans either. This is an important point, provided equal efficacy can be demonstrated by the i.m. or s.c. route, since it is easier to treat patients on an ambulatory basis with such routes of administration. Hawkins *et al.* presented their results on Phase I evaluation of recombinant IFN-αA and -αD in 1983 (1983a). Toxicity and side effects were as expected from other studies. Important factors limiting the dose employed in studies using the recombinant IFNs were fatigue and anorexia. It was suggested in these studies that one should begin to use mass units (mg) for expression of IFN amounts. It is obvious that such a concept has limitations when different preparations are used, and it does not really tell how many active IFN molecules are in the preparations. Therefore, it is suggested that if mass units are used, one should also mention the corresponding number of antiviral international units as well as the purity.

An excellent review on the pharmacokinetic studies made up to that time with IFN was published in 1981 by Bocci (1981a). A statement for future therapy made at that time was that it was impossible to know whether one should aim at short- or long-lasting IFNs when therapeutic preparations are made available for trials against malignancies. The catabolism of IFNs has more recently been reviewed by the same author (Bocci, 1982). Billiau (1983) has also reviewed the pharmacokinetics after injecting human IFNs into animals and humans. It is clear that IFN given by infusion or by i.m. injections will give rise to the most steady serum level. If large quantities are required at any time, one will have to inject IFN i.a. or i.v. Which of these alternatives is the best choice in any state of a disease is at the moment unknown. For additional information on IFN kinetics, see Section V below.

Most of the original work on IFN-α pharmacokinetics in humans was done with natural IFN-α produced in Finland (cf. Stewart, 1979a; Scott 1982). Emödi's group in Switzerland also did early pharmacological work on patients receiving exogenous human leukocyte IFN-α therapy (Emödi *et al.*, 1975a). These preparations were mostly used for clinical antiviral work and not for treating tumors (Emödi and Rufli, 1977; Emödi *et al.*, 1975b, 1976). It seems that natural human leukocyte IFN-α is mainly catabolized in the kidneys (Bino *et al.*, 1982). The pharmacokinetic results after i.m. injections of human lymphoblastoid IFN did not seem to show any differences from what was noticed after natural human leukocyte IFN administered in a similar manner (Priestman *et al.*, 1982).

For clinical IFN work, it is important to know that the liver is a catabolic site, especially for glycosylated IFNs (Bocci *et al.*, 1982).

By site-specific mutagenesis of IFN-β, it has been possible to pro-

duce an IFN-β protein by a mutant with properties that have been modified for clinical trials. This product has been named IFN-β serine 17, while natural IFN-β has cysteine at position 17. This product has been employed in Phase I clinical trials (Mark *et al.*, 1983). The IFN has been given both i.m. and i.v., and further Phase I trials at several institutions are planned. At the time of writing, no details on pharmacokinetics are available.

Phase I studies on partially pure human natural IFN-γ with a specific activity of at least 10^6 IU/mg of protein was presented by Gutterman *et al.* (1982c). This semipurified natural human IFN-γ was given to various patients with metastatic cancer. The first nine patients received identical doses by the i.m. route in doses ranging from 1.5×10^5 to 9.6×10^6 IU. The interval between doses was 72–96 hours. A second group of nine patients received identical doses but by the i.v. route as a push infusion over 5 minutes. In the i.m. study, IFN-γ could only be detected in the serum on two occasions, despite the fact that several blood samples were drawn for serum concentration determinations. After i.v. administration, there was a clear dose response in the serum, and the maximum titer could be seen at 2 minutes, with a half-life range of 10–15 minutes. Side effects included symptoms similar to the ones seen by giving natural IFN-α to patients. It was difficult to establish any relationship between IFN dose and myelosuppression.

In 1982, Weck *et al.* presented comparative work using cloned IFN-α, -β, and -γ in comparative experiments. Their general conclusions were that IFN-β and IFN-γ were relatively more species specific than IFN-α1 and IFN-α2. Furthermore, it was concluded that antiviral titers of these IFNs differed depending on the cell line used for testing. After injecting IFNs i.v., it was found that there was a rapid clearance from the circulation at about the same rate by using all preparations. In contrast, when i.m. injections were given, IFN-β or IFN-γ were present at very low concentrations in the blood, whereas injection of α2 gave rise to significant serum levels. The major types of the human IFNs tested also had different effects on mitogenic responses, mixed lymphocyte reactions, and responses to sheep red blood cells. It was not possible to correlate effects on any of these parameters to antitumor responses. Cantell *et al.* (1984) injected rabbits i.m. with human IFN-α and IFN-γ. Blood levels and kinetics were similar whether the IFNs were given alone or in combination at various doses.

IFN has difficulties in penetrating the blood–brain barrier (Jordan *et al.*, 1974; Habif *et al.*, 1975). IFN has therefore already been administered intrathecally (see Ruutiainen *et al.*, 1983). A rather large con-

centration can be accomplished in the bone marrow (Orlowa *et al.*, 1980).

Bocci (1983) has a long time suggested that IFNs probably should be administered intralymphatically, since in natural situations IFN levels are higher in the lymph than in the plasma. After i.m. injections, most of the IFN is absorbed by the blood capillaries. It has also been shown that IFNs undergo renal filtration and tubular uptake and degradation. A system for delivering IFN by the lymph has been reported (see Bocci, 1983).

In the future, it will probably be increasingly important to study IFN receptors *in vivo*. Maxwell *et al.* developed a sensitive assay for IFN receptors by the use of IFN conjugated to β-galactosidase, and this is at present being investigated at the clinical level (Maxwell *et al.*, 1983). New techniques are clearly warranted in this area.

IV. Anti-IFN Antibodies

We tested 20 patients with osteosarcoma treated by exogenous human natural leukocyte IFN-α for up to 18 months. Eleven patients were free from tumor growth during the treatment, while nine developed metastases. Blood samples were taken regularly from these patients to see if neutralizing antibodies could be detected against natural IFN-α. All patients formed antibodies toward contaminants in the IFN preparations, but there were no patients developing antibodies to the IFN (Ingimarsson *et al.*, 1981). Today, this experiment is being repeated with monoclonal antibodies, since we now know that the class of IFN-α contains many different IFNs, and, hence, we might not have detected antibodies reacting with certain subtypes only.

The first observation that a human being injected with IFN developed antibodies to the injected material was reported in 1981 when Vallbracht *et al.* treated a boy with nasopharyngeal carcinoma with natural IFN-β and during the course of IFN therapy found that IFN-neutralizing activity appeared in the serum of the patient. The activity was shown to be due to the presence of IgG antibodies directed against the IFN-β. Since that time, several other patients have been reported to have antibodies to various types of IFN preparations. The first time it was demonstrated that a person who had not been injected with any human IFN had neutralizing antibodies to these substances was in 1981, when Mogensen *et al.* reported on a herpes zoster patient who was injected with IFN-α. Serum samples were taken before the first injection on two occasions, and when they were tested it was

found that they contained antibodies able to neutralize IFN-α activity. The authors raised several interesting questions in their short article, but the most important question is probably if the antibody contributed in any way to the dissemination of the zoster infection.

In 1983, Trown *et al.* reported that IgG antibodies to natural human leukocyte IFN had been detected in the serum of three patients with cancer. These antibodies were found in two of these patients before the treatment with IFN was commenced. In the third patient, detectable antibodies developed during the course of treatment. Six recombinant human leukocyte IFN subtypes and one recombinant hybrid human leukocyte IFN was also tested and it was then found that the neutralizing titers were different against different subtypes of leukocyte IFN. In 200 normal donors, no antibodies were found, but by radioimmunoassay, antibodies to IFN-αA were found in 3 of 50 samples of serum from cord blood. One of these 3 sera also contained neutralizing antibodies. It is interesting that human amniotic fluid normally contains IFN-α (Lebon *et al.*, 1982). Trown *et al.* (1983) also found IFN-α antibodies in a patient with SLE.

V. Side Effects and Toxicity

All of the IFNs used so far have been shown to have toxic effects and in various clinical trials to cause so-called "side effects" (Billiau, 1983). The most common side effect is fever (Table II), but it has been learned more and more through work, especially with recombinant IFN, that other side effects seem to be more dose limiting and more serious for the patients. The pharmacokinetics and toxicity seen after administering different IFN preparations have been subjects in an excellent review by Scott (1982). Side effects of long-term treatment with human leukocyte IFN have been the subject of a thesis (Ingimarsson, 1980). For a recent review on adverse reactions registered after IFN-α administration, see Miller *et al.* (1984).

We started to give concentrated human leukocyte IFN-α to 11 patients with malignant tumors in 1970, and a report was written in 1973 (Strander *et al.*, 1973). The preparation was at that time given at a dose of $1–3 \times 10^6$ IU i.m. three times weekly. No serious toxic effects were seen. No anti-IFN antibodies could be revealed in these studies. It was found that long-term IFN administration at a high dose can be achieved in humans (Strander *et al.*, 1973). In the early IFN trials, an impure preparation was used for injecting the patients. Later, a semipurified preparation containing between $1–5 \times 10^6$ IU/mg of protein was used in Sweden. When patients with juvenile laryngeal papillo-

TABLE II
SIDE EFFECTS REPORTED IN PATIENTS IN CONNECTION WITH IFN ADMINISTRATION

Clinical	Laboratory
Common	Common
Fever	Reversible lymphopenia
Headache	
Malaise	
Myalgia	
Chills	
Anorexia	
Fatigue	
Less common	Less common
Cardiac toxicity	Thrombocytopenia
Rigors	Anemia
Nausea	Increase in hepatocellular enzymes
Vomiting	Granulocytopenia
Local inflammation	EEG manifestations
Urticaria	Anti-IFN antibody formation
Arthralgia	Hypocalcemia
Debilitation	Hyperkalemia
Stupor	Serum creatinine elevation
Peripheral neuropathy	Urea nitrogen increase
Diarrhea	
Labial herpes simplex recurrence	

matosis or osteosarcoma were injected with these preparations, it was found that three side effects—fever, shivering, and coryza—were most common after the semipurified preparation was used at a dose of 3×10^6 IU per i.m. injection. Some of the initially reported symptoms disappeared when the IFN was semipurified (Ingimarsson *et al.*, 1979a). A decrease in the concentration of lymphocytes, monocytes, and granulocytes in the blood was seen in most patients tested 24 hours after an i.m. injection of semipurified natural human leukocyte IFN-α (Einhorn *et al.*, 1980b). In the earlier studies with natural human leukocyte IFN-α, it could be seen that hemoglobin concentrations rose in patients after withdrawal of IFN-α treatment (3×10^6 IU i.m. three times weekly) and the mean sedimentation rate fell significantly at the same time (Ingimarrson *et al.*, 1980c).

In the anti-tumor trials, toxicity has been reported for higher IFN doses (see Smedley and Wheeler, 1983). When using, for example, IFN-α at a dose of 3×10^6 IU by i.m. injections daily or three times weekly, the treatments can clearly be made ambulatory. However, when the doses are increased, there are several side effects and toxic

symptoms appear which cause dose limitations. The most common of these are pyrexia, headache, myalgia, fatigue, anorexia, nausea, paresthesia, effects on blood cells, and effects on the liver. The central nervous system (CNS) toxicity reported also has to be taken into consideration. Horning *et al.* (1983) treated 17 patients with disseminated cancer with human natural leukocyte IFN-α in doses of $3–50 \times 10^6$ IU daily for 30 days. It was found that doses above 18×10^6 IU were intolerable by giving this preparation i.m. because of fatigue and weight loss. There were some minimal responses in 3 of the 17 patients but no really clinically significant responses. Two patients who were treated with the highest doses also received a human leukocyte IFN-α preparation obtained from a second source. This preparation gave rise to less toxicity. Based on the toxicology studies and the serum IFN levels, it was concluded that different human leukocyte IFN-α preparations prepared in a similar manner differed in their biological properties when given *in vivo* to patients. A highly purified preparation of natural human leukocyte IFN-α was given i.m. and was shown to give rise to the same types of side effects found in patients receiving partially purified IFN preparations (Scott *et al.*, 1981). It was concluded from that study that the side effects caused by the leukocyte IFN preparations are due to the IFN molecules themselves. It seems that a similar IFN preparation also retains its anti-tumor activity (Ling, Einhorn, Secher, Einhorn, and Strander, unpublished observations).

Several reports on side effects have been presented in connection with the lymphoblastoid IFN studies. Rohatiner *et al.* (1981a), at the Bartholomeuw Hospital in London, treated 18 patients with various hematologic maligancies with human lymphoblastoid IFN given i.v. Two patients received the IFN by i.v. push (5×10^6 IU/m^2); the other 16 received it by continuous i.v. infusions for 5 days. Clinical toxicity was dose related, and severe pyrexia, general malaise, and anorexia were especially registered. High IFN concentrations could be detected in the blood. It was concluded by Priestman and Lucken (1981) that lymphoblastoid IFN was inherently pyrogenic. Indirect symptomatic treatments also indicated that it might cause the release of endogenous pyrogens. In another study, human lymphoblastoid IFN was given to 17 ambulatory patients with cancer and single injections of $1–5 \times 10^6$ IU i.m. caused fever, chills, malaise, myalgia, and headache (Laszlo *et al.*, 1982). Effects were seen on NK cell activity, but there was no activation of monocyte function. Weck *et al.* (1984) studied 15 patients with refractory CLL who received human lymphoblastoid IFN. The patients were given doses of $1–8 \times 10^6$ IU/m^2 followed

1 week later by 5 daily similar doses. The IFN dose was then escalated to 10×10^6 IU/m^2 daily during the third week. Side effects and IFN titers were determined in all patients, and these data will provide a base for further anti-tumor studies using this group of patients. Forty-three patients with malignancies were treated with high- or low-dose lymphoblastoid IFN therapy (Silver *et al.*, 1982, 1983a). The patients were randomly assigned to either a low-dose (2×10^6 IU of the IFN per m^2 i.m.) or a high-dose strategy (5×10^6 IU/m^2 i.v. by continuous infusion over 24 hours, then escalating by 5×10^6 IU/day as tolerated over 10 days, repeated every 28 days). Toxic symptoms consisted mainly of fatigue, but the most important dose-limiting toxicity was granulocytopenia. In 31 evaluable patients, 9 (29%) showed some kind of tumor response. The clinical experience using the Wellcome human lymphoblastoid IFN up to March 1983 was reviewed by Toy (1983c). An extensive Phase II clinical investigational program has been developed.

Rohatiner *et al.* reported a Phase I study with human lymphoblastoid IFN administered by continuous i.v. infusion to patients with acute leukemia and other types of tumors (1981c). Bolus injections were given to patients at a dose of 5×10^6 IU/m^2 and later to 37 additional patients by continuous i.v. infusion. The doses ranged from 5 to 200×10^6 IU/m^2 per day, and the treatment persisted for 5, 7, or 10 days. Common side effects due to the IFN therapy were registered. Myelosuppression was seen in all patients, and transient rises in alkaline phosphatase and transaminases in serum were observed. There was also dose-limiting CNS toxicity, hyperkalemia, and hypercalcemia at the highest dose. It was considered that the maximum safely tolerated dose was 100×10^6 IU/m^2 administered for 5 days. At that dose, considerable toxicity was also encountered. In six patients, a fall in the number of circulating leukemic blasts was noticed, and in one patient a decrease in blast bone marrow infiltration was evident. That patient went into partial remission. It was concluded that to achieve good serum levels one should give human lymphoblastoid IFN by continuous i.v. infusion, and by doing this one could actually maintain a high serum concentration for long periods. Rohatiner *et al.* (1982a) reported on the CNS toxicity of IFN therapy at the Third International Congress for Interferon Research in Miami. Eight of 11 patients receiving human lymphoblastoid IFN or recombinant IFN-α2 by continuous i.v. infusion at a dose of 100×10^6 IU/m^2 per day for 7 days showed evidence of CNS toxicity. Reversible electroencephalogram (EEG) abnormalities were observed in all patients injected. The abnormalities were greatest between Days 6 and 11, and a return to

normal occurred by 2–3 weeks. Lambda waves showed a parallel increase in number, height, and duration. The toxicity was not due to electrolytic disturbances. Since the side effects seen with the lymphoblastoid IFN have generally been the same as with pure natural leukocyte IFN preparations given at equivalent doses (Priestman, 1980), it would be interesting to know whether the EEG changes also occur with low-dose administration of human natural leukocyte IFN-α. The gross effects have been confirmed by using leukocyte IFN-α (Färkkilä *et al.*, 1984).

The results obtained at the National Cancer Institute, using recombinant and lymphoblastoid IFN-α in patients with disseminated cancer, were presented as Phase I trials in 1982 (Oldham *et al.*). Side effects were rather similar to the ones seen with natural IFN-α. A large series of Phase I trials with recombinant IFN-α2 was started in the United States in October 1981 and completed in July 1982. More than 150 patients were treated with doses up to 200×10^6 IU. The IFN was given i.v., i.m., or s.c. (Rudnich, 1982). The same side effects were reported as with natural and lymphoblastoid IFNs. The effects were dose dependent. Severe fatigue and confusion were the most disturbing toxicity signs. Edelstein *et al.* (1983a) treated 10 cancer patients who were part of a Phase I study with recombinant IFN-α2 s.c. at a dose of 10×10^6 IU weekly. This schedule was acceptable for 9 of the 10 patients. There were some responses, but details were not given in the short abstract of the AACR meeting. A point emphasized by the authors was that 10×10^6 IU twice weekly seems to be an acceptable schedule using this type of IFN s.c. Hofmann *et al.* in 1983 reported on six patients with various disseminated malignant tumors, who received human IFN-α2 in a Phase I study. The IFN was given in escalating doses up to 200×10^6 IU as 30 minute i.v. infusions every other day. Side effects were dose dependent and severe but could be controlled by paracetamol treatment. Hepatotoxicity, thrombocytopenia, and marked hyperglycemia developed in a few patients. All of the side effects subsided on treatment cessation. It was concluded that IFN-α2 can be given in a high dose, but if more than 30×10^6 IU is given rapidly, the side effects will become uncontrollable. Scott *et al.* (1983) treated six healthy volunteers with single i.m. injections of an ascending dose schedule of human IFN-α2 prepared from *E. coli*. At a dose of 3×10^6 IU, all of the volunteers had febrile reactions. There were rises in serum 11-hydroxycorticosteroids, and there were falls in plasma zinc levels at the highest dose. Indomethacin did not alter these changes.

In studies of recombinant IFN-αA and -αD, there was a positive

correlation between the antiviral activity of the IFN preparation and the acute clinical toxicity (Borden *et al.*, 1982e). It also became evident from these studies that the species-specific antiviral activity of an IFN preparation does not predict other biological properties studied after administering the preparation *in vivo*. Furthermore, Hawkins *et al.* (1984b) found that IFN-αD was better tolerated than IFN-αA. When it came to studies on factors that could be of some importance for the anti-tumor activity, there were no differences, however. Neurological side effects after giving recombinant human IFN have also been reported (Smedley *et al.*, 1983). Madajewicz *et al.* (1982) treated nine patients with i.m. doses of up to 100×10^6 IU of recombinant IFN-α2. the maximum dose that could be tolerated by the injected patients varied between 10 and 100×10^6 IU. CNS toxicity was a problem in these studies (i.e., confusion, lethargy, and restlessness). There were some signs of an anti-tumor effect in three of the patients. Comparisons of side effects obtained by giving effective anti-tumor therapy to various patients by employing different IFN preparations should be undertaken in the future.

Natural human IFN-β given i.m. to patients at daily doses of up to 20×10^6 IU caused reactions rather similar to the ones seen after injection of natural IFN-α, including febrile reactions and lymphopenia. Interestingly, after intradermal challenge, some patients developed an allergic state of the reaginic type, but there were no allergic symptoms (Billiau *et al.*, 1979). On a daily schedule, it seems that $\sim 1-2 \times 10^7$ IU/day would be the maximum that patients can tolerate when various IFNs are given by the i.m. route, or in the case of IFN-β by the i.v. route. The exception is recombinant IFN-αD (for a discussion, see Borden, 1983b).

Matsuki *et al.* (1983) concluded, after studies in which they used natural human IFN-β on 10 patients with malignant bone and soft tissue tumors, that i.v. drip infusion with IFN-β with premedication of non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin may be the best method for application of human IFN-β. Skin temperatures were increased in patients receiving the IFN-β. It is unclear whether the fever itself might exert some effect on the tumor disease under such circumstances. Hawkins *et al.* (1984c) administered naturally produced IFN-β from human foreskin fibroblasts by i.m. and i.v. routes to 18 patients with advanced cancer. After a single i.m. injection, there developed fever, enhancement of NK cell activity, and depression of the white blood cell count in the absence of detectable antiviral activity in the serum. When the preparation was given i.v., the dose-limiting toxicities at 10^7 IU were fever, rigor, and

fatigue. It was found that administration of 10^7 units of IFN-β divided equally between a bolus injection of 10 minutes and a 3-hour infusion was well tolerated and resulted in a high initial peak and a lower level of IFN subsequently. This schedule was the one the authors recommended for further Phase II studies. It is emphasized that the level of IFN present in the serum has to be interpreted with caution in clinical studies employing the IFN-β.

At the American Society of Clinical Oncology (ASCO) meeting in 1984, Borden *et al.* (1984a) presented data on 20 patients who were evaluable for toxicity after receiving i.v. injections of up to 60×10^6 IU of substituted recombinant IFN-β. Up to this dose, the IFN preparation was given i.m., but i.v. it was given even up to doses of 400×10^6 IU. This is the IFN-β which has a serine substitution for a cysteine at the amino acid position 17, as mentioned previously. Thirteen patients subsequently received daily i.v. injections of the maximum dose. Dose-limiting toxicity occurred in only two patients who received 400×10^6 IU i.v. An anti-tumor effect was seen in two patients with non-Hodgkin's lymphoma. Using this IFN, it will be important in the future to see if there is development of anti-IFN.

Heyman *et al.* (1983) conducted a Phase I trial of natural IFN-γ on patients with various disseminated malignancies. These were late-stage patients who were refractory to other types of treatment. The patients experienced dose-dependent fever, chills, fatigue, and anorexia upon i.v. injections. Low levels of IFN-γ could be detected in the serum. Also, immunomodulatory effects were seen at the doses employed. Doses up to 20×10^6 IU/m^2 were given to the patients in this preliminary report. In another study, partially purified human natural IFN-γ was given to patients with metastatic cancer (Gutterman *et al.*, 1984). Nine patients were injected by the i.m. route. Doses ranged from 1.5×10^5 to 9.6×10^6 IU. There was no evidence of IFN-α or IFN-β activity in the preparations. The material was free of interleukin 1 and interleukin 2 as well as lymphotoxin. Some endotoxin was present. Minimal side effects were registered, and no antiviral activity could be detected in the patients' sera. Fifteen patients were then injected by i.v. bolus infusion. This time, the doses ranged from 1.5×10^5 to 54×10^6 IU. Serum half-life was found to be dose-dependent, with increasing time by increasing dose.

Eight patients were then treated with 6-hour infusions, during which 3×10^6 IU was given by i.v. bolus followed by 4×10^6 IU hourly for 6 hours. Serum levels of IFN-γ ranged from 40 to 60 IU over a 6-hour period. Granulocytopenia could be demonstrated at 24 hours, and this was sustained during the infusion period, which lasted for 10 days. There was also a marked increase in serum β_2-microglobulin.

These studies emphasize that in order to achieve a consistent serum antiviral activity, the preparation has to be given by continuous i.v. infusion. Whether this is important for anti-tumor activity or not has not been studied so far. Harvey *et al.* (1983) injected natural IFN-α to 12 patients with various malignancies and reported toxicity already by injecting moderate doses, concluding that constitutional symptoms were dose limiting. At the ASCO meeting in 1984, van der Burg *et al.* presented data on patients who had received recombinant IFN-γ with a specific activity of more than 3×10^7 IU/mg of protein, which had been injected for 4 consecutive weeks by i.v. bolus injections to groups of three patients with doses of up to $81 \times 10^6/m^2$. Fever constituted the predominant side effect, and the temperature reached 40°C in some patients. Fatigue and somnolence were also registered. Anti-IFN antibodies could not be detected, but IFN was found in the serum after giving doses higher than 9×10^6 IU/m^2, which gave measurable serum levels up to 12 hours (van der Burg, Edelstein, Clarke, Rudnick, Dawson, and Gerlis, Poster C248, ASCO meeting, Toronto, 1984).

Since some CNS toxicity had been seen in connection with various IFN trials, and since such side effects were the major dose-limiting factors when high doses of lymphoblastoid IFN were given by continuous i.v. infusion to patients, Rohatiner *et al.* (1983b) decided to do a study on the CNS side effects. Eleven patients were investigated, all of whom had leukemias or lymphomas. Seven of the patients received lymphoblastoid IFN, and four patients were treated with recombinant IFN-α2. They were all treated by i.v. infusion for 7 days with a daily inoculum of 100×10^6 IU/m^2. One patient received a higher dose for 5 days. All patients had flulike symptoms, and seven of the patients showed drowsiness. When EEGs were performed, a severe, reversible abnormality was demonstrated in all patients. This was also true for patients not showing signs of CNS toxicity. Transient hepatic dysfunction was observed in all patients. EEG changes were suggestive of encephalopathy, but the degree of abnormality did not reflect the clinical state and did not correlate with the concentration of IFN found in the serum of various patients. Similar findings have been made in patients receiving chemotherapeutic agents (Schäffler *et al.*, 1982). The changes were reversible, and the clinical implications are not presently known.

Mattson and Holsti (1983) discussed their results with treatment of non-small-cell carcinomas and small-cell carcinomas of the lung with natural IFN-α. Especially interesting in this context are their patients receiving natural IFN-α for small-cell carcinomas in cases in which

irradiation had been given to the primary tumor. In that study, extremely large doses had been given (100–200 × 10^6 IU of natural leukocyte IFN-α per 24 hours) in order to give rise to measurable amounts of IFN in the cerebrospinal fluid. Their observations are discussed in Chapter 10, Section X.

One drawback in the English studies has been the findings that even with low-dose IFN treatment there were gross changes in the patients' EEGs, even though, at least initially, neurological symptoms were absent (Honigsberger *et al.*, 1983). The underlying molecular mechanism for the CNS toxicity in IFN-treated patients has not yet been determined. These side effects are important, and cognitive changes have been noted in several Phase I and Phase II clinical trials of cancer patients receiving IFN (see, for example, Maltson *et al.*, 1984a). Such changes include concentration difficulties, disorientation, anxiety, agitation, somnolence, and objectively, EEG changes (see above). Mayer *et al.* (1984) have now started to define the cognitive changes noted on IFN therapy given at the National Cancer Institute. It will be interesting to see how these changes follow the different doses of IFN employed and also if there are different reactions caused by different IFNs.

The cause of fever developing in IFN-treated patients is unknown. Ackerman *et al.* (1984) found no evidence for *in vitro* stimulation of any endogenous pyrogen by either recombinant IFN-α or IFN-β. It has been proposed that early reactions to IFN-α are probably mediated by histamine release and late reactions by prostaglandin synthesis (Scott *et al.*, 1980). Myalgia has been reported by many IFN-treated patients. The release of prostaglandin E_2 induced by leukocytic pyrogen can cause the type of myalgia that accompanies fever (Baracos *et al.*, 1983).

Clinical IFN toxicity might of course be potentiated when IFN is used in combination with other drugs and especially chemotherapeutic agents. Priestman reviewed in 1982 the clinical studies going on with IFN in the United Kingdom (Priestman, 1982). An interesting study that he discussed was a combined study in which the lymphoblastoid IFN was given with a chemotherapeutic regimen consisting of cyclophosphamide, vincristine, 5-fluorouracil, and hydrocortisone. Breast cancer patients were randomized to receive human lymphoblastoid IFN i.m. 3 × 10^6 IU daily, from Days 1 to 28 and therafter 3 × 10^6 IU three times each week. When data were compiled in 1983, it was seen that there was severe myelosuppression with life-threatening infections in 2 of the 14 patients receiving the combinations. There was also excessive clinical toxicity, so this study was

terminated (T. J. Priestman, personal communication). Sangster *et al.* (1983) reported specifically on a 43-year-old man with malignant testicular teratoma who had been treated with heavy chemotherapy. When the patient then received lymphoblastoid IFN, he developed cutaneous vasculitis and ischemic skin changes. Again, caution has to be advocated when IFN is given to patients who receive extensive chemotherapeutic treatments. Especially, one should probably be cautious with the combination of doxorubicin and IFN, since one has to take cardiac toxicity into consideration (see Bristow *et al.*, 1982). This is an area that requires further investigation.

VI. IFN and Disease

Lymphomas constitute a tumor type that can appear at a rather high rate in immunodepressed individuals (see Allison, 1970). The main reason for the high frequency seen in this group of patients is, however, unknown. An interesting virus in this context is EBV, which can produce various lymphoproliferative diseases in a variety of immunodeficient patients (see Purtilo *et al.*, 1981), and it can be stated that people with the greatest possibility of developing EBV-associated tumors are the ones with genetically determined immunodeficiencies (see Purtilo, 1981). It will be extremely interesting to see if IFNs play a role in these diseases and whether the IFN system can be affected in such a way that oncogenesis can be restricted in such patients. The IFN system is probably extremely important at various stages in the development of diseases by a lymphotropic pathogen like the EBV (see Sugden, 1982).

Another reason for using IFN in patients with induced immunodeficiencies, such as renal transplant patients, is that there is substantial evidence that EBV can cause lymphoproliferative disorders after renal transplantation (Hanto *et al.*, 1981). This probably occurs since EBV-transformed B lymphocytes escape normal control mechanisms in the impaired host.

It would be logical to use IFN therapy in cancer patients also in order to prevent some of their infections. For a review on the immune compromised host, see Hughes *et al.* (1983), and for a discussion of the role played by IFN in viral infections, see Sonnenfeld and Merigan (1979b). IFNs could also be advocated together with irradiation treatments due to the well-known fact that immune dysfunction can be induced by such treatments (see Doria *et al.*, 1980). In this context, it is of interest that Tálas *et al.* (1979) found that endogenous IFN production can have a radioprotective effect in animals. It should also be

mentioned that, by employing a screening system, Cesario and Slater (1980) could show that some therapeutic concentrations of antineoplastic agents could affect, and sometimes to a large extent diminish, the antiviral effects of human IFN. These studies were done with both natural IFN-β and IFN-α. This has therapeutic implications and could be one of the reasons why viral infections tend to be more serious for patients having malignant diseases and who are in addition being treated with antineoplastic agents. Sugiyama and co-workers made a series of investigations on the cellular immune response of patients with oral cavity cancer before and after treatment with irradiation and 5-fluorouracil (reviewed by Sugiyama and Nakai, 1980). It was found, as expected, that irradiation and 5-fluorouracil were immunosuppressive. The IFN-producing ability was also lowered in these patients, and the IFN production by their leukocytes stimulated by PHA and *Corynebacterium parvum* was significantly decreased. It will be interesting in the future to see what role the IFN system really plays in patients in whom the immune response is reduced.

The production of various IFNs in individual patients probably varies extensively (see Valle *et al.*, 1975). This could mean that single patients, irrespective of their disease, might require different types of IFNs as well as different doses to obtain optimal effects on any particular disease being treated. Treuner *et al.* (1983b) showed in healthy adults and children that there were no great differences in their IFN production of IFN protection. Among 41 healthy individuals and 63 patients with malignant disease, no defect was found in IFN production. These investigators found that spontaneous IFN production and the level of plasma IFN was higher in patients with malignant disease than in healthy people. They could also see that children undergoing chemotherapy has lower production of both IFN-α and IFN-γ and that children in remission after chemotherapy showed the same pattern as control patients (healthy individuals).

It has been reported that some children have deficient production of leukocyte IFN-α, although this is difficult to prove, depending on the natural contact of the IFN system with various viral infections (Isaacs *et al.*, 1981). In a recent study, however, children with recurrent respiratory infections did not seem to have deficient production of either IFN-α or IFN-γ, and Chadda *et al.* (1984) suggested that routine screening for IFN production in such patients is not likely to be rewarding. The PBMCs in renal transplant patients seem to have diminished IFN-γ-producing capacity (Weimar *et al.*, 1983a).

De la Peña *et al.* (1975) reported that the immunodepression seen in cancer patients is paralleled by their ability to produce IFN. They also

found that levamisol could induce IFN formation. It is known that prostaglandin E_2 is present in very high concentrations in cancer tissue and at the same time that the tumors in experimental systems can sometimes be affected by prostaglandin synthetase inhibitors (Goodwin *et al.*, 1980). In humans, overproduction of this type of prostaglandin can be responsible, at least in part, for depressed immunological reactions in patients with some neoplastic diseases.

It has been suggested that the production of IFN may contribute to immunological aberrations in patients with immune diseases (Hooks *et al.*, 1979). Also, the concept of presence of antibodies to IFN in humans is an important area where much future work is desirable (for a review, see Panem and Vilćek, 1983). Neutralizing antibodies have been found in normal sera, in cord blood, and in various diseases. Especially, some patients with autoimmune diseases have antibodies. Results in 1983 suggested that acid-labile IFN-α seen in patients with autoimmune diseases might represent a novel type of IFN-α with special properties (see Preble and Friedman, 1983a). Others have found acid-stable IFN-α in SLE (Ytterberg and Schnitzer, 1982). IFN has been proposed to be the cause of lymphopenia in SLE (A. Schattner *et al.*, 1982, personal communication). The concepts of acid-labile IFN-α have been discussed extensively (cf., for example, Green and Spruano, 1984; Preble *et al.*, 1984). It is intriguing that patients with active SLE have defects in their Fc receptor-specific clearance and that this defect correlates with their disease activity (see Frank *et al.*, 1983). In Table III I have listed various symptoms and laboratory findings in SLE which *could* be explained by the presence of IFN. ER^- cells from 61% of patients with SLE do not show increased cyto-

TABLE III
SYMPTOMS AND LABORATORY FINDINGS IN SLE WHICH MIGHT BE RELATED TO IFN

Symptoms	Laboratory parameters
Focal or diffuse glomerulonephritis	Leukopenia
Fever	Anemia
Malaise	Thrombocytopenia
Muscle pain	
Growth retardation	
Lymphoadenopathy	
Muscle atrophy	
CNS symptoms	

toxicity after IFN-α treatments. Those patients who did not exhibit enhanced cytotoxic responses had higher disease activity than responding patients (Fitzharris *et al.*, 1982). Similarly, ER^- cells from half of the patients treated with IFN-γ failed to show increased cytotoxicity. It is well known that the NK cell-mediated cytotoxicity is abnormal in a number of human disease states, but it has also been found that the cellular response from, for example, SLE patients to exogenous IFN, is impaired (see Sibbitt *et al.*, 1983). These authors suggested that a decreased NK cell activity in SLE patients might be due to impaired release of a soluble cytotoxic factor.

Some other common clinical symptoms could also perhaps be due to maladies of the IFN system, and it has already been advocated that selective defects in IFN-γ secretion by PBMCs can lead to clinical syndromes (Virelizier *et al.*, 1978). It has been shown that IFN levels detected in virus-infected patients correlate to the fever observed during the virus infection itself. This would imply that it is likely that IFN could be the substance responsible for the characteristics of some disease states (Falcoff *et al.*, 1983). Hooks *et al.* reported in 1982 on a patient with a disease consisting of proliferation of T cells with Fc receptors for IgG. It was found that this patient's lymphocytes could spontaneously produce IFN-γ *in vitro*. The peripheral lymphocytes of this patient consisted of 95% of Tg cells that had a morphology of T cell CLL and were normal cytochemically and in their chromosome pattern. The majority of the Tg cells could mediate ADCC.

Recombinant leukocyte IFN-αA given at a dose of 10^8 IU i.m. three times per week to patients with mycosis fungoides gave rise to a clinical syndrome and histopathology in a patient consistent with acute interstitial nephritis and minimal-change nephropathy. The patient was treated up to the time when nephrotic syndrome became apparent. The patient was then given 9×10^6 IU three times weekly, followed by 50×10^6 IU three times weekly. Therapy was discontinued when the proteinurea recurred (Averbuch *et al.*, 1984). Renal injury has also been reported in other patients receiving recombinant leukocyte IFN-αA (Sherwin *et al.*, 1982a). The pattern of injury seen in the mycosis fungoides patients resembles what can be seen after other drug therapies. It has been suggested that such changes could be caused by an altered T cell function.

Neighbour *et al.* (1981) found that leukocytes of multiple sclerosis (MS) patients produced significantly less IFN in response to measles virus stimulation *in vitro* than those of normal individuals. Such an anomaly was found to be general and not specific for the measles virus and it was observed consistently with inducers of both virus-induced

IFN and IFN-γ. Merrill *et al.* (1983b) could show that PBMCs from MS patients produced more prostaglandin E and E_2 *in vitro* than controls, and this correlated with lower levels of NK cell activity and endogenous IFN production. It appears in these systems that at least some of the prostaglandin-producing cells are adherent monocytes/macrophages. In MS, it seems that depression of IFN-induced natural killing by prostaglandin E is greater than in other neurological diseases or in control patients. This is thought to be due to the prostaglandin effect on IFN-induced recycling (Merrill *et al.*, 1983a). NK function can be affected in MS patients in a normal manner (Rice *et al.*, 1983), but the human IFN-γ response of peripheral blood leukocytes to Con A is depressed (Vervliet *et al.*, 1983b). However, it should be emphasized that in the patient-derived cultures, whenever a response was achieved this was not lower than in controls. IFN-α production, after induction by Sendai virus, was not abnormal in cells from MS patients. It would be important to study similar parameters also in patients with amyotrophic lateral sclerosis (ALS).

Abnormalities in the production of and response to IFN have been reviewed (L. B. Epstein *et al.*, 1982). It is clear that production of IFN can be abnormal in various immunodeficiency states, in immunosuppressed patients, and in connection with some congenital infections. The abnormalities and also the characterization of various IFNs produced in such patients will be subject to intensive research in the future and must be considered to constitute very important topics. Also, in the response to IFNs there have been observed abnormalities like those, for example, in cells from patients with trisomy 21 (Down's syndrome). These patients show an increased sensitivity to IFNs. Some cell lines do not exhibit these chromosome 21 dosage effects when tests are made for antiviral and anticellular activities (Zhang *et al.*, 1982). In the future, it will therefore be advisable for investigators trying to treat patients with chromosome 21 trisomy to test the particular patients under study for IFN sensitivity *in vitro*. In general, cellular receptors for human IFN-α have been reported to be more extensively expressed on the peripheral lymphocytes from patients with Down's syndrome (Mogensen *et al.*, 1982). Abb (1983) has found that the production of IFN-α and IFN-γ is reduced in the peripheral blood of patients with acute leukemia, but the IFN response of lymphocytes in disorders with decreased resistance to infections seemed not to be affected in a few cases in which IFN-α was tested (Strander *et al.*, 1970; Einhorn, 1980).

The role of IFN in various autoimmune diseases has been the subject of a lot of work (see above and Hooks and Detrick-Hooks, 1982;

Preble *et al.*, 1982; Panem *et al.*, 1982), but more recently the problem of the acquired immunodeficiency syndrome (AIDS) has received special attention. Eglin *et al.* (1984) found that increased levels of IFN-α are consistently present in patients with the more aggressive form of Kaposi's sarcoma seen in Zambia in young adults. They found no association with raised IFN-α levels in older Zambians who have the more benign form of Kaposi's sarcoma. Elevated levels of the acid-labile form of IFN-α have been found in homosexual men with AIDS (Eyster *et al.*, 1983). These latter authors have suggested that this type of IFN can be used as a marker to identify affected asymptomatic members of high-risk groups. De Stefano *et al.* (1982) looked at the IFN system in homosexual patients. Sera from 91 homosexual men were tested. Of these, 27 patients had Kaposi's sarcoma, and of these, 17 had significant titers of human IFN in their sera (63%). Ten of 35 patients with lymphadenopathy also had significant titers in their serum (29%). Three of 4 patients with other clinical symptoms also had titers. Of 25 healthy subjects, only 2 (8%) had detectable IFN concentrations in their sera. The IFN present had the properties of human IFN-α, and it was also found that the IFN-α detected was inactivated at pH 2 and therefore in this respect appeared to be similar to the IFN that is seen in patients with SLE. The role played by IFN in the development of these various diseases is at present not known. The priority in studies of AIDS is of course to determine the cause of the syndrome (Curran, 1983). It is interesting that it has been suggested that measurement of serum IFN levels in individuals at high risk for AIDS may be of diagnostic value (for references, see Abbott *et al.*, 1984). Data seem to indicate that the measurement of circulating IFN-α in high-risk populations may be indicative of AIDS and to some extent also predictive.

An area now receiving increasing attention is the one dealing with human papillomavirus (HPV)-associated diseases. For a discussion of the types of HPV, see Howley (1982). The association between HPV and various tumor diseases in humans is firmly established (see Gissmann, 1984) after much work was devoted in the 1970s on the possible role played by HPV especially in the development of squamous cell carcinomas (see Zur Hausen, 1978). The papillomaviruses are now being studied extensively, and there are already model systems being developed in tissue culture. Papillomavirus antigens are found abundantly in cervical dysplasia (Kurman *et al.*, 1981), and DNA from HPV 16 can hybridize with DNA prepared from human cervical cancers in 61% (Dürst *et al.*, 1983). HPV 16 DNA prevails in the malignant tumors. Indirect evidence suggests that this virus hypothetically might

need a helper virus in order to cause malignant transformation of benign tumors. HPV-associated, nonirradiated juvenile laryngotracheal papillomatosis can give rise to bronchogenic squamous cell carcinoma (for literature, see Runckel and Kessler, 1980).

It seems that papillomas induced by repeated carcinogen applications arise from many more cells than tumors induced by the carcinogen-promoter regimens (Reddy and Fialkow, 1983). This should be considered in the treatment of these diseases. In the bovine papillomavirus Type I system, tumorigenicity of sarcomas possessing this virus has been found to be compatible with low levels of the expression of the transforming region of the bovine papillomavirus Type I (Jaureguiberry *et al.*, 1983). Maybe such a system could be used as a model for IFN therapy of these diseases. IFNs have been used often on HPV-associated papillomas which consist of the following entities: common warts (verruca vulgaris), plain warts (verrucae planae), genital warts (condylomata acuminata), bladder papillomas, and laryngeal juvenile warts [juvenile laryngeal papillomatosis (JLP)]. The possible role for IFN in the treatment of these diseases has been discussed by Scott (1983a), in whose article there are also discussions concerning spontaneous resolution of these benign tumors. Scott emphasizes that double-blind placebo-controlled or comparative trials employing IFN should be done on large numbers of patients. Such trials have been initiated in the United States, and it will be interesting to follow the results over the next few years.

CHAPTER 7

ANIMAL TUMOR MODELS

The early work of Gresser's group on the anti-tumor effects on IFN preparations in mice was of great importance for the development of anti-tumor IFN therapy in humans (Gresser and Bourali-Maury, 1972; Gresser *et al.*, 1967, 1969). An important experiment was also reported in 1972. R3 strain female mice, with a high spontaneous frequency of mammary carcinomas, had a delay in the development of tumors when they were treated weekly from 6 weeks of age with mouse IFN (Came and Moore, 1972). This lay the groundwork for studies on mammary carcinoma in humans (Borden *et al.*, 1981). Much more has to be done in mouse tumor models, and it is only to be hoped that experimental systems in animals for the testing of tumors treated with IFN will be expanded (see Chirigos, 1981–1982; Bekesi and Robez, 1981–1982). Results suggest that the mouse systems employing IFN-α, -β, and -γ could have relevance to the human situation (Fleischmann *et al.*, 1984b). Most animal IFN models have been developed in murine systems. Mouse IFN preparations have in the past contained mixtures of IFN-α and IFN-β. Now these IFNs can be obtained separately, and, in addition, IFN-γ of the murine type is becoming available.

In the murine system, extremely small amounts of IFNs are sometimes adequate for exerting anti-growth effects on various cells (Buffet *et al.*, 1978). Indirect evidence presented by S. L. Lin *et al.* (1983) suggests that IFN-α can inhibit the expression of the transformation-related phenotype in Rous sarcoma virus-transformed rat cells by selectively reducing the synthesis of the virus-transforming gene product. An extremely interesting model system for IFN treatment of cells containing papillomavirus is the one employing the bovine papillomavirus transformation of mouse cells and to study the inhibition of this transformation by IFN and the reversion of established transformants by IFN exposure (Turek *et al.*, 1982). It will be exciting to follow the development of this model system.

Let us now directly discuss *in vivo* work performed in experimental animals. Early work has been reviewed by Stewart (1979a) and will not be presented here.

Lymphomas and leukemias can be affected by IFN treatment in

animal models. Gresser *et al.* (1976) established that daily administration of potent virus-induced mouse IFN begun after the clinical diagnosis of lymphomas in AKR mice could increase average survival by ~100%, and the therapeutic effects compared favorably with results that had been reported using chemotherapeutic drugs. Lee *et al.* (1983) defined the optimal treatment conditions for L-1210 leukemia in mice by one IFN, namely, the highly purified hybrid human leukocyte IFN-αAD. They concluded that their treatments prior to tumor inoculations were without effect, while treatments from the third day posttumor inoculation were most effective. It was better to give IFN every third day than to give it more frequently. It was also confirmed that IFN-resistant cells in culture could react toward the IFN *in vivo*, and these authors thought indirect actions by IFNs probably play an important role for IFN effects *in vivo* against tumors.

The most well-studied system for IFN resistance is the L-1210 lymphoma subline (Gresser *et al.*, 1974). The IFN-resistant variants of this originally sensitive tumor originated by spontaneous random change. These cells have been invaluable for studies on the mechanism of action of IFN therapy in the treatment of murine tumors. Gresser *et al.* (1972) developed an L-1210 system in which cells were originally sensitive to virus-induced IFN but could be made resistant. When mice bearing L-1210-sensitive cells were treated, there was a somewhat greater protective effect by IFN than in mice inoculated with L-1210-resistant cells and injected with IFN. Resistant cells lack IFN receptors (Aguet, 1980). On the other hand, the IFN also worked on the L-1210-resistant cells in the sense that the resistant tumors could definitely be inhibited by IFN *in vivo*. The interpretation of these results would be that a strong IFN effect is probably mediated by mechanisms when it does not matter whether the tumor cells *in vivo* are IFN sensitive or IFN resistant.

Tomida *et al.* (1983b) described how the survival time of mice implanted with differentiation-inducible mouse myeloid leukemia cells can be affected by treatment with IFN. An interesting animal model for studies of human acute myelocytic leukemia has been developed in rats in which the leukemia model has been used for IFN treatment of minimal residual disease (Hagenbeek *et al.*, 1983). IFN-α treatment of such animals seems to be promising for eradication of minimal residual disease. It is clear that when IFN therapy is interrupted in animals bearing tumors, there is usually a reactivation of the malignant process. One such example is the Rauscher murine leukemia virus-induced erythroleukemia in mice in which continued application of IFNs produced by mouse L cells can cause complete inhibition

of the virus-induced erythroleukemia but whenever the IFN therapy is halted again there is a reactivation of the leukemic process (Hekman *et al.*, 1981).

The Friend leukemia system in mice has been used as a model for IFN therapy. Belardelli *et al.* (1982a) demonstrated that IFN was as effective in mice inoculated with IFN-resistant Friend leukemia cells, and this suggested to the authors that the IFN-induced anti-tumor activity was to a large extent mediated by the host. The authors suggested that IFN induces a host-mediated anti-tumor effect by mechanisms that are not mediated by easily recoverable soluble factors or by known cytotoxic cells and that the mechanism that is important for the host-mediated effect remains unknown (Belardelli *et al.*, 1982b). In the Friend erythroleukemia systems, Belardelli *et al.* (1983) were able to show that administration of highly purified IFN to DBA/2 mice inhibited the growth of both IFN-sensitive and IFN-resistant leukemias implanted subcutaneously. It was more effective to give the IFN at the site of tumor inoculation. Tumor cell necrosis was obvious in the absence of any host cell infiltrates. The IFN inhibited the growth of subcutaneous tumors, induced complete tumor regression in some mice, and was able to inhibit the development of tumor metastases in liver and spleen.

In an important series of experiments Gresser *et al.* (1983) injected three different mouse strains with antibody to mouse IFN-α/β This antibody enhanced the i.p. transplantability of six different murine tumors both as manifested as an increase in the percentage of tumor bearing mice and as a decrease in the survival time. The effects were noticed with antibodies produced in different animals, and the anti-IFN globulin was equally effective in mice inoculated with IFN-sensitive and IFN-resistant tumor cell lines. In experiments employing Friend erythroleukemia cells, it could be seen that the enhancing effect was observed over a wide range of tumor cell inocula, that the effect was related to the antibody dose given, and that it was most pronounced if the antibody was already administered at the time of tumor cell injection. In the Friend erythroleukemia system, it could also be seen that enhancement was observed after s.c. injections of the tumor cells. The suggestion made by these authors is therefore that endogenous IFN production is present and that it plays a role in the inhibition of murine tumor growth in immunocompetent mice. In the case of Friend leukemia cells, it was observed that both IFN-resistant clones and wild-type, IFN-sensitive Friend leukemia cells show specific saturable binding sites for mouse IFNs with similar affinity con-

stants. On the other hand, (2'-5')A synthetase activity is not inducible by the IFN-resistant variants (Affabris *et al.*, 1983).

The importance of the IFN system in tumor development in experimental animals can be exemplified by studies on infections in mice (Inglot *et al.*, 1979). It was shown that in long-term experiments administration of potent anti-IFN IgG to 4- to 5-week-old and also to 1-year-old mice can transform a neoplastic viral disease that is benign and regressing in some mice into a malignant and lethal one. The authors therefore concluded that endogenous IFN plays a role in the balance between host defense and tumor development in the animals.

Mouse IFN-α/β caused inhibition *in vitro* of methylcholanthrene-induced mouse sarcoma cells (Wivel and Pitha, 1982). These cells were less tumorigenic in the animals. IFN caused a reduction in the tumor-specific transplantation antigen but increased the expression of H2 antigens. In this system, functional T cells are probably very important for defense against the mouse sarcomas. MSV-induced tumor cells are sensitive *in vitro* both to lysis by NK or NK-like cells and to the growth inhibitory effect exerted by murine L cell IFN. It was therefore surprising that growth of the tumor *in vivo* could be either inhibited or enhanced by IFN (Murasko *et al.*, 1983). If the IFN was given systemically at the same time as the tumor challenge, there was enhancement, while if it was injected at the site of tumor inoculation every day following tumor challenge, there was an inhibition of tumor formation and growth. The authors also studied the radioresistance of this system and were able to show that the inhibition of the MSV-induced tumor growth by IFN has a radioresistant component. Furthermore, they demonstrated that the enhancement of the induced tumor formation by IFN is dependent on an interaction between radiosensitive populations of cells, one possibility being that these would be lymphoid cells.

In an interesting comparative study, C57BL/6 female mice were inoculated with murine osteosarcoma cells and treated with virus-induced IFN or IFN-γ preparations (Crane *et al.*, 1978). A 7-day course of $3–6 \times 10^4$ IU/day of the virus-induced IFN completely inhibited or delayed the appearance of these tumors in the experimental animals. On the other hand, it seemed that when IFN-γ was used in the same system, 100-fold smaller amounts of IFN were required to inhibit development of the tumors.

This result also leads to clinical interest of IFN-γ therapy of human osteosarcoma. In a murine model in C3H/HeN mice, Satomi and coworkers demonstrated that IFN and poly(I)·poly(C) therapy reduced

pulmonary metastases of Dunn osteosarcomas after the "primary transplanted" tumor had been excised. There was also prolongation of survival. The elimination of tumor cells from the lung correlated in these studies very well with *in vitro* NK cell activity (Satomi, 1983; Satomi *et al.*, 1983). Also, melanoma metastases could be affected. In these studies, the IFN primarily exerted its anti-tumor and antimetastatic effects directly on the tumor cells (Mishima *et al.*, 1983). In mouse systems working with fibrosarcomas, it could be demonstrated that the antiproliferative activity of IFNs is probably only responsible for a small part, if any, of the therapeutic effect (Kataoka *et al.*, 1983). In a model system in C57BL/6 Je mice, Davies and Field (1983) studied the effect of poly(I)·poly(C)-poly-L-lycine, a potent IFN inducer, on the growth and development of osteosarcoma in mice. A significant anti-tumor effect could be achieved by injection of the inducer immediately after tumor implantation, followed by four subsequent treatments. If more osteosarcoma cells were injected or treatment was initiated after development of palpable tumors, the results were not as good. The conclusion drawn from these studies would be that optimal therapy in this particular system results from repeated treatment prior to development of extensive tumor burdens. To summarize, sarcomas in experimental animals can be affected in most systems by systemic IFN application.

In a neuroblastoma system in mice, Tebbi *et al.* (1983) found that when daily injections of 5×10^6 IU/kg of body weight for 5 days or 1.5×10^7 IU/kg of body weight for 10 days were given to neuroblastoma-bearing mice, there was an increase in survival as compared to controls when virus-induced IFN was employed.

Potentiation between IFNs has been reported in animal models after having shown that mixed preparations of mouse virus-induced IFN and IFN-γ cause a greater level of antiviral protection than would be expected on the basis of individual activities. Fleischman *et al.* (1979, 1980) also reported that anti-tumor effects obtained by using virus-induced IFNs in inbred mice were potentiated by the injections of mouse IFN-γ preparations.

Combinations of IFNs with other substances have also been used in experimental models. In an interesting paper, Bourgeade *et al.* (1980) reported that IFN-γ had less antiviral activity than IFN-$\alpha 2/\beta$ in transformed embryo cells in the murine system. In nontransformed cells, however, the IFNs had similar activity. When the phenotype of MSV-transformed cells was reversed by sodium butyrate, sensitivity to the antiviral action of IFN-γ was restored. Several experiments were also performed that indicated that the actions of the various IFNs differed.

It has been advocated many times by Chany that one should compensate for the immunodepression due to IFN in the treatment of tumor diseases. For example, the T cell cytotoxicity increase after IFN treatment is known to be diminished by the IFN action on the target cells. Therefore, one might have to balance all of the components involved in the system in *in vivo* situations (see Chany *et al.*, 1982a). Chany and Cerutti (1982) reported that a combination of IFN and arginine butyrate is effective in treating mice with sarcomas. Optimal results, however, were obtained when a single injection of *Corynebacterium parvum* was added to compensate for the repressive effects on the immune system considered to be due to the IFN injections. The parvum injection was then followed by nine daily alternating shots of arginine butyrate and IFN, which led to an optimal anti-tumor effect. Cimetidine can reduce tumor formation in mice (Gifford *et al.*, 1981), and there are also scattered reports on anti-tumor effects in humans (see Armitage and Sidner, 1979). There is evidence now from experimental systems that the conjunction of IFN and synthetic immunomodulators such as cimetidine can give rise to delayed tumor development in experimental animals (Cerutti and Chany, 1983). In the particular experimental model used by these authors, there was no effect, however, demonstrable on the final survival rate. In an interesting series of experiments, Chany *et al.* have treated AKR mice, developing spontaneous leukemia normally in almost 100%, with *Corynebacterium parvum*, IFN, and arginine butyrate (1983). It was found that when all three substances were combined, the mean survival time could be increased and the final survival rate could also be improved. When *Corynebacterium parvum* or IFN were used separately, they were completely ineffective in this system.

Coordinated immune stimulation, for example, employing *Corynebacterium parvum* and the IFNs (Chany and Cerutti, 1983), will be interesting to see applied to the human system, especially on cases in which the immune system is also known to be affected, as in AIDS. On the other hand, it has been shown that in some experimental systems, such as the B60 malignant melanoma system in mice, the IFN anti-tumor effects might be exerted through direct anti-tumor action (Bart *et al.*, 1980).

In the murine system, it has been shown that IFN treatment of animals in combination with local hyperthermia can increase the anti-tumor effect on solid tumors (Yerushalmi *et al.*, 1982). Likewise, in model experiments performed in a murine system *in vitro*, Dritschilo *et al.* (1982) could mimic a clinical situation in the sense that cells continuously exposed to small doses of IFN in tissue culture were

potentiated by irradiation. The results could be interpreted to mean that the effect was due to an inhibition of the ability of the cells to accumulate sublethal radiation injuries.

It has to be determined how effective the combination of IFNs and chemotherapeutic agents can be made clinically, and hence animal studies in this area should be undertaken on a large scale. Chirigos and Pearson showed in 1973 that systemic leukemia in mice responded better to combination therapy with chemotherapeutic agents and IFN than with single-agent therapy. In this particular system, mice were first given BCNU treatment to reduce the tumor load. IFN was then given to the animals when they had a small tumor burden. Given in this way, the combination could be shown to have a synergistic anti-tumor effect (Chirigos and Pearson, 1973). This area was not much further developed, probably on account of the provocative experiments of Gazdar, who in 1972 showed that several transplantable mouse and rat tumors showed a significantly enhanced growth rate by pretreating animals with various IFN inducers. It has to be remembered, however, that these treatments were made prior to transplantation and that the effects of the treatments used on the immune system were not studied. These original findings have to be taken into consideration, however, in the design of clinical studies. Large-scale studies *in vitro* and animal work *in vivo* on the effects of combining IFNs and cytotoxic drugs are now being performed (see, for example, Namba *et al.*, 1983). Cyclophosphamide is especially being used in combination with IFNs in experimental models (see Kidowaki *et al.*, 1983). Gresser *et al.* (1978) treated female AKR mice that had definite peripheral lymphadenopathy and splenomegaly with cyclophosphamide, cyclophosphamide and IFN, or nothing. The combination of cyclophosphamide and daily IFN treatment caused prolongation of survival in these mice while the controls developed lymphoma.

Combination therapy using IFNs and chemotherapy can also be suggested based on work performed by Youn *et al.* (1982) treating mice with an immunomodulator, poly(A)·poly(U), which is known to induce IFN, and cyclophosphamide. Tumor inhibition was most extensive in the animals receiving the combined approach. Slater *et al.* (1981) showed in the murine L-1210 leukemia system that addition of virus-induced IFN to all methotrexate-containing regimens increased the mean survival time of the mice. This survival increase was sustained through four transfer generations, despite resistance developing to the antimetabolite.

In the mouse neuroblastoma system, it is clear that combined therapy with murine virus-induced IFN and cyclophosphamide is more

effective than the treatment of mouse neuroblastomas with either agent alone (Tozawa *et al.*, 1982). A liposarcoma model in rats exemplified what many investigators have found; namely, that treatment with IFN in animal systems is sometimes ineffective after the tumor has been transplanted into the animals. In the model in question (Marquet *et al.*, 1983b), a rat fibroblast IFN was given to animals that had spontaneous, transplantable liposarcomas. The combination of cyclophosphamide and IFN could not lead to additional retardation of growth as compared to single-drug therapy. Actually, cyclophosphamide used on its own was the best type of treatment. The combination systems probably have to be studied much more extensively in order to achieve optimal benefit.

To determine the anti-cancer activity of IFN combined with chemotherapeutic drugs in an experimental system, Schabel *et al.* (1982) injected *cis*-platinum or mitomycin C to mice bearing P388 cells. Mouse IFN was then injected at a dose of 10^6 IU daily i.p. after the chemotherapeutic drugs had been given. The IFN preparation consisted of a 1:1 mixture of IFN-α and IFN-β. It was concluded from these experiments that the IFN preparation gave rise to cytostatic effects but did not reduce the number of P388 cells. There was an increase in life span if the IFN was used in combination with the chemotherapeutic drugs. The sequence of the experiment suggested that the IFN preparation was holding a drug-sensitive tumor burden static during a time period when normal cells have to recover from dose-limiting toxicity caused by the chemotherapeutic regimen. This is an interesting model and suggests one way to apply IFN therapy at the clinical level.

De Clercq *et al.* (1982) developed an experimental system in BALB/c mice in which IFN-β, IFN-γ, and the IFN inducer poly(I)·poly(C) were evaluated for effects on spontaneously growing mammary carcinomas. When the tumors in the animals had reached a palpable size, the mice received three i.p. injections of IFN for 6 weeks. A reduction in tumor size was achieved by IFN-β, IFN-γ, and poly(I)·poly(C). Cyclophosphamide as a positive control gave rise to a similar reduction. When IFN-β and IFN-γ were combined, there was a greater antitumor effect than with either treatment alone. Except for inhibiting primary tumor growth, the IFNs were also able to reduce the incidence of lung metastases. No complete remissions were obtained in this experimental system. In some interesting experiments performed in animal models on combinations of IFN-α, IFN-β, and IFN-γ on different tumor model systems, also in combination with cyclophosphamide in some experiments, Heremans *et al.* (1983a,b) made some

important conclusions. They found, as had been found earlier (1) that IFNs were able to cause an increase in survival time but rarely caused tumor regression, (2) that high doses of the IFNs were required in the animal model systems, (3) that IFN-γ combined with the other IFNs led to a greater anti-tumor activity than when either IFN type was used alone, and (4) that the combination of IFNs and cyclophosphamide in these systems was superior to either treatment regimen alone. The following tumors were employed in these studies: B16 (malignant melanoma), TA3 (spontaneous mammary adenocarcinoma), and Sp2/0-Ag14 (a myeloma cell line). It has to be remembered, however, that in some systems, even enhancement of tumor growth has been reported by combining IFNs and chemotherapy (see above and, for example, Marquet, 1983a).

The mouse bladder tumor MBT-2 has been used as a model for the study of cytotoxic drug sensitivity of human bladder carcinomas. A potent IFN-inducer poly(I)·poly(C) was used in that model by Borden *et al.* (1984c). In this system, 10^5–10^6 tumor cells were implanted per mouse. It was interesting that the tumor graft reduction was more pronounced in mice inoculated with high numbers of MBT-2 cells. The treatment had to be continued for a long time in order to be effective. Cyclophosphamide had an inhibitory effect on these tumors in the mice and was found to have an additive effect on what could be achieved with the poly(I)·poly(C) treatment, but the toxic effects were likewise increased.

An interesting article on the relevance of animal tumor models to human tumor immunology has been written by Herberman (1983). IFNs can exert extensive effects on the immune system of injected animals. Lindahl *et al.* showed equivocally that murine IFNs could enhance the expression of surface antigens on murine leukemic cells as measured by alloantibody-absorbing capacity (Lindahl *et al.*, 1973). King and Jones (1983) have shown in the murine system that IFN-γ can induce Ia antigen expression and increase H2 antigen expression on murine macrophage tumor cell lines. Experiments by V. E. Miller *et al.* (1983) indicate that the increase of natural resistance to IFN-treated tumors do not involve NK cells or macrophages, and these authors suggested that IFN may enhance host anti-tumor resistance by increasing tumor reactivity to antibodies. Uenishi *et al.* (1983) presented data, employing the nude mouse system, which indicate that NK cell activity is not essential for the anti-tumor effect of IFN mediated by the host. Their data suggest the existence of regional tumor resistance of the host induced by IFN, and they presented data that no

antiproliferative agents induced by the IFN treatment could be found in the serum. The suggestion from their work is that there is an undefined, host-dependent, anti-tumor mechanism directing the outcome of work employing human tumor cells transplanted into the nude mouse—in this case, human nasopharyngeal carcinoma. On the other hand, there is some evidence that IFNs, for example, IFN-αA and -αD, probably can affect survival by immunostimulation (Kohl, 1983).

Paciucci *et al.* (1983) made investigations that suggested that administration of exogenous IFN or interleukin 2 to tumor-bearing hosts, with the aim of strengthening the protective cytotoxic mechanisms against the tumor, might have been self-limiting on account of competing effects on the cytotoxic and target tumor cells *in vivo*. It is interesting that the response of mice to IFN-γ inducers was decreased after the animals had been injected with tumor cells or cell-free tumor ascitic fluid (Matsubara *et al.*, 1980). The *in vivo* induction of the IFN-γ seems to be impaired by factors with molecular weights of less than 10,000. In a model system, Bruley-Rosset and Rappaport (1983) treated young and aged C57BL/6 mice with IFN at a dose of 10^4 IU i.p. No augmentation of cytotoxicity mediated by the NK cells was found. The IFN treatment rather reduced this activity. No evidence was found for involvement of suppressor cells. The capacity of spleen cells to generate cytotoxic T cells after allogeneic stimulation was increased in the IFN-treated mice. These authors concluded that contaminating molecules present in their IFN preparations could have deleterious effects, since mock IFN preparations increased the percentage of tumors in mice. This exemplifies one of the drawbacks of using semipurified preparations.

It has been shown that an acid derivative of vitamin A (transretinoic acid) can enhance local tumor growth in experimental tumor systems in mice and also partially reverse the protection exhibited by IFNs against tumor growth and mortality in the animals (Baron *et al.*, 1981). This again emphasizes the necessity for being cautious in applying combination therapies.

What is the cause of IFN resistance in some animal systems? Aguet *et al.* (1981b) showed that the mechanisms underlying IFN resistance probably belong to several categories. Some cells are resistant because they lack specific binding sites (like the IFN-resistant mouse L-1210 cells), while others are resistant depending on other mechanisms and irrespective of the presence of specific receptor sites. It could even be shown that (2′-5′)A synthetase was induced in resistant mouse embryonal carcinoma cells.

The anti-tumor effects exerted by IFN in the mouse systems have been reviewed by Gresser (1983). There is no really clear-cut answer to the mechanisms behind the effects of IFNs on tumors in mice. Several possibilities exist, and it is even likely that effects are exerted mainly by mechanisms of which we are presently unaware. With this incomplete knowledge in our minds, let us turn to the treatment of tumors in patients.

CHAPTER 8

TREATMENT OF HUMAN PAPILLOMAVIRUS-ASSOCIATED TUMORS

I. Local Treatment of Human Papillomavirus-Associated Tumors

In 1979 Scott and Csonka injected warts with small doses of natural IFN-β, and there was a suggestion that IFN inhibited the growth of the warts. Local injections with natural human leukocyte IFN into warts caused regression also in the hands of Ho *et al.* (1981). Circulating NK cell activity was elevated in patients after prolonged treatment. This was a study in which complete resolution of various warts in a single patient was not achieved, but it could be seen that intralesional injections had an effect, whereas systemic therapy had not. In the systemic treatment, the dose was 2.4×10^6 IU given to an adult patient, and it was given twice weekly, which, according to the experience in juvenile laryngeal papillomatosis patients, would be on the borderline for giving a clear effect (Haglund *et al.*, 1981). Pazin *et al.* (1982) treated two patients with extensive wart formation, who had been stable for 2 years or more, with human natural IFN-α. Intramuscular administration produced softening and decreased scaling of the warts in both patients. Double-blind placebo-controlled intralesional injections resulted in progressive disappearance of the warts treated with the IFN preparations. Even a dose–response relationship was established. Intramuscular injections caused a small effect. A dose of 3.8×10^4 IU locally per injection produced a definite but modest response.

Niimura *et al.* (1983) treated 80 patients who had bilateral common warts of the extremities with either natural human IFN-β or placebo consisting of human serum albumin and lactose mixtures. Treatments were given at weekly intervals and injections were given intralesionally. Each patient received 10^5 IU of the IFN into the warts on one side and placebo injections on the matching extremity. Everything was done in a randomized way and the code was maintained by a controller until the experiment was completed. Sixty-four patients were treated to completion. More than 81% of the IFN-treated extremities were cured or responded effectively to therapy. Only 17% of the placebo-treated lesions responded in this way. No adverse effects

were registered. This demonstrates the effectiveness of IFN preparations on papillomatous disease.

Uyeno and Ohtsu (1982) injected human natural IFN-β into 49 patients with verruca vulgaris, 8 patients with verruca plana juveniles, 2 patients with condylomata acuminata, and 2 patients with molluscum contagiosum. In the first group, there was a response rate of ~82% and in the second, 14%. There were good responses among the few treated lesions with condylomata and molluscum. The tumors were treated i.t. or subtumorally in volumes of up to 0.1 ml per patient. The concentration of the IFN was 0.3×10^6 IU/ml. It was concluded that the treatment was remarkably effective. Uyeno and Ohtsu then turned to other diseases of the skin. In a case of cutaneous metastases of malignant melanoma with eight metastatic skin lesions, there were degenerations of tumor cells in seven of the lesions and no evidence of any tumor cells in the eighth lesion after treatment. On a case with skin metastases from gastric cancer, the treatment was without effect. Complete disappearance of tumor cells occurred in a lesion of breast cancer in the skin, and there was also a complete remission of a lesion containing lymphomatoid papillosis. A case of parakeratosis Mibelli was more difficult to evaluate, but in a case of cutaneous T cell lymphoma, injections of IFN into lesions seemed to cause almost complete tumor disappearance. The results were considered encouraging by the Japanese workers.

A Yugoslavian group presented three series (Ikić *et al.*, 1975a–c) of patients with condylomata acuminata treated with human natural leukocyte IFN-α preparations. In the first study, 36 of 40 patients had complete regressions after treatment locally with ointment containing human leukocyte IFN preparations. The condylomata acuminata were unchanged in the remaining four patients. It was concluded that this type of treatment was well tolerated and that there was possibly an anti-tumor effect in these patients. It was then decided to do a double-blind study with human leukocyte IFN, and this was also reported in 1975. A similar type of ointment was employed. Regressions were achieved between 4 and 12 weeks in all 10 IFN-treated patients. Regression was also seen in 3 of 10 patients receiving placebo. The difference is significant, and it was concluded that ointment containing human leukocyte IFN had an effect on condylomata acuminata. In the last series of investigations, the same group studied the use of a cream instead of an ointment in some patients to see whether a quick regression of the condylomata acuminata could be achieved. Beneficial effects were achieved in 20 of 40 patients regardless of whether they were treated with ointment or cream. The results were poorer

than in the previous studies. It is of interest that this time, 32 of the 40 patients were males. It is difficult to know why the results seemed to be poorer in the latter study, since according to the Yugoslavian investigators this could not really be attributed to the way the IFN had been prepared.

Vesterinen *et al.* (1984) treated eight patients who had vaginal flat condylomata with an IFN cream containing a semipurified human leukocyte IFN preparation. IFN activity could be recovered from the cream. Five of eight patients in a double-blind controlled trial showed regressions of their condylomata, while no change was noticed in the other three. No complete cures were achieved, however, and in two of the responding patients there was a relapse within 2 months after ending the treatment. In the placebo group, there were two patients showing progression, and three patients exhibited no change. It was concluded from that study that vaginal flat condylomas can respond to local application of a cream containing IFN. Dose and schedule have to be determined through future studies employing this type of approach. Gall *et al.* (1984) treated 15 evaluable patients with lymphoblastoid IFN in an attempt to cure patients with condylomata acuminata. The IFN was given i.m. at a dose of 5×10^6 IU/day for 21 days, then three times per week for 2 weeks, and then for an additional 6 weeks with the same dose if a complete response had not occurred. If there were still warts at this time, additional intralesional therapy was administered twice weekly for 4 weeks. Of the 17 treated patients, two left the study. Nine of the 15 remaining patients showed a complete response, and five showed a partial response (response rate 93%). Only one patient was a failure. Six patients were treated with intralesional therapy, and, of these, two showed a complete response (33%) and three had partial responses. One of the six withdrew from the study. It will be interesting to follow this group of patients and to see for how long a period these patients have to be treated in order to prevent recurrences.

In 1978 Nola *et al.* had started to treat patients with urinary bladder papillomatosis with human leukocyte IFN preparations. Eight patients with recurrent papillomatosis were treated, and the results showed that a long remission could be achieved with human leukocyte IFN alone or in combination with electroresection. It is difficult to evaluate the order of the regressions, but the authors claimed that there was regression in patients who had had recurrences at short intervals, bleedings stopped, signs of cystitis disappeared, and the size of tumor masses was reduced. The number of eventual complete regressions was not stated.

II. IFN Treatment of Juvenile Laryngeal Papillomatosis

Evidence has accumulated that human papillomavirus (HPV) is involved in the development of juvenile laryngeal papillomatosis (JLP) (Mounts *et al.*, 1982). Concerning the cloning and characterization of HPV DNA from a laryngeal papilloma, see Gissman *et al.* (1982). Morphological development of the viruses in human JLP is difficult to follow due to the scarcity of the viral particles that can be detected (Lundquist *et al.*, 1975). Over the last few years, the cultivation of HPV-containing cells *in vitro* has meant much for the development of this area (see Steinberg *et al.*, 1982). For a general discussion on the prognosis of patients with JLP treated by conventional means, see Cancura (1977).

IFN treatment of severe JLP, especially when recurring in children and young adults, has taken place at the Karolinska Hospital since the beginning of 1976 (Cantell and Strander, 1977; Einhorn and Strander, 1978a, 1984; Ingimarsson *et al.*, 1979a; Haglund *et al.*, 1981, 1982; Strander 1981–1982; Strander, 1982b, 1983a; Strander and Einhorn, 1982a). Case descriptions of the first seven patients have been given in detail by Haglund *et al.* (1981). Tumor progression occurred in all of these cases before treatment. The patients were given 3×10^6 IU i.m. of natural human leukocyte IFN-α three times per week. Then the tumors decreased in size. When treatment was discontinued, growth recurred, and when the patients were treated once again, the tumors vanished. It was concluded that exogenous natural human leukocyte IFN can affect the clinical course of JLP. The optimal schedule for therapy is still being worked out at the Karolinska Hospital, but it seems that long-term treatments are important and that the patients can then in most cases finally be released from the IFN therapy. A few patients—at the moment, 3 of 12—still have to take IFN therapy continuously. It will be interesting to follow the very large-scale trials in Canada and the United States and to develop the best possible schedules for therapy.

To date, all of the more severe cases in Sweden have now been treated. At the Karolinska Hospital, there are at present 12 patients with this diagnosis who have received human natural leukocyte IFN-α injections (Haglund, Lundquist, and Strander, unpublished observations). Ten of them are boys, between 2 and 22 years of age, and with a debut of their diseases at the ages of 1.5–21 years. There are two females, 4 and 12 years old, who had their disease at the ages of 2.5 and 4 years. Two of the patients have had tracheostomies at the

initiation of treatment. For five of the patients, the treatment has lasted between 1 and 2 years, for two patients between 2 and 3 years, for one patient between 3 and 4 years, and for four patients the treatment has lasted for more than 4 years. Nine of the 12 patients have complete remissions, and these patients require neither IFN nor operations. One patient has a partial remission and is still receiving IFN. Two patients have had intermittent good effects of the IFN therapy but at the moment they can no longer be considered to have partial remissions, since the tumors, despite being between 4 and 4.5 years of treatment, are still rather active. At present, we are discussing whether we should intensify the treatment of these patients. The standard treatment is the same as previously, 3×10^6 IU three times weekly, which might be a borderline dose based on previous work.

Bomholt (1982, 1983) treated, in Roskilde, Denmark, eight patients with recurrent laryngeal papillomatosis with semipurified natural human leukocyte IFN-α. The patients were given $1–4 \times 10^6$ IU daily or every second day for 150 days. After an initial 3-week period of treatment, papillomas were removed in the patients microsurgically, and then the patients were continued on the IFN. At the end of 5 months, a recurrence of small papillomas was found in one of the patients. After the treatment was stopped (7–11 months) there were additional recurrences in four patients. Most of the patients in this study were adults, and their papillomatosis had lasted from 2 to 31 years. It was concluded in this pilot study that IFN can also change the history of laryngeal papillomatosis in adult patients (Bomholt, 1983).

Others have also found that IFN therapy really seems to have an impact on severe juvenile laryngeal papillomatosis (see White, 1983). Leventhal and co-workers (1982) treated patients with JLP with lymphoblastoid IFN at a dose of 3×10^6 IU i.m. daily for 4 weeks and then three times weekly for 5 months. The patients were 3–8 years old. The dose was well tolerated, and there were no local toxicities. Symptomatic side effects were the ones expected. When it became clear that such young patients can take these doses of lymphoblastoid IFN, it was decided to do a multicenter randomized study on 60 patients to study the effects on both the regrowth rate and papillomata regression. The trial was constructed as a crossover study, and the study design was to randomize patients to 6 months of observation with surgical reevaluations either before or after a 6-month period of IFN treatment. The study is ongoing, and results should soon be available.

Fourteen patients with JLP were treated in Houston, Texas, with systemic administration of semipurified IFN-α (Goepfert *et al.*, 1982).

Initially, patients were given 2×10^6 IU of IFN-α per m^2. The IFN was given i.m. The frequency of administration was reduced to three times weekly when the papillomas stabilized or decreased over two successive endoscopic examinations. Of the 14 patients, 12 completed a minimum of 7 months of IFN-α therapy. At the time of the report, there were 12 evaluable patients, of whom five showed complete response (42%), three had partial remission (25%), and the remaining four had some kind of response considered moderate or slight by the investigators. The effects of the IFN therapy were noticed within 6–12 weeks of therapy. Some of the IFN used in the Texas study was purchased from Finland, and some was purchased from New York State. Comparisons were made between the preparations regarding responses and toxicity (Sessions *et al.*, 1984). There was no significant difference in response of the papillomatosis to these two IFN preparations. There was some suggestion that maybe the New York product was associated with less toxicity, but it is difficult to evaluate, especially since one then has to do extremely strict determinations to see that both preparations really contain the same number of IFN molecules and antiviral units.

In 1983 data were presented from Iowa City concerning treatment of 19 patients with JLP with partially purified natural human leukocyte IFN-α (McCabe and Clark, 1983). All of the patients suffered from moderate to severe respiratory papillomatosis. Intramuscular injections were given three times a week and, depending on the age of the children, $3–10 \times 10^6$ IU was given each time. Six of the 19 patients became free of papillomatosis lesions after treatment periods ranging from 1.5 to 12 months. Seven of the patients had minimal residual disease, four had moderate disease that did not require laryngoscopy, and two were considered not to have responded to the treatment. Toxicity was of a nature that had been reported previously by other groups. As stated by others, the authors considered it most important to find out how long the treatment has to continue to render the patients free of papillomatosis. Lusk *et al.* (1984) summarized the Iowa City experience. Nineteen patients have so far been entered into a prospective study, and a response rate of 76% has been achieved (13 of 17 evaluable patients). Of the 13 responders, nine still maintain their response, while two have worsened and require frequent laser endoscopies. The remaining four patients have moderate to severe disease and require frequent rescue treatments. Forty-seven percent, i.e., 8 of 17 patients, are off IFN and require some kind of removal of papillomas now and then in order to maintain an open airway. Noth-

ing more has been found as far as side effects are concerned, and there were no serious disturbances reported on the liver function tests. The authors also mention that two large-scale trials have been undertaken on this type of disease, one sponsored by the National Institutes of Health, in which patients are treated with natural leukocyte IFN-α, and one sponsored by the Wellcome Foundation, in which the patients are treated with lymphoblastoid IFN-α (see above and Weck *et al.*, 1983).

Lodemann *et al.* (1984) treated four children with JLP with HuIFN-α and there was a response in three (one complete and two partial responses). The dose was 1–3 $\times$ 10^6 IU i.m. daily or three times a week. IFN treatment caused a rise in (2′-5′)A synthetase levels in the blood lymphocytes. An elevated synthetase activity was necessary, even though not always sufficient, for successful therapy, the authors concluded. These investigators' measurement of this activity might be a good monitor for estimating IFN activity. Of special interest is a report cited in a review by Göbel *et al.* (1981), in which natural IFN-β was found to be ineffective in patients with laryngeal papillomatosis while, on the other hand, a clear dose-dependent response was observed with natural IFN-α.

Schouten *et al.* reported in 1983 that a squamous cell carcinoma of the bronchus developed in a patient during his course of JLP. What makes this case report important was the fact that the patient had received IFN of the natural type, both IFN-β and IFN-γ, at a dose of 3 $\times$ 10^6 IU three times per week i.m., and later two times per week. The IFN caused regression of the patient's papillomas, and he was in complete remission when he developed the carcinoma. If this can be generalized, it means that IFN at this dosage and using these schedules have some effects on benign tumors but not on a carcinoma. It is interesting that our group has also seen the development of squamous cell carcinoma in the tongue of a patient who received immunosuppressive therapy due to a kidney transplant. He had been injected with natural IFN-α because of extensive wart formation. His warts regressed on IFN therapy, but very close to the termination of the IFN treatment, he developed a squamous cell carcinoma (Strander, unpublished observation). Whether IFN itself plays a role in such developments remains unknown since this patient, for example, received extensive immunosuppressive therapy. In future treatments aiming at the eradication of JLP, IFN therapy should probably be combined with CO_2 laser excision and podophyllum painting (Dedo and Jackler, 1982).

III. Systemic IFN Treatment of Other HPV-Associated Tumors

One patient with plantar warts present from childhood responded to treatment with natural IFN-α that was given at a dose of 3×10^6 IU/day (Strander and Cantell, 1974). The warts reappeared approximately 1.5 months after the discontinuation of IFN therapy. The patient was originally treated for carcinoma *in situ* of the uterus, and the effect on the warts was registered as a by-product. This observation was important for our later work on papillomavirus-associated diseases. The case convinced us that IFN therapy might have an effect on such diseases.

Under certain circumstances, it is clear also that condylomata acuminata can be affected by systemic IFN treatment, as in a patient given 3×10^6 IU of semipurified human natural IFN-α by i.m. injection (N. Einhorn *et al.*, 1983a). Until now, 11 patients with condylomata acuminata have been treated at the Karolinska Hospital (P. Ling, personal communication). There were two complete and three partial responses (45%) among these patients. So far, there have been no responses in the group having simultaneous dysplastic changes. The studies of Gall *et al.* (1984) were mentioned in connection with local condyloma treatment, but the larger number of their patients were treated systemically with lymphoblastoid IFN-α with good results (see Section I).

Two studies have been done in Israel using natural human IFN-β in the treatment of condylomata acuminata (Schonfield *et al.*, 1984). In the first study, which was open, different IFN-β preparations were employed on 16 female patients in order to find out which was the most suitable one. In a second part of the study, a double-blind placebo trial was performed on 22 patients given injections of 2×10^6 IU IFN-β i.m. or placebo for 10 consecutive days and followed up for 3 months. Lesions disappeared in 9 of 11 patients in the IFN-β-treated group (response rate, 82%) while two remissions were registered in the placebo group. The results were evaluated ~5 weeks after completion of the course of injections. After an additional 3 months, eight of the nonresponders were given a course of IFN-β, and all of these patients responded to treatment. None of those who responded has so far had a recurrence and the disease-free period is now at least 12 months. That a systemic response was achieved by injecting the IFN-β i.m. could be seen by measuring changes in the (2′-5′)A synthetase levels in the peripheral white blood cells. These results must be considered promising but need confirmation.

Christophersen *et al.* (1978) treated 10 patients with various tumor diseases with exogenous i.m. natural semipurified IFN-α therapy,

usually with a dose of 4×10^6 IU daily for 1 month and then three times weekly. The 10 patients reported on were treated for 2–28 months. Side effects consisted mostly of fever. It is difficult to evaluate these cases. There were three cases among the treated patients who had bladder papillomas. They showed normalization of their bladder urothelium. There was a regression of papillomas whenever they were smaller than 1 cm, while the larger papillomas tended to be stationary during treatment. Further Danish results on the treatment of bladder papillomas with IFN-α were reported in 1981 (Osther *et al.*). The results were again considered encouraging.

CHAPTER 9

REGIONAL TREATMENT OF OTHER TUMORS

I. Intra- and Peritumoral IFN Therapy

It is always difficult to evaluate the effects of intralesional, perilesional, and topical administration of various drugs. A summary of how such treatment has been given to tumor patients by using preparations containing IFN was presented by Ikić (1983). As has been discussed elsewhere, such treatments have been used for cervical intraepithelial neoplasia of the uterus, invasive cervical carcinoma, head and neck cancers, urinary bladder papillomatosis, breast cancer, pleural cancer, and condylomata acuminata. The idea behind these experiments is that high doses of IFN give rise to a direct anti-tumor effect and, in addition, that the therapy causes infiltration of the tumor with lymphocytes and macrophages. These studies deserve consideration, of course, but in this area properly controlled trials are required in order to see which effects are due to the IFNs and which are not. Our experience over the years is limited to treatment of two patients with squamous cell carcinomas of the head and neck and three patients with malignant melanoma. In these cases, injections were made with 3×10^6 IU i.t. There were no partial or complete responses (Strander, unpublished observation). Knezević *et al.* (1979) injected patients locally in the tumor area with semipurified leukocyte IFN-α. They treated patients with cervical cancer and head and neck carcinomas. They could see that following local application there was activation of the regional lymph nodes. Their conclusion from this work was that their preparation strongly increased the active defense of the lymph nodes and that there was also an insulation of tumor cells due to formation of hyaline masses around necrotic tumor areas. It is difficult, however, to interpret these results in an exact manner because of the fact that leukocyte IFN was only available in an impure state at the time of these investigations. This criticism can be applied to much of the work on local therapy.

Horoszewicz *et al.* found with human natural IFN-β that pyrogenic responses could be avoided (Horoszewicz, 1978b; Horoszewicz *et al.*, 1980). They established that their IFN-β had an anti-growth effect on many different tumor cell lines, and they also found by using the nude

mouse model that a correlation was obtained between the results in the mice and the effects on the human tumors in tissue culture (Horoszewicz *et al.*, 1980). The IFN-β was injected as in other studies by the same authors at a dose of 5×10^5 to 1×10^6 IU daily directly into subcutaneous metastatic lesions of patients having malignant melanoma, breast carcinoma, or prostatic carcinoma. They saw nodule infiltration of lymphocytes and macrophages in the tumor and regression of some injected tumors but no complete remissions. It is possible, that IFN-β could be used most advantageously after being injected locally. In 1975 D. B. Habif at Columbia University reported anti-tumor effects on local mammary carcinoma growth by the use of natural IFN-α given i.t. (personal communication).

Sawada *et al.* (1982) treated 6 malignant solid tumors: 2 neuroblastomas, 2 Wilms' tumors, 1 malignant teratoma, and 1 thoracic rhabdomyosarcoma. The natural IFN-α in this case was given either i.m. or i.t. or both. It is difficult from the description given to say how strong the effects were on the tumors, but there were significant reductions, according to the authors, of some tumors receiving the local treatment. In the same paper, the authors looked at the effects of i.p. IFN administration on mice bearing neuroblastomas and saw a prolongation of survival of the animals. Different malignant tumors receiving local IFN therapy are described in the following section.

II. Local Treatment of Malignant Melanoma

Horoszewicz *et al.* (1978a) injected semipurified natural human IFN-β, 5×10^5 IU daily, into cutaneous and subcutaneous metastatic lesions in three patients with malignant melanoma. By local injections, partial regression was achieved. When subcutaneous nodules in three patients with breast cancer were injected by a similar schedule, heavy infiltration of lymphocytes was revealed, and local tumor regression was also seen in these patients (Nemoto *et al.*, 1979). In the local treatment of tumors, it is difficult to speculate on mechanisms, but in the Roswell Park Memorial Institute experience there was heavy infiltration with lymphocytes (Horoszewicz *et al.*, 1978b). This had also been found upon local administration of IFN in the Yugoslavian studies discussed in Chapter 8, Section I. Nola *et al.* (1979) reported on two patients with malignant melanoma treated locally with human leukocyte IFN preparations. In one case there was complete regression, and in the other patient there was a 75% local regression. Ishihara *et al.* (1982) treated patients with malignant melanoma by i.t. administration of human natural IFN-β. Initial doses were $3–6 \times 10^5$

IU, depending on the size of the tumor, and the dose was then increased to a maximum level of 6×10^6 IU.

As a rule, injections were given every other day. The treatment consisted of injecting the IFN into cutaneous and subcutaneous metastatic lesions of malignant melanoma. Eight patients were treated, and effects were demonstrable in seven. There were at least four partial responses. In the injected lesions, there was local mobilization of lymphocytes. In these studies, it would have been important to see effects of injected control preparations. These investigators had, however, used many other types of agents, such as picibanil, bestatine, and vincristine, and they had also injected saline locally without seeing any infiltration of lymphocytes. It has to be emphasized, however, that the preparations in almost all i.t. work in the world so far have been made with semipurified IFN preparations containing many substances other than IFN.

Ishihara *et al.* (1983) have updated their results on treatment of malignant skin neoplasias with intralesional administration of IFN-β or IFN-α. The IFN-α employed was either human lymphoblastoid IFN or recombinant IFN-αA. All of the preparations were injected locally at a dose of 3×10^5 IU with the IFN-α and IFN-β mixtures or 1.5×10^6 IU in the case of IFN-αA. The doses were then escalated to 6×10^6 IU for the mixtures and to 9×10^6 IU for the recombinant IFN. Of 17 cases with malignant melanoma treated with IFN-β, there were four complete responders and seven partial responders (response rate, 65%). In addition, there were two cases with minimal responses and two cases with no change. It is of interest that, among the other malignant skin tumors injected, there was one case of squamous cell carcinoma showing a complete response. The cases with the other diagnoses were so few, however, that it is difficult to make conclusions. With the lymphoblastoid IFN in malignant melanoma, one of four cases showed partial response, but there were also two minimal responses and one case showing no change during treatment. With the recombinant IFN, there was one partial response, and one patient who showed no change on intralesional therapy. It was the impression of the authors that smaller tumors responded better. No severe reactions occurred on these treatments.

In 1982 an interesting report was presented by Borgström and coworkers in the *New England Journal of Medicine*. They combined 4–12×10^6 IU of natural IFN-α given as daily i.m. or i.t. injections with oral cimetidine (1000 mg/day) on the presumption that the cimetidine would work as a histamine antagonist and stop the activation of suppressor cells by IFN. In this study, there were initially six patients

treated, and no objective tumor regressions were registered during the time of IFN therapy alone. When IFN and cimetidine were combined, there were two patients showing complete remissions, one patient having a partial remission (response rate, 50%), and a stationary status was established in the fourth patient. This study has continued and is now being expanded also in collaboration with other centers to see whether these effects can be achieved by IFN alone or if only the combination is able to cause these impressive regressions. By 1983, 20 patients had received combined treatment (Flodgren *et al.*, 1983a). Again, it was concluded that IFN treatment alone, administered i.m. or i.t., was ineffective. At this time, eight patients with metastases confined to the skin and the subcutaneous tissue had been treated, and five of these had shown complete tumor regressions (63%). There was also one patient showing an extensive partial regression having skin disease and a complete regression of a pulmonary metastasis on a radiogram (the lung metastasis was not, however, cytologically confirmed). Three additional patients showed stable disease status. The treatment was well tolerated. An explanation for the excellent results is that one can get better effects using this type of treatment when patients with cutaneous rather than visceral disease are treated. This is an interesting hypothesis, but since the results reported are so extraordinary, it will also be necessary to document in more detail what the addition of the cimetidine to the IFN treatment really can accomplish. It will be interesting to also see what results other groups might achieve with similar treatment. Flodgren *et al.* have continued their studies on metastatic melanoma with IFN-α, and in 1983 they reported four complete and two partial regressions after giving a combination of IFN and cimetidine in malignant melanoma (Flodgren *et al.*, 1983b). At that time, there were still 20 evaluable patients given the doses presented above (response rate, 30%). These investigations are now being extended. Borgström *et al.* (1983) have also responded to questions concerning their combination therapy with cimetidine and natural human leukocyte IFN-α for malignant melanoma patients in a letter to the editor of the *New England Journal of Medicine*. They agree that they cannot rule out the possibility that cimetidine alone might be responsible for some effect seen in malignant melanoma. However, they refer to an ongoing Swedish study in which seven patients with malignant melanoma treated with cimetidine have all shown tumor progression. Clearly, this area requires additional studies, and such are on their way.

Hill *et al.* (1983b) also treated patients with malignant melanoma with a combination of IFN-α and cimetidine. Intralesional IFN-α

therapy at 6×10^6 IU for 5 days weekly and with cimetidine given at a dose of 300 mg orally q.i.d. resulted in two complete and three partial regressions in 16 evaluable patients (31%). In addition, there was one minor response, and four patients had stable disease. This group believes that the addition of cimetidine increases response rates with IFN-α in the treatment of disseminated malignant melanoma. Hill *et al.* (1983c) updated their work on the combination of natural IFN-α and cimetidine as a combination for generalized malignant melanoma patients. Natural human IFN-α was given intralesionally at a dose of 6×10^6 IU 5 days per week together with cimetidine 300 mg orally four times daily. Six of the 32 patients treated so far had responses (19%), of which three were complete and three were partial. The patients showing responses had cutaneous, subcutaneous, or lymph node metastases. The longest complete response has lasted 1 year so far. The authors conclude that in their experience the response rate of the IFN–cimetidine combination is at least comparable to what has been achieved with 5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboxamide (DTIC), which is probably the most common chemotherapeutic agent used in this disease. In this group of patients, a randomized trial should be performed. The results would be of interest, since there are always patients who have localized inoperable malignant melanomas that create clinical problems. Therefore, it would be interesting to also know how these patients respond to a combination of local irradiation, cimetidine, and local IFN administration.

III. Local Treatment of Breast Cancer

The Columbia University and the Roswell Park Memorial Institute experience has been discussed in Sections I and II. Nola and coworkers (1979) treated four patients with breast carcinoma by crude human leukocyte IFN in Yugoslavia. Two of the patients had recurrent breast carcinoma and two had primary carcinoma. In four cases there were partial regressions of the tumor upon local administration of the crude IFN. All four patients were later operated on. To summarize, the few breast cancers injected locally with IFNs seem to have shown a response.

IV. Local Treatment of Cancer of the Uterine Cervix

The Yugoslavian IFN group in Zagreb applied semipurified crude human leukocyte IFN-α locally to patients with cervical cancer (Ikić *et al.*, 1975d; Krušić *et al.*, 1977, 1979; Singer *et al.*, 1979). In their first report, they treated 10 patients with cervical cancer of the uterus with

human leukocyte IFN. They could see a drop in the activity of β-glucuronidase activity in vaginal secretion of treated patients. In 8 of 10 patients, the enzyme level was reduced to normal values. The international classification was not followed with regard to clinical responses. In 1977, the same group reported data on 12 patients with cancer of the uterine cervix. Again, it is difficult to evaluate the clinical results by standard criteria. The investigators had treated 12 additional patients, and when they analyzed the results achieved on these patients and the 10 previously treated, they noted that there was conformity in regression of Papanicolaou tests. In 1979, the group had treated a total of 37 patients with cancer of the cervix. There was hyperactivity of the lymph nodes in the treated patients upon treatment. Some metastases were subsequently registered in 2 of 27 patients topically treated with the human leukocyte IFN during the time of observation. To nine of the patients, the human leukocyte IFN had been applied both topically and i.m. In these studies, the overall response was assessed on the basis of histological and biochemical investigations, and it is difficult to evaluate the clinical relevance of such findings when employing present-day criteria of tumor responses. Singer *et al.* (1979) reported on 31 patients with cervical precancerosis who were treated locally in the vagina with an IFN-containing powder. They observed regressions of pathological changes, and their conclusion was, also on the basis of virological findings, that in cervical carcinoma patients human leukocyte IFN therapy is perhaps beneficial at some stage of precancerosis development. Later data are not available on these patients.

Møller *et al.* (1982) treated six patients suffering from moderate to severe dysplasia or carcinoma *in situ* of the uterine cervix with a gel in which they had incorporated purified natural human leukocyte IFN-α. The patients received a small amount of the gel for 6 weeks. Only minor clinical improvements were seen, but after an additional 6 weeks there were responses in all patients, and three of the responses were classified as complete.

V. Local Treatment of Neurological Tumors

Sawada *et al.* (1981) reported that 10 intralesional injections of natural leukocyte IFN-α at a dose of 3×10^5 IU were able to reduce neuroblastoma masses.

At the University of Lund, Sweden, two patients with advanced malignant glioma were treated with natural human IFN-α by local application (Osther *et al.*, 1981). The patients had a Grade 3 astrocy-

toma in the left parietal lobe, and a Grade 3–4 astrocytoma in the right temporal lobe. Both tumors had been resected, and chemotherapy had been given to the patients. Both tumors had relapsed based on computed tomography (CT) scanning. A Ricksham reservoir was placed, and the IFN was injected through the catheter tip into the center of the tumor, 4×10^6 IU of IFN-α was given daily, and simultaneously 4×10^6 IU was given i.m. Both tumors seemed to grow during treatment, and after 2 weeks, an operation was performed. Both of these patients died, but the interesting finding at postmortem examination was that the tumors had become necrotic and surrounded by granulation tissue. In one case in which the survival was longer, the tumor was totally encapsulated and insulated from the remaining brain tissue. The surface of the tumor consisted of granulation tissue, with a well-developed demarcation zone toward the surrounding tissue.

Nagai *et al.* (1981–1982) used human natural IFN-β to treat patients with brain tumors by systemic or local routes. In a preliminary report, they had one complete response and four partial responses when the IFN was injected via an Ommaya's reservoir into nine patients with malignant brain tumors. This gives the tremendous response rate of 56% in these severe cases. By systemic administration, no IFN could be detected in the cerebrospinal fluid. It has to be remembered, though, that in this study the volume of the tumor was visualized on a CT-scan, a method that is discussed below in connection with IFN therapy (Boëthius *et al.*, 1983).

Nakamura *et al.* (1982) treated four patients with malignant brain tumors (three glioblastomas and one astrocytoma) with IFN-β. Injections were first given i.v. but were then given directly into the tumor by way of an Ommaya's reservoir. The IFN was administered daily, the dose of IFN started at 30×10^4 IU/day, and the treatment period extended up to 30 weeks. In one of the four cases (one of the patients with glioblastoma), there was a partial regression as measured by CT scanning, and at the same time, improvement was seen in neurological signs. The effect here turned quickly into a stable state that lasted for 2 months before progression. Sano *et al.* (1982), in Phase II studies by a cooperative study group of 10 institutions, treated 42 cases with glioblastoma and malignant astrocytoma by i.v. or i.t. administration of $1–6 \times 10^6$ IU/day of IFN-β for more than 8 weeks. Seven cases (17%) showed complete or partial response. In seven cases of medulloblastoma, in addition, there were three cases (43%) showing complete or partial response. In five cases of other gliomas, there were two (40%) that showed complete or partial responses. Altogether, 22% of all the patients treated showed complete or partial response (Sano *et al.*,

1982). Nakagawa *et al.* (1983) reported on 13 patients (10 adults and 3 children), selected at random, who were treated by giving 10^6 IU of IFN through an Ommaya's reservoir or by intrathecal injections. The IFN preparation contained semipurified natural IFN-α, and it was administered either one or two times weekly to seven patients or every day for 1 month, followed by 1 month's suspension of administration, to the other six patients. The total dose administered was 12×10^6 IU in the weekly group and 25×10^6 IU in the daily group. No serious side effects were seen. No tumor regressions were registered in the first group of patients, but in two of the six patients given daily injections a decrease of tumor volume was documented and they were considered to have partial remissions. It again has to be considered that CT scans of IFN-treated patients might be difficult to interpret (Boëthius *et al.*, 1983). Also, other Japanese investigators have done large studies on human brain tumors looking for effects of IFN treatments (see Ueda *et al.*, 1983). The IFN used in these studies was natural IFN-α that was semipurified. In these investigations, the patients were treated with IFN alone to primary metastatic brain tumors and the effects were registered by objective means. In 1983, 2 of 10 cases had shown partial regression upon systemic administration (20%), while 2 of 4 cases had shown partial regression upon local administration. The other main Japanese approach in the treatment of brain tumors has been the use of IFN-β in combination with irradiation and chemotherapy. In such studies, it is naturally more difficult to evaluate whether clinical effects are due to IFNs or not.

VI. Local Treatment of Head and Neck Tumors

Padovan and co-workers reported in 1979 on the treatment of cancers of the head and neck region with semipurified human leukocyte IFN-α preparations (Padovan *et al.*, 1979). They concluded, after topical administration, that tumors could be made to regress and also that human leukocyte IFN preparations could inhibit metastatic dissemination of these tumors. In their paper, they did not use the response criteria that are nowadays internationally employed, but on the basis of the description of their patients they saw at least partial remissions in 10 of 13 patients with carcinoma of the mucosa or of the skin in the head and neck region. They also achieved partial remission in at least 8 of 14 patients with basalioma of the same region. There was also a response in a patient having leukoplakia. The same group of authors reported in 1975 on the successful IFN treatment of basocellular carcinoma of the skin. The effects of IFN therapy on head and neck

tumors in Yugoslavia was reviewed by Padovan *et al.* in 1980. They had treated 30 patients who had skin and head and neck tumors with i.t. or peritumoral injections. The IFN was injected at a dose of 3×10^5 IU. Sometimes an ointment containing 3×10^4 IU of natural human leukocyte IFN-α per gram was applied. It is difficult to evaluate the results, since many of the patients had also received other treatments, but the authors concluded that several patients showed disappearance or regression of their tumors and that the treatment reduced the percentage of recurrences and metastatic dissemination. Depending on the fact that other treatments were employed in some cases, the role played by IFN therapy against these particular tumors has to be evaluated with caution.

Sato *et al.* (1983b) administered human natural IFN-β topically in premalignant lesions arising in the oral mucosa. IFN-β was prepared in water-soluble gel form. Fourteen oral lesions including eight lichen planus and six leukoplakias that showed erosions were treated. The erosive lesions disappeared in eight patients. In the other six patients, white coatings and streaks could not be completely resolved by therapy. In cases in which only the gel was administered, there were no effects. The authors concluded from this pilot study that it would be interesting to extend these studies on the use of IFN treatment on premalignant lesions in the mucosa in the oral cavity (Sato *et al.*, 1983b). This would be an important disease entity to treat if there were clear-cut effects with IFN therapy, since there is always the risk of development of carcinoma in these patients (Einhorn and Werzäll, 1967).

There are hints suggesting that there might be an effect of bacillus of Calmette–Guerin (BCG) injected in combination with chemotherapy in the treatment of head–neck cancer (Taylor *et al.*, 1983). Such an effect could conceivably be due to the presence of induced IFN. This is probably especially true when the BCG is injected intralesionally (Bier *et al.*, 1981).

VII. Local Treatment of Lung Tumors

Jereb and co-workers (1977) injected crude human leukocyte IFN into six patients with breast cancer who had unilateral pleural carcinosis. All patients had previously been treated for their primary tumor either by surgery or irradiation and for recurrent disease with different types of conventional therapies. It was seen that malignant cells in the pleural cavity disappeared after the second, third, or fourth applications. One of the patients developed a severe anaphylactic reaction,

and some antibodies (directed against the IFN molecules?) were found in her serum. The remaining five patients experienced fever and malaise. Two of the four patients alive at the time of the report were disease free in the injected area 4–5 months after the last application of human leukocyte IFN.

Six patients with mesothelioma were treated with IFN at a dose of 3×10^6 IU intrapleurally twice weekly for 1–4 months (Weimar *et al.*, 1981). In addition, one patient received IFN-β i.p. followed by IFN-α treatment i.m. One patient without detectable pleural infusion received i.m. natural IFN-α only. Five patients were treated with natural human IFN-α i.p. after saline had been injected i.p. twice weekly for a control period of 2–4 weeks. Intrapleural IFN administration was well tolerated, fever being the main side effect registered. It is difficult to evaluate exactly the therapeutic effects, since everything was registered by cytological investigations of the pleural fluids. This can admittedly be difficult to quantify. In three of the patients, there was disappearance of tumor cells and there were histiocytic–lymphocytic reactions in five of the patients. When tumor cells became undetectable, the effect was associated with an increase in pleural fluid production and in the lactate dehydrogenase (LDH) level. To my knowledge, investigations with IFN in this disease have not been pursued elsewhere.

VIII. Local Treatment of Bladder Tumors

A Japanese series has been initiated by Mishina *et al.* (1983) on patients with bladder tumors in whom IFN is injected locally into the bladder at various doses, extending from 6×10^4 to 2×10^5 IU weekly. No regressions have been seen so far. Instead, these authors started a series of patients treated with a combination of IFN and chemotherapeutic drugs. This series is ongoing, and no results are available as yet.

Twenty-three patients with superficial bladder cancer having either biopsy-proven carcinoma *in situ* changes or recurrent, noninvasive, low-grade, transitional cell carcinoma were treated with recombinant IFN-α2 (Torti *et al.*, 1984). The IFN was instilled intravesically for 2 hours weekly during a period of 8 weeks after cystoscopy and biopsy had been performed. There was no maintenance therapy. Seventeen patients were evaluable on this schedule. Complete response was seen in six of eight (75%) of the carcinoma *in situ* patients, but in none of the transitional cell carcinoma patients. One of the complete responders later relapsed. It does seem as though this IFN preparation

has activity when given locally on the *in situ* state but not on invasive carcinoma of the bladder. Some other results on bladder tumors have been presented in Chapter 8, Section III, and Chapter 10, Section I.

IX. Intraarterial IFN Therapy

Hawkins *et al.* (1982) treated 23 patients with malignant melanoma, colon carcinoma, or breast carcinoma having hepatic metastases (18 patients) or nonhepatic metastases (5 patients) by direct i.a. injection into the tumors of $3–30 \times 10^5$ IU of natural IFN-α daily for up to 28 days. Such direct administration was achieved without untoward toxicity. Much less of the IFN appeared in the systemic circulation 2 hours after the injection was given i.a. as when given i.v. A two-peak pattern was registered. No patients exhibited partial or complete response. It was mentioned that two patients with melanoma metastases showed some decrease in tumor size. A livido reticularis reaction (rash) was reported in two of the patients receiving IFN-α daily through catheters.

CHAPTER 10

SYSTEMIC THERAPY OF INDIGNANT DISEASE

I. Systemic IFN Therapy of Tumors—Screening Trials

After it was reported that high-dose IFN therapy can be given to tumor patients (Strander *et al.*, 1973), anti-tumor screening trials have been performed at many institutions. As an example, natural human leukocyte IFN-α was prepared in China in the late 1970s. In the beginning, the cells employed were umbilical cord blood leukocytes, but then production was made from cultures containing buffy coat leukocytes or from tissue cultures containing lymphoblastoid cells. Partially purified human IFN-α was available for clinical trials in 1980, and it was first applied for the treatment of malignant tumors in that year (see Peiwei, 1983). Want Peizhong *et al.* reported on a patient with nasopharyngeal carcinoma showing a minimal response after i.m. injection with 2–4 $\times$ 10^6 IU of natural human IFN-α daily together with intranasal drops containing 9 $\times$ 10^5 IU for 50 days. Wang Lanzhi *et al.* injected four cancer patients, and Shi Penzda *et al.* treated three cases of primary liver cancer also with i.m. injections of IFN-α but combined with cytosine arabinoside (ara-C). Also, the combination of treatment with IFN and *Corynebacterium parvum* has been used in China. On account of the small-scale IFN production, few results are available from China at present. Poorly differentiated nasopharyngeal carcinoma is a common disease in southeast China, and IFN treatments have been initiated in such patients (G. DeThé, personal communication).

In 1980 Gutterman *et al.* reported on results accomplished on 38 patients with advanced breast cancer, malignant lymphoma, and multiple myeloma, injected i.m. with semipurified natural leukocyte IFN-α. The patients were injected daily for 4–26 weeks with 3–9 $\times$ 10^6 IU. If a response was achieved, the patients were maintained on 3 $\times$ 10^6 IU three times weekly. Seventeen of the patients had breast cancer, and partial regression was seen in seven (41%) of these. There were 11 lymphoma patients, and in six of these, tumor regression was achieved (55%). Regressions were seen in the multiple myeloma group in 6 of 10 patients (60%). An interesting finding in this series was that there were significant differences between the disease groups in the 6-hour IFN serum levels. For the breast cancer patients,

the data showed 133 units/ml and for the myeloma/lymphoma patients, 65 units/ml. The reason for this significant difference has still not been revealed.

Osther *et al.* (1981) treated nine patients with various forms of malignancies. These patients were termed "heavy tumor load" patients and there were three responders (Stage IIB and IIIB cervical carcinoma of the uterus and Stage IVB Hodgkin's lymphoma). It should be emphasized, however, that two carcinoma patients received irradiation in addition, but one of the patients showed a 50% regression before the start of irradiation. It is, therefore, probably fair to say that in this pilot study, two patients showed partial regression. Eleven other patients were in remission at the start of the IFN-α treatment and of these, four maintained their state of remission during IFN treatment. Details on these patients were, however, not presented. Of seven patients treated for bladder papillomas (Grade II), one patient went into complete remission and three patients showed partial regression (response rate, 57%). None of the patients had serious side effects. Hill's group in Dallas has also treated patients with various malignancies (Hill *et al.*, 1981a,b). In 1981 they presented data on 27 patients with malignancies other than leukemias who had received human natural leukocyte IFN-α. Of 19 evaluable patients, measurable responses were seen in seven. There was, however, only an extensive response seen in a patient with bladder carcinoma in whom complete remission was achieved. Usually, these patients received natural semipurified human leukocyte IFN-α i.v. in divided daily doses of $0.5–2 \times 10^6$ IU/kg of body weight for periods of up to 2 months. There were exceptions to this rule, however, and a patient with ovarian cancer received a single dose of 50×10^6 IU of IFN-α i.p. Side effects in these studies resembled those reported earlier by giving human leukocyte IFN-α.

At the Cleveland Clinic, Budd *et al.* (1983, 1984) used natural human leukocyte IFN-α in 15 patients with malignancies. Three patients had colorectal cancer, two had gastric cancer, two had breast cancer, two had renal cell carcinoma, and there was one case each of lymphoma, multiple myeloma, malignant melanoma, adenocarcinoma of presumed ovarian origin, squamous cell carcinoma of the soft palate, and fibrosarcoma. The IFN was given once daily on Days 1–5 and 8–12. Five patients received 3×10^6 IU per dose, five received 9×10^6 IU per dose, and five received 15×10^6 IU per dose. One patient who received 15×10^6 IU developed severe chest pain and died of ventricular fibrillation. He had no previous history of cardiac disease. Unfortunately, no autopsy was made. Toxicity was, otherwise, similar to

what had been reported in other studies. There were three minor responses in patients having multiple myeloma, breast cancer, and renal cell carcinoma. No partial regression was seen and it was concluded that the IFN could be safely given at the dose ranges employed, but there is then the question of the patient who died of cardiac disease. The dose-limiting toxicity in these studies employing purified IFN was leukopenia.

Talpaz *et al.* (1984) treated patients with human natural leukocyte IFN-α at doses of 0.4–6.4 $\times$ 10^6 IU/m^2 i.m. daily for 2 weeks in combination with DFMO at a dose of 1.5 or 2.5 g/m^2 per orally (p.o.) q.i.d. for 14 days. At the combination of the highest IFN and DFMO doses, there was severe toxicity. At lower doses, the combination was tolerated. There was reversible damage of the hearing, as revealed by investigations of the audiograms in 13 of 18 treated patients. Antitumor responses were evaluable in 16 patients who had been on the study for at least 4 weeks. There were seven patients who had metastatic malignant melanoma, and there was one complete and one partial response (response rate, 29%) and, in addition, a minor response. There was an objective response in one of two patients with metastatic colorectal cancer, and there were minor responses in two of four patients with renal cell carcinoma and in one patient with large-cell carcinoma of the lung. There were also signs of activity against a chemotherapy-resistant CML case. This study does not permit calculations of response rates, but the combination clearly showed antitumor activity and it is now being used in Phase II investigations.

Sarna *et al.* reported at the ASCO meeting in 1982 on seven patients who were treated with human lymphoblastoid IFN. They were initially injected with 0.75 $\times$ 10^6 IU every 12 hours and the doses were then increased to 12 $\times$ 10^6 IU. The IFN was given i.m. Toxicity was severe, but not overwhelming. Partial response was seen in a patient with renal cell carcinoma at 0.75 $\times$ 10^6 IU and in a patient with diffuse histiocytic lymphoma at 1.5 $\times$ 10^6 IU. Since toxicity was tolerable, this initiated the further University of California—Los Angeles (UCLA) series of investigations on the IFN treatment of patients with malignancies (Sarna *et al.*, 1982). In addition, Sarna *et al.* reported in 1983 (1983b) on 33 patients with advanced malignancies who were treated with semipurified human lymphoblastoid IFN. The patients received doses of 0.75–50 $\times$ 10^6 IU i.m. every 12 hours for 7-day therapy courses. These courses were repeated every 4 weeks. Toxicity was similar to what had been reported by others in similar trials. Included were 11 patients with breast cancer, 7 with sarcoma including 1 with Kaposi's sarcoma, 3 with lymphomas, 3 with myelomas, 2 with

Hodgkin's disease, 2 with chronic lymphocytic leukemia, 2 with renal cell carcinomas, and 1 patient each with lung adenocarcinoma, colon adenocarcinoma, and an unknown primary. Partial responses were seen in 3 patients (1 with renal cell carcinoma, 1 with diffuse histiocytic lymphoma, and 1 with Hodgkin's disease). There were minimal responses in 4 patients (1 with chronic lymphocytic leukemia, 2 with multiple myeloma, and 1 with breast cancer). There were no correlations between dose levels and response. It was concluded that IFN had a modest therapeutic efficacy in these patients. It is interesting that these investigators did not see any response in any of the 11 patients with breast carcinoma.

Knost *et al.* (1982) injected human lymphoblastoid IFN by i.v. infusion into patients with a variety of malignancies. The patients received a total of five cycles over a 5-week period. Each cycle consisted of 6-hour infusions given on 5 consecutive days followed by a 2-day rest period. In the beginning of each 5-day cycle, the dose was escalated from 0.1 to 50×10^6 IU daily for 5 days. Toxicities were similar to the ones reported in similar studies. High levels of serum IFN were achieved after the large-dose infusions. A patient with nondifferentiated carcinoma achieved partial remission. Knost *et al.* reported in 1983 on the effects of highly purified human lymphoblastoid IFN given to patients with various disseminated malignancies refractory to standard therapy. Twenty-nine of the patients in the study received the IFN by the i.v. route, and 11 received the preparation in the study by the i.m. route. Each patient received doses escalated from 10^5 to 5×10^7 IU. The side effects observed were the same as those registered in other studies. They were clearly dose dependent and were less intense in the i.m. group than in the i.v. group. The side effects became severe at a dose of 3×10^7 IU. Of the patients receiving the preparation i.v., two showed partial response (one patient with an anaplastic carcinoma of undetermined origin and one patient with nodular poorly differentiated lymphocytic lymphoma). The study showed that the human lymphoblastoid IFN could be administered in escalating doses to 30×10^6 IU daily for 5 days by either route.

Kimura (1983) has reviewed the Phase II studies performed with human lymphoblastoid IFN for solid tumors and hematological malignancies in Japan. Most of the patients received 3×10^6 IU of the IFN i.m. daily. In some patients, however, the dose was escalated to 6 or even 9×10^6 IU over a period of at least 4 weeks. The pharmacokinetics after injecting the human lymphoblastoid IFN was similar to what had been reported by other investigators. In the treatment series of solid tumors, there was a total of 191 patients: 81 patients with renal

cell carcinoma, 26 patients with breast cancer, 12 patients with gastric cancer, 9 patients with colorectal cancer, 9 patients with hepatoma, and 54 other patients with various malignancies. In 54 evaluable patients with renal cell carcinoma, there were 4 complete and 6 partial responses (18.5%). In addition, there were 6 minor responses. This is interesting, especially since there were 26 stable patients. Eleven of 21 evaluable breast carcinoma patients likewise showed stable disease. Otherwise, there were no regressions except in the miscellaneous solid group, in which there were 3 partial responses in 36 evaluable patients. In all of the material with solid tumors, there were 13 of 136 evaluable patients who showed response (9.5%). In the treatment of hematological malignancies in this study, a total of 180 patients entered. There were 71 patients with myeloma, 70 with lymphoma, 28 with acute leukemia, and 11 with chronic leukemia. Of 66 evaluable patients with multiple myeloma, there were 1 complete and 13 partial responses (21%). In addition, there were 16 minor responses and 23 patients showing stable disease. Among the lymphomas, there were 2 complete and 7 partial responses (18%) and, in addition, 5 minor responses in 51 evaluable patients. There were no signs of activity on acute and chronic leukemia patients (16 and 8 of these were evaluable, respectively). Treatments continued for 2–77 weeks. It is difficult to evaluate how long the responses lasted. For a drug used in Phase II studies, the results must be considered interesting. These studies were multicenter trials being performed by using the human lymphoblastoid IFN in a study group scattered over Japan.

Human lymphoblastoid IFN produced in hamsters was used in a study on metastatic renal cell carcinoma and multiple myeloma by Yoshikawa *et al.* (1983). The dose was $2.5–5 \times 10^6$ IU/day i.m. A 72-year-old male patient with multiple lung metastases from renal cell carcinoma showed complete response and a multiple myeloma (IgG-κ type) patient appeared stable during treatment. In a third patient, there was a slight decrease in the IgE level (it was an IgE-κ-type myeloma). No changes were seen in his osteolytic lesions. This study is going to continue. Silver *et al.* (1984) used lymphoblastoid IFN on patients with various forms of malignancies by randomly assigning the patients to either a low-dose (2×10^6 IU/m^2 i.m.) or a high-dose regimen (5×10^6 IU/m^2 i.v. by continuous infusion over 24 hours and then escalating the dose by 5×10^6 IU/m^2 per day, repeated every 28 days). It was found that the maximum tolerated dose was $\sim 18 \times 10^6$ IU/m^2 but that there was wide individual variation. Forty-eight patients have been entered into this trial, and as yet there is no statistically significant difference in the prognosis by the different ways of administra-

tion. Of 27 patients with breast carcinoma treated, there was one partial response (4%), four minimal responses, five patients showing stabilization, and the rest progressed. Of 13 patients with ovarian carcinoma, there was one partial response (8%), three minimal responses, and one patient showing stabilization. There was no correlation between response and effects on NK cell activity changes.

The experience at the National Institutes of Health (NIH) using recombinant IFN-αA and human lymphoblastoid IFN on cancer patients was also summarized at the UCLA meeting in March 1982 (Sherwin *et al.*, 1982a). At that time, it was reported that, of 76 evaluable patients treated with a recombinant IFN-αA, there was a total of nine partial remissions in patients with various diseases. Of the 14 evaluable patients treated with human lymphoblastoid IFN, there were two partial remissions. Comparative work was not done, but it was concluded that both types of preparations had anti-tumor activity in humans. In 1982 Gutterman *et al.* (1982b) reported that 16 patients with advanced cancer had been treated with recombinant purified IFN-αA. Preparations had been given i.m. in doses of $3–198 \times 10^6$ units. The interval periods were 72–96 hours. The serum concentrations achieved were similar to the ones obtained with natural IFN. Also, the side effects were similar to those seen with other IFN preparations. Three of the patients developed antibodies to the recombinant IFN. Seven of the 16 evaluable patients showed evidence of some kind of anti-tumor effect, according to the authors. Of 16 patients treated, one partial remission was achieved (6%). It is interesting that this is a patient who previously had shown complete remission after being treated with semipurified natural leukocyte IFN-α. This patient had a poorly differentiated lymphocytic lymphoma. On the basis of these studies, it cannot be said whether recombinant leukocyte IFN-αA can achieve clinical anti-tumor effects similar to the natural human leukocyte IFN-α. Also in 1982, Horning *et al.* reported their experience with recombinant IFN-αA given to eight patients with advanced cancer. They studied both clinical and immunological effects in their patients. Doses from $3–198 \times 10^6$ IU were given by i.m. injection in this Phase I study. They studied effects on lymphocyte preparations, and it was especially noticed that β_2-microglobulin was increased in peripheral blood lymphocytes in the treated patients. The side effects were extensive at the higher doses. Their pattern, however, was very similar to the one obtained by injecting patients with natural IFN-α. All of the patients had been treated previously with chemotherapy, radiotherapy, or chemotherapy and IFN. There were some signs of

anti-tumor effects according to the authors, but there were no complete or partial remissions.

A large Phase I trial employing recombinant leukocyte IFN-αA treatment in patients with refractory disseminating malignant tumors was reported in 1982 by Sherwin *et al.* (1982b). Eighty-one patients received IFN by i.m. injections three times weekly for 28 days. There was an escalation of doses in various patients to 136×10^6 units per injection. Toxic reactions resembled those seen previously with natural leukocyte IFN-α. Also, the pharmacokinetics were similar with the subtype A and the natural IFN-α. It was concluded that the recombinant preparation was active *in vivo* since there were nine partial responses among 76 evaluable patients (12%). Of the nine patients who responded, there were five with non-Hodgkin's lymphoma.

Kirkwood *et al.* (1983a) presented immunopharmacological data in a Phase I study employing recombinant IFN-α2 given by the i.m. route in cancer patients. Similar side effects to those found in other studies were noticed. NK cell activity was augmented significantly in the treated patients. It was interesting to see that the effects on NK cell activity and T cell subsets were optimal at less than the maximally used dosage of the IFN-α2. There were partial responses in a few patients—in two melanoma patients and in one patient each with myeloma, Hodgkin's disease, mycosis fungoides, and diffuse histiocytic lymphoma. Responses in these patients were seen at doses of $10–100 \times 10^6$ IU daily.

Ng *et al.* (1983) tried to correlate anti-tumor responses and various parameters in patients with advanced cancer (21 with colorectal cancer and 9 with breast cancer) who were treated with recombinant IFN-αA at doses of $9–50 \times 10^6$ IU/m^2 every other day for 3 months. The patients had undergone very little or no prior treatment, and their performance status was good. It was concluded that the recombinant IFN-αA caused measurable, reproducible, and sustained increase in some parameters that might be relevant for anti-tumor immune activity. However, it was not possible to correlate these effects with any tumor responses, since there were very few such responses in the total material. In 1983 Abrams *et al.* reported data in a Phase I trial with recombinant leukocyte IFN-αA given as a highly purified preparation with a specific activity of 2×10^8/mg into s.c. and nodular metastases in refractory patients with disseminated malignancies. Up to 86×10^6 IU was given per lesion. In one patient with melanoma, there was a minor regression observed, but in the other patients there were no signs of response. Edelstein *et al.* (1983b) reported on 27 patients with

various types of malignancies of nonreticuloendothelial type that were treated with recombinant IFN-α2 as a single i.m. injection to determine the maximum tolerated dose. It was found that 100×10^6 IU caused severe fatigue which was dose-limiting. All doses above 3×10^6 IU produced the flulike syndrome well known from earlier IFN studies. In their paper, the authors stated that 3 of 19 available patients had partial remission and 12 had disease stabilization.

In a paper presented in 1983, Quesada and Gutterman described the effects achieved by injecting recombinant IFN-αA into 37 patients with metastatic cancer. The doses employed were escalated from 9 to 86×10^6 IU. The preparation was given by i.m. injection twice per week. Peak serum concentrations were obtained 6–8 hours after injections, i.e., resembling what is found when natural IFN-α is injected. The side effects, which were reversible after discontinuation of the treatment, included the ones usually seen by administering natural leukocyte IFN at high doses to patients. Antibodies to the subtype A of the IFN-α were detected in three patients as long as treatment was continued. One patient was especially interesting since a drastic reduction in the αA concentration in the serum coincided with relapse of the disease. Partial remission was achieved in three patients: two with nodular lymphocytic poorly differentiated lymphoma (NLPDL) and one with cutaneous T cell lymphoma. Duration of the response was 18 or more weeks in the two patients with NLPDL and about 4 weeks for a cutaneous T cell lymphoma patients. Minor responses lasting from 13 to 20 weeks were found in an additional patient with NLPDL, one patient with Waldenström's macroglobulinemia, one patient with breast carcinoma, and one patient with renal carcinoma. Of the 37 patients treated, 16 reached at least the 68×10^6 IU level. There was a total of 11 lymphoma patients, 9 breast carcinoma patients, 9 colon carcinoma patients, 4 renal carcinoma patients, 3 sarcoma patients, and 1 patient with ovarian carcinoma in these studies. The data indicate that perhaps lymphomas should be one of the choices whenever IFN therapy is considered for trials at the clinical level.

A general research group for the clinical evaluation of IFNs was organized in Japan by the Ministry of Health and Welfare. The group studied the efficacy of recombinant leukocyte IFN-αA as an anti-tumor agent. There are already available Phase I and Phase II reports employing this IFN (see Taguchi *et al.*, 1983). The IFN injected was a recombinant IFN-αA preparation in which IFN had been purified to 98%. In nine medical centers, Phase I studies were initiated and single i.m. injections were given containing up to 100×10^6 IU per

injection. Serum IFN levels and adverse reactions were similar to those reported in other studies. Phase II studies were then made on eight subgroups of cancer patients. The studies were performed at a total of 129 medical centers. So far, 405 patients have entered the studies. For breast cancer, there were 16 evaluable patients and no responders. The results on stomach cancer, colon cancer, and lung cancer are not available, since too few patients have entered so far. For bone and soft tissue cancer, there were 28 evaluable patients with two partial responses (7%) and one minor response. For multiple myeloma, there were six partial responders among 28 patients (response rate 21%). For renal cell carcinoma, there was one complete response and four partial responders (response rate, 13%) among 38 evaluable patients. In addition, there were four minor responses. There were three partial responses among 21 patients having brain tumors (14%), and two minor responses were seen in this group too. In melanoma, there was one partial response in 16 evaluable patients (response rate, 6%). Two minor responses were also seen. For malignant lymphoma of the skin, the results are interesting, since complete remission has so far been achieved in all of three evaluable patients. Serum IFN levels were dose related, and major adverse reactions were like those seen with other preparations. What causes concern is that serum anti-IFN antibodies were detected in 25 of 153 tested patients (16%). However, no severe clinical reactions explainable by antigen–antibody reactions were reported. Injections were given by the i.m. route.

At the NIH IFN workshop in 1983 (Foon *et al.*, 1984a), a Phase II efficacy trial using recombinant leukocyte IFN-αA was reported on in patients who had failed at least one standard combination chemotherapeutic regimen. The patients received recombinant leukocyte IFN-αA at a dose of 50×10^6 IU/m^2 by i.m. injection three times weekly for 3 months or longer. Sixty-four patients had been evaluated in the autumn of 1983: 41 with NHL, 9 with CLL, and 14 with cutaneous T cell lymphoma. Objective evidence of anti-tumor activity was found in 16 of 25 patients with favorable-histology NHL (response rate, 64%). There were 2 complete and 14 partial responders. In cutaneous T cell lymphoma, 8 of 11 patients showed partial response, which would indicate a response rate of as high as 73%. Two of six patients with unfavorable-histology NHL had brief responses, and this was also true of three of eight patients with CLL. Duration of the response seemed to be rather hopeful for the favorable-histology NHL cases and possibly also for the cutaneous T cell lymphoma cases. Side effects were as reported previously in similar studies. It will be exciting

to see whether this group will combine IFN therapy with its monoclonal antibody system (Foon *et al.*, 1983a).

An interesting approach to the treatment of advanced malignancies was initiated by Welander *et al.* (1984). They based their studies on findings from clonogenic assay studies indicating that cytotoxicity is related both to peak levels of recombinant IFN-α2 and duration of cell exposure to this treatment. Doxorubicin, which was also used in these studies, is a cytotoxic drug that is dependent on cell exposure time. A system was set up in which simultaneous i.m. injection of 10×10^6 IU/m^2 and i.v. infusion of 10×10^6 IU/m^2 of recombinant IFN-α2 was given. One hour later, an i.v. infusion of doxorubicin was made at a dose of 20 mg/m^2 given over 2 hours. The patients received courses of this regimen three times, once weekly. After 2 weeks of rest, the responses were evaluated. There were 14 patients who had received the treatment. There was some nausea and vomiting, but generally, the patients seemed to tolerate the treatment well. No cardiac toxicity was reported. Of six patients who were evaluable for tumor response, there was partial remission in one with ovarian and two with cervical cancers, but progression in two ovarian cancer patients and one with colon carcinoma. This type of treatment is to be continued, and more studies are to be made on its clinical efficacy.

IFN-β has also been used for screening tests. IFN-β prepared from the clonal line M-108, which is derived from an SV40-transformed human skin fibroblast, was used on 12 patients with different types of advanced cancer (McPherson and Tan, 1980). The IFN was administered i.m. in doses up to 10×10^6 IU three times weekly for 2 weeks. At this dose, the IFN-β could be detected in the plasma. Also, bolus injections given i.v. were followed by an immediate peak of plasma activity. Continuous infusion over 2 hours was found to give a dose-related level of activity. There was a 21-year-old man in this study with acute myelogenous leukemia (AML) who had blast cells trisomic for chromosomes 8 and 21. His peripheral blast count decreased dramatically from 59 to 0% and increased again when the IFN therapy was withdrawn. The other patients, seven with solid tumors and the remaining four with hematologic malignancies, did not show any response during this short-term trial.

The work being done on the administration of IFN-β in humans up to 1981 was summarized by De Somer (1982). Data on the Belgian experience with IFN-β in patients can also be studied in other articles (Billiau and De Somer, 1980; Billiau *et al.*, 1979b). Two metastatic osteosarcoma patients resistant to chemotherapy were treated with 3.5×10^6 IU of IFN. Likewise, a neuroblastoma patient was treated with

1.7×10^6 IU and three myeloma patients were treated with $3–10 \times 10^6$ IU. There were also five patients with metastatic skin epithelioma in the head–neck region, three patients with metastatic breast cancer, and one patient with nasopharyngeal carcinoma who were treated in Belgium and partly also in collaborative studies with French investigators. Some small metastatic nodule regression in a breast cancer patient, regression of the nasopharyngeal carcinoma, and some stabilization of the myeloma (light-chain disease) in one patient were observed. The i.m. injections were given at a dose of $3.5–5.9 \times 10^6$ IU daily or every other day for 1–3 weeks. There was also some regression of small verrucae in one renal transplant patient and some effect also on two patients with laryngeal papilloma. There was partial regression of a papilloma in a third patient given a short course of IFN-β treatment and some regression of condylomata was also observed. So, all in all, these trials were encouraging on the benign tumor variants. Toxic effects were similar to the ones reported for IFN-α. However, the results obtained in the malignant diseases brought about a rather pessimistic picture. De Somer ended his article with the words "IFNs remain in the stage of potent drugs in search of a disease."

Furue (1982) injected human IFN-β into 17 patients with malignant diseases. He gave single i.v. administration, i.v. drip infusion, or i.m. injections. The results from these studies were that with the natural IFN-β employed, usually at a dose of 3×10^6 IU, therapeutic effects could only be obtained by i.v. injections. There were three cases of complete remission in which IFN was administered subsequent to or in combination with chemotherapy. It cannot be said how big a part the IFN treatment played, and combination studies clearly have to continue in a controlled manner in order to give additional information. An increase in NK cell activity could also be seen when similar doses ($3–6 \times 10^6$ IU) of human natural IFN-β were given i.v. daily to tumor patients (Ogawa *et al.*, 1982). An increase in the delayed-type hypersensitivity reaction to recall antigens was also observed in most of the patients after human IFN-β treatment. Ogawa *et al.* treated 17 cancer patients with $3–6 \times 10^6$ IU of natural human IFN-β i.v. daily. One patient with CLL showed partial remission, and there were three patients showing stable response. Otherwise, no beneficial effects were registered. The side effects included the ones normally seen after injection of natural IFN at this dosage. Ezaki *et al.* (1982b) presented data on patients with various malignant diseases, most of whom had previously been treated by other means, injected with either natural human IFN-β or natural IFN-α at doses of $3–6 \times 10^6$ IU daily. The IFN-β was given i.v. and the IFN-α i.m. Twenty-nine pa-

tients were treated with the IFN-β and 21 with the IFN-α—50 patients altogether. Of 27 evaluable patients treated with IFN-β, there were two partial remissions (one in CLL and one in multiple myeloma). In addition, there were nine patients with stable disease. With the IFN-α, there were two partial remissions (both patients having multiple myeloma) and 10 patients with stable disease. By comparing these two types of IFNs, so far these investigators have not seen any difference in the anti-tumor spectrum and total toxicity. Chills and leukopenia were perhaps more frequent in the IFN-β-treated patients, whereas gastrointestinal problems, malaise, and thrombocytopenia were more common in IFN-α-treated patients. NK cell activity was increased with both types of IFNs but reached the highest activity in the peripheral blood at 24 hours after initiation of the IFN-β treatment and at 48 hours after injections of IFN-α (Ezaki *et al.*, 1982b).

Koyama at the National Medical Center Hospital in Tokyo has summarized the results of a cooperative clinical study employing human IFN-β at a dose of $3–12 \times 10^6$ IU/day given by i.v. infusions, i.m. injections, i.t. injections for skin malignancies, and local administration for brain tumors (1983). Pharmacokinetic studies were performed, and anti-tumor effects were evaluated in patients having a total of 307 malignant tumors. IFN could be detected in the serum and toxicity was as expected on the basis of previous studies. In the treatment of malignant brain tumors, 10 institutions throughout Japan participated. The dose in these cases was $3–6 \times 10^6$ IU in the case of i.v. drip infusion and $1–6 \times 10^6$ IU for local administration. Here, the IFN was administered into the cerebral ventriculus via an Ommaya's reservoir. Of 28 cases treated, there were three responses (11%). When the patients were treated by infusion and the Ommaya's reservoir was used, there was one complete response and five partial responses among 32 cases (19%). Dermatological institutions also participated in this multicenter trial. Especially promising results were obtained in 20 cases with malignant melanoma with metastasis in which there were one complete and nine partial responses among 23 cases (response rate, 44%). In the other tumors treated, the results were more difficult to evaluate. All skin tumors made up a response rate of 33% in 43 cases with 2 complete and 12 partial responses (see Koyama, 1983). These clinical studies are now being extended. The same group found 4 partial responses among 11 multiple myeloma patients treated (response rate, 36%).

Kato *et al.* (1983) treated 84 patients with various malignant tumors with human IFN-β in Japan. Highly purified IFN preparations were

employed. In an i.v. administration group containing 35 patients, 3–5 × 10^6 IU of IFN-β was given weekdays over a period of 2–12 weeks. In the second group, 19 patients were administered 10 × 10^6 IU three times a week for 2–9 weeks. In the third group, three patients were given 50 × 10^6 IU twice a week for 4–9 weeks. In the fourth group, local treatment was performed in which the dose was 3–5 × 10^6 IU administered weekdays for 2–4 weeks. This group consisted of 18 patients. In the fifth group, finally, a single dose of 10^7 IU was administered three times a week for 2–5 weeks. Fifty-seven of the patients were given the IFN-β by the i.v. infusion route, while the remaining patients were given the IFN by local infusions. In the i.v. group, none of the patients showed complete response but 3 of 57 (5%) showed partial response (2 cases of malignant lymphoma and 1 case of renal cell carcinoma). In the 27 patients receiving local IFN-β, 3 of 27 (11%) were rated as complete responders and 3 of 27 (an additional 11%) were partial responders, making up a response rate of 22%. In addition, 2 patients had a minor response. In the third group, there was 1 partial response in a renal cell carcinoma patient. In the fourth group, there was a breast cancer patient showing complete response and 1 case of mycosis fungoides showing complete response. No relationship was seen in these studies between total dosage and anti-tumor effects. The local administration gave rise to smaller side effects. Anti-tumor effects could be demonstrated in patients with mycosis fungoides, breast cancer, renal cell carcinoma, malignant lymphoma, and bladder carcinoma. Extensive case histories have not been given so far. To summarize, there were 57 evaluable patients with various malignancies, of whom 3 had partial responses and 7 had minor responses after i.v. infusion. Among 27 patients given local infusions, there were 3 complete responders, 3 partial responders, and 2 minor responders. Only 5 of these patients showed progression during treatment. It is interesting that the patients receiving local administration showed a tendency to have a lower incidence of side effects. Whether this is related to the distribution of IFN in the patients is not known.

McPherson *et al.* (1984) have extended their Phase I studies on the use of human IFN-β on human cancer patients. In four patients, human IFN-β was given at a dose of 2.5 × 10^6 IU/m^2 over 30 minutes by constant i.v. infusion for 4 days, Monday to Thursday, each week. In addition to the i.v. infusion, three of the patients received the IFN-β intralesionally. One patient received the IFN i.v. in doses up to 100 × 10^6 IU/day, and he also received the IFN-β intralymphatically and s.c. In the remaining three cases, the IFN-β was given in escalating doses

by i.v. infusion over 30 days for 4 consecutive days each week, with the doses being doubled each week as long as toxicity so allowed. There was some suppression by intralesional IFN-β in one patient with locally advanced recurrent nasopharyngeal carcinoma, and in two other patients receiving i.v. treatment, there were signs of stable disease. There were no partial regressions.

At Tokyo University, Furue *et al.* (1983) injected natural IFN-α and IFN-β and recombinant IFN-α into rabbits and saw that the clearance curve seemed to be similar for the various IFNs after i.v. administration. If, however, i.m. or s.c. injections were made, lower blood concentrations were found for IFN-β. Similar studies were done in patients with advanced cancer. After single injections, it was difficult in the patients to show effects on the various immune parameters studied. Thirty-one cases with advanced cancer were treated; there were six patients with stomach cancer, four with malignant lymphoma, three with liver cancer, three with sarcoma, and one each with colon cancer, renal cell carcinoma, malignant melanoma, multiple myeloma, recurrent cervical cancer, and cancer of the esophagus. Six of the cases were treated with i.m. injections of IFN-α, and 25 cases were treated with i.v. administration of IFN-β. In 23 of the 31 cases, IFN was given on its own. The dose of IFN was $3–6 \times 10^6$ IU daily or 2–3 times per week. In the 23 cases treated with IFN only, there was a minor response in a malignant melanoma patient, who, however, received intrahepatic arterial infusion of IFN-β. Otherwise, there were no responses. Side effects were similar to those reported in other studies. It was concluded that human IFNs have minimal activity at these doses in the treatment of advanced cancer patients.

Human natural IFN-γ was administered i.m. with escalating doses ranging from 1×10^4 to 1×10^6 daily for 8 weeks for tumor patients (Osther *et al.*, 1983a). Side effects were similar to those with IFN-α, but were perhaps less pronounced. There were three transient minor responses in nine patients treated with various malignancies but no partial responses. Hill *et al.* have done a Phase I trial on natural IFN-γ and presented the data in 1982 (Hill *et al.*, 1982). Side effects were similar to those seen with IFN-α and there were some signs of tumor regression in two of three breast cancer patients and one of two malignant melanoma patients. In 1983 the experience at the Cancer Center at Wadley, Dallas, using natural IFN-γ (Georgiadis and Johnson, 1981; Johnson *et al.*, 1981) was summarized (Osther *et al.*, 1983b). Eleven patients with biopsy-proven cancer were treated with natural IFN-γ prepared by these methods. Two patients died quickly because of their bad general condition, not because of the IFN-γ injections.

The starting dose was 10^4 IU escalated to 1×10^6 IU. Of the other nine patients, three had disseminated breast cancer, two had disseminated colon cancer, two disseminated malignant melanoma, one had cervical carcinoma, and one had lung cancer. One of the three patients with breast cancer showed a minor response, two patients with colon cancer showed stable disease, and one of the two patients with malignant melanoma showed a minor response. It was then decided to start Phase II trials. Ten patients were involved in this trial but two were excluded shortly after the start of the trial because of their bad general condition. Of the remaining eight patients, two had CLL, one had plasmacytoma, and two had disseminated breast cancer. One of the patients with breast cancer showed a minor response, while the CLL patients showed stabilization, one of them actually showing a 30% decrease in absolute lymphocyte count. Information is not yet available about the other patients. If these results hold up, it seems to be interesting to continue these studies with natural human IFN-γ. In another report, semipurified natural human IFN-γ was given to five patients with malignancies at 0.5, 1.2, and 4×10^6 IU/day i.m. for 5 days per week for 2 weeks (Hill *et al.*, 1983b). Side effects were similar to those previously seen with IFN-α. In 12 so far evaluable patients in the 1983 report, there was complete response in one patient with renal cell carcinoma and stable disease was achieved in one patient each with breast cancer and malignant melanoma. The serious side effects that limited the dose in this study consisted of constitutional symptoms. Human IFN-γ has also been used in a Phase I trial at the Wadley Institute in Dallas (Osther *et al.*, 1984). They used natural semipurified human IFN-γ which was administered i.m. in escalating doses from 10^4–10^6 IU daily for a period of 8 weeks. Nine patients with various malignancies were treated and three (two with breast cancer and one with malignant melanoma) showed minor responses. There were no partial regressions. These trials are being continued.

In Mexico, three patients with malignancies—one each with medulloblastoma cerebellum, meningioma, and prostatic carcinoma—were treated with human IFN-γ (Peddinani and Savery, 1983). Unfortunately, no details were given concerning the IFN preparation but it was prepared from a natural source. Symptomatic improvement was reported on these three patients. In a preliminary report, van der Burg *et al.* (1984) injected purified recombinant IFN-γ produced in *E. coli* into patients with various malignancies. Eleven patients were started on doses up to 9×10^6 IU/m^2. No tumor responses were seen in these preliminary studies. Let us now turn to more specific treatments aiming at anti-tumor efficacy in particular diseases.

II. Systemic Treatment of Leukemias

Falcoff *et al.* treated in 1966 leukemic children with a few thousand IU of concentrated human leukocyte IFN-α. In these early studies, it was found that low-dose IFN injections could be given repeatedly to tumor patients. In a series of papers, Hill and associates reported their results in the treatment of leukemias with human natural leukocyte IFN-α (Hill *et al.*, 1979, 1980a,b, 1981a,b). Over the years, five patients with acute lymphocytic leukemia (ALL), three with acute granulocytic leukemia (AGL), and one with blastic transformation of chronic granulocytic leukemia (CGL) received high-dose i.v. injections of semipurified human natural leukocyte IFN-α. The patients receiving high-dose IFN treatment ($0.5–5 \times 10^6$ IU/kg of body weight) responded in an excellent fashion, with a decrease in leukemic cells in their peripheral blood. Each of the five ALL patients, two of the three AGL patients, and the CGL patient responded. One patient continued on a standard AGL protocol and then went into complete remission. This was the first demonstration that a reduction in the number of leukemic cells in the peripheral blood could be achieved by injecting patients with high-dose IFN therapy. The results obtained in the treatment of leukemic patients by the group at the Wadley Institute in Dallas have been amply reviewed (Hill *et al.*, 1980b). It is suggested from these investigations that in order to achieve positive induction results with human natural IFN-α, one has to use high initial doses. This group's results on the immune response by injecting patients with semipurified natural IFN-α have also been presented (Khan *et al.*, 1980b). In 1981 they also reported on two complete responses in bladder carcinoma and a minimal response in renal cell carcinoma patients and again reviewed their experience with the leukemias (Hill *et al.*, 1981–1982).

There have been several anti-tumor studies performed with semipurified human leukocyte IFN-α prepared by investigators in Moscow (see Kuznetsov *et al.*, 1979). Soloviev *et al.* (1979) treated 12 children with different types of acute leukemia . All patients were in their first acute period. They found that when IFN was inoculated into the bone marrow (at a dose of $1–3 \times 10^6$), bone marrow biopsy immediately after inoculation showed that 1250–5000 IU/ml could be detected. The authors also found IFN in the serum 2 days after inoculation at a concentration not exceeding 50–200 IU/ml. Subsequent to IFN treatment, these patients received chemotherapy. Soloviev *et al.* again used semipurified natural human IFN-α for treating patients with hematologic malignancies in a 1980 report. They had found in experi-

mental work in animals that a dose of 10^6 IU/kg of body weight did not penetrate into the bone marrow. The same observation was made in humans. Ten hours after i.m. inoculation, no IFN could be detected in the bone marrow in children who had received 6×10^4 IU/kg of body weight i.m. Therefore, it was decided to inoculate IFN directly into the bone marrow. After these injections of 2×10^6 IU, IFN could still be detected in the marrow of some patients 2 days after IFN injections. Fourteen patients were treated, all of whom were children. Thirteen had different types of acute leukemia, and one child had lymphosarcoma. All of the patients were severely ill and had clinical signs of their first acute period of the disease. The amount of blast cells varied between 13 and 93% in 11 of the children. Five patients had lymphocytosis. Half of the children had a total infiltration of blast cells in the bone marrow, while the other half had between 36 and 56% of blast cells. The children were divided into three groups, one group consisting of five patients with acute leukemia injected with IFN i.m. They received 5×10^4 IU/kg of body weight with 12-hour intervals between injections. They received a total of $16–21 \times 10^6$ IU. All of the patients showed clinical improvement during the period of IFN therapy. All of the children, however, retained their infiltration with blast cells in the marrow. After 2.5–3 weeks, chemotherapy was given to all the children. In the second group, seven children with acute leukemia received injections of IFN into the bone marrow three times a week. The dose was 10^5 IU/kg of body weight per day. The treatment persisted for 1.5–4 weeks. Again, all patients showed clinical improvement during IFN therapy, and in three, there were partial remissions, as confirmed by myelograms. Chemotherapy was then started. In the third group, there were two patients who received IFN injections by the i.m. route and also into the bone marrow. Both of these patients responded with partial remissions. The authors concluded that acute leukemia in children can be affected by IFN therapy and that chemotherapy can be instituted after a period of prior IFN treatments.

Marty *et al.* reported in 1981 on their results in Phase I/II trials with semipurified natural human IFN-α given to patients with previously untreated AGL. The IFN was administered every 8 hours for 5 days in seven patients or by slow infusion in one patient. When possible, the patients received a second similar course 2 weeks after completion of their first course. Five patients received just one course. Two patients showed stable disease or possibly a minor response and the remaining seven cases were failures. This French group could not demonstrate any effect by giving IFN-α at these doses (10^4 to 5×10^5 IU/kg of body

weight per day for 5 days) as remission induction therapy. The authors emphasized the need for a general methodology in the use of biological response modifiers in clinical trials.

The preliminary results obtained by the London group in the treatment of leukemias with IFN were presented in 1981 (Rohatiner *et al.*, 1981a,b; see also the article by Malpas, 1983, and data presented here in Chapter 6). The previous investigations by the group at St. Bartholomuew's Hospital in London was followed by another report in 1983 (Rohatiner *et al.*, 1983a) in which 14 patients with AML, who did not respond to conventional chemotherapy, received human lymphoblastoid IFN at the maximum tolerated dose of 100×10^6 IU/m^2 by continuous i.v. infusion for 7 days. Ten patients were evaluable for response. A transient decrease in bone marrow infiltration was seen in two patients. The results were considered negative, although there was a fall in circulating blast cells in three patients. These were short-term studies in which it could be clearly seen that complete remission was difficult to achieve with this type of regimen. A model was developed in which bone marrow aspirates from 23 patients with AML were cultured with human lymphoblastoid IFN. Dose-dependent growth inhibition was observed *in vitro* (Rohatiner, 1984).

An interesting short-term study was performed by Dow *et al.* (1984) on human lymphoblastoid IFN treatment of six children with acute nonlymphocytic leukemia in relapse, two children with myeloblastic crisis of Philadelphia chromosome (Ph^1)-positive CML, and one juvenile CML patient resistant to chemotherapy. The patients were treated with the IFN as a continuous infusion for 10 days, and five patients received 15×10^6 IU/m^2 per day, two patients received 10×10^6 IU, and two other patients received 30×10^6 IU. A colony inhibition test was made on all of the infused patients, and their marrow mononuclear cells were cultured with or without IFN in agar. Enough cells from six patients were obtained to allow *in vitro* cultivation studies. The IFN employed produced dose-dependent inhibition of colony formation. A similar finding was found *in vivo*, showing that 15×10^6 IU/m^2 per day was not enough while 20 or 30×10^6 caused a decrease in blood blast counts. There was no effect on the bone marrow. A direct correlation was found here in the short-term system between the high levels of IFN that had to be used both *in vitro* and *in vivo* to achieve detectable anti-tumor effects.

Bratt *et al.* (1984) treated a 32-year-old female with Down's syndrome who had developed ALL with human natural leukocyte IFN-α at a dose of 3×10^6 IU daily i.m. The number of leukemic cells in the blood showed a decrease, and the proportion of blast cells in the bone

marrow decreased slightly. A large fraction of these cells became vacuolized. After a short initial response, the patient showed disease progression, and IFN was withdrawn after a little more than 2 months of treatment. The response of the patient's lymphocytes to mitogenic stimuli and the NK cell activity in the peripheral blood increased during IFN therapy. The patient then reacted with complete remission to vincristine/prednisone treatment. It is interesting that although this patient had cells containing three copies of chromosome 21, there was a response that did not tally with exceptional IFN sensitivity. The importance of the chromosome 21 influence has been noted in Chapter 6, Section VI.

Normal myeloid stem cells, CML stem cells, and acute leukemia cells can be inhibited by IFNs (Gresser *et al.*, 1970; Williams *et al.*, 1981; Gidáli *et al.*, 1981). Therefore, Talpaz and Gutterman started to treat patients with CML with natural IFN-α (Talpaz *et al.*, 1982; Talpaz and Gutterman, 1983). At the time of the IFN Rotterdam Conference in 1983, hematological remission had been registered in five of seven patients. Giving 9×10^6 IU daily in three patients resulted in all three achieving hematologic remission. After having attained remission, the patients were given 3×10^6 IU daily as maintenance. This group reported in 1983 (Talpaz *et al.*, 1983a,b) on nine patients with refractory CML with severe symptomatic thrombocytosis who had been given natural semipurified human leukocyte IFN-α. A significant decline in the platelet counts was seen in all treated patients. Two patients were treated for an extended period of time, and here one noticed disappearance of cells with an extra Ph^1 chromosome and an apparent remission of blast cells in the bone marrow in one patient. The patients received 9×10^6 IU of the IFN-α daily by i.m. injections. This caused marked toxicity requiring cessation of treatment in four patients. The most severe reaction consisted of severe muscle pain. It is interesting in this study that leukocytes and platelets responded differently to chemotherapy and to IFN in the sense that the platelets were more sensitive to the cytoreductive effect of the IFN, while chemotherapy more effectively depressed leukocyte counts. This might indicate that a proper combination perhaps should be looked for between IFN and chemotherapy in order to achieve therapeutic control of CML. The side effects and especially the neuromuscular pain could be treated with steroid and indomethacin. Whether such symptomatic treatments might affect the anti-tumor effect of the IFN preparations has not been studied in detail, however.

Talpaz *et al.* (1983c) also reported on seven patients with CML injected with partially purified natural human leukocyte IFN-α at a

dose of 9–15 × 10^6 IU daily by i.m. injections. Hematologic clearance of the disease was seen in five patients. In the responding patients, a reduction was also seen in white blood cell count, serum B12, and LDH levels. Three patients had enlarged spleens, and they all showed a decrease on treatment. The responding patients were later maintained on an IFN-α dose of 3 × 10^6 IU daily. The follow-up time in this report was not very long. This should be an interesting disease to treat with IFN, not only because of the clinical effects but also in order to study the oncogene activation inherent in the proliferative advantage of the Ph^{1+} cells.

At the meeting of the American Society of Hematology in 1983, information was given on 25 evaluable patients in the benign phase of CML and treated with semipurified natural leukocyte IFN-α at a dose of 3–9 × 10^6 IU i.m. daily (Talpaz *et al.*, 1983b). Six patients had been treated previously with single-agent chemotherapy; the other patients were previously untreated. In 22 of the patients (88%), normalization of peripheral blood parameters was achieved. The spleen returned to normal size in four and decreased significantly in size in 10 additional patients of 15 with splenomegaly. The responding patients had been maintained for a medium of 10+ months. In one patient, blast transformation occurred after 2 months. It is interesting that in 7 of the 11 patients who had been followed for more than 10 months, there was a decline in the number of Ph^{1+} cells in the bone marrow. It remains to be tested whether human IFN-α can have an effect on blast transformation, as suggested by the authors.

Mathé *et al.* (1981) treated nine patients with B cell chronic lymphocytic leukemia with semipurified natural human IFN-α. The IFN was administered in 10-day cycles separated by 10- to 15-day periods of rest. Doses were escalated in seven patients. During the first cycle, 1.5 × 10^6 IU was given every day. Later, the daily dose was escalated to 3 and further to 6 × 10^6 IU/day every day for 3 months or longer, depending on the results of the treatment. Incomplete reduction was seen three times in tumor masses and a reduction in peripheral lymphocytosis was observed in seven patients. Dose escalation did not lead to further reduction of lymphocytosis but was already seen at 1.5 × 10^6 IU daily. During the intervals between courses, there were usually relapses of lymphocytosis. Two of four patients treated by the continuous treatment schedule showed responses; one was a partial response and the other patient showed disappearance of cutaneous lesions and improvement in blood cell counts. Therefore, there were clearly signs of activity by using this kind of treatment. NK cell activity was raised in treated patients. There were no indications that this

rise was dose dependent. In 1982 Huang *et al.* presented pharmacokinetic studies on patients with chronic lymphocytic leukemia who had been given human lymphoblastoid IFN (Huang *et al.*, 1982a). Twelve patients were given IFN at doses of 3×10^6 IU/m^2 or greater. IFN could be detected in the serum as long as 24 hours after injections. At that time, there were no presentations made concerning antitumor responses. More detailed data were presented at the AACR meeting in 1982 (Huang *et al.*, 1982b) concerning seven patients with refractory CLL treated with doses of $1–6 \times 10^6$ IU/m^2. The schedule consisted of single injections followed by a week of rest and then daily injections from Monday to Friday for 2 weeks. One of the two patients with far advanced Stage IV disease who received 3×10^6 IU/m^2 showed an objective response to single injections. This was considered a partial nodule regression. This patient also had a partial regression on subsequent therapy. The remaining five patients had less progressive disease, and there was more than a 75% reduction in lymphocyte counts in two patients. There was at least a 75% reduction in node measurements in one patient, and there were minor responses in two patients. Of five patients who had some kind of response, three exhibited stable disease 2–4 months after completion of the study. These response figures look hopeful in this group of patients but unfortunately, the responses were of short duration in most of the cases.

Uematsu *et al.* (1983) treated 10 patients with adult T cell leukemia with human lymphoblastoid IFN. Half of the cases were treated simultaneously with steroids. The first three patients were given 3×10^6 IU daily, one case was given 6×10^6 IU daily, and the remaining six cases received, by dose escalation schedules, 3 to 9 or 12×10^6 IU daily. There were two partial responses (response rate, 20%). The first responding patient received 3×10^6 IU/day for 51 days i.m. However, the treatment of this case had to be terminated because of a generalized erythroderma caused by the IFN preparation. The second patient that responded received the IFN every third day on a dose escalation schedule up to 9×10^6 IU per day. Here, the treatment was finally discontinued due to liver dysfunction. Therefore, the administration of IFN-α finally had to be terminated in all cases. It will be interesting to follow the future trials with IFN in this disease, which was first described in Japan in 1976.

In 1983 at the American Society of Hematology meeting, it was reported by Quesada *et al.* (1983a) that of 11 patients with hairy cell leukemia, treated with semipurified natural IFN-α at a dose of 3×10^6 IU daily i.m., 10 (91%) showed remission. There were three patients with complete remissions (response rate, 27%) and seven patients

with partial remissions (response rate, 64%). Remission was always achieved within 2–4 months. Remissions at the time of the report had been maintained from 3+ to 12+ months. This was an important finding since it could imply that IFN-α might become first-choice therapy in the treatment of hairy cell leukemia. This paper was followed by a more extensive report in 1984 (Quesada *et al.*, 1984a), in which the results of treatment of seven patients who had progressive hairy cell leukemia were reported. According to strict criteria for response, three patients had complete response and four had partial response. It would be interesting to also know, however, what happened to the other patients who were reported on at the American Society of Hematology meeting. Clearly, regardless of the absolute final figures, these important observations suggest that IFN might become the therapy of choice in a human malignancy. If so, this could be a disease model in which one might study in detail some of the biological effects after IFN administration to humans. At the M. D. Anderson Hospital in Houston, additional patients with hairy cell leukemia have now been injected with IFN-α (Quesada *et al.*, 1984b). There are a total of 16 patients treated, 13 with prior splenectomy. All patients had progressive disease prior to IFN therapy. In this series, there were three patients with complete response and nine patients with partial response (response rate, 75%), and there were three additional patients with minor responses. Five patients received IFN for only up to 4 months, and further improvement might be possible in these patients. Once remission was obtained, there was a loss of infection in the responders. Nine patients with hairy cell leukemia were treated with semipurified human natural IFN-α in Sweden (H. Hagberg, B. Glimelius, B. Simonsson, M. Björkholm, A. Åhre, and G. Alm, personal communication). One case has been newly diagnosed while eight of the patients had their hairy cell leukemia diagnosed previously. Five of the patients had been splenectomized. Three patients had received intermittent chlorambucil treatment without effect. Lymphocytes from seven of the patients were positive when stained with tartrate-resistant acid phosphatase. Human natural IFN-α was given in semipurified form 5 days per week i.m. at a dose of 3×10^6 IU. Eight of nine treated patients improved in their peripheral blood values so that partial remission was established (response rate, 89%). One patient remained unchanged. One patient still has involvement of bone marrow. This response rate is remarkable.

It will be very interesting to study IFN effects in hairy cell leukemia more extensively. This is partly due to the rearranged heavy and light chains found in this disease and also due to the fact that it might

constitute a malignancy that is intimately involved with the B cell differentiation process (see Korsmeyer *et al.*, 1983). Likewise, it will be interesting to follow up various prognostic factors in hairy cell leukemia and to see how they relate to the response of these patients to treatment with various IFNs (see Hersh *et al.*, 1982). The data accumulated from the studies made so far indicate that host defense parameters seem to play an important role during the course of hairy cell leukemia. It will have theoretical implications to follow the human T cell leukemia viruses (see Kalyanaraman *et al.*, 1982) in patients with hairy cell leukemia treated with IFN.

III. Systemic Treatment of Lymphomas

Horning has summarized the results obtained by IFN treatment of malignant lymphomas (1984). The conclusion from these studies consists of a clear demonstration that both human natural and recombinant IFN-α exhibit activities against nodular lymphomas and that the best results so far have been obtained with relatively high doses of IFN. Another reason for treating lymphoma patients is the presence of herpes zoster/varicella infections. They are common both during initial development and during the course of the lymphomatosis disease (see Goffinet *et al.*, 1982).

Blomgren *et al.* (1976) treated a case of Hodgkin's disease (lymphocytic predominance, Stage IVB) with natural human leukocyte IFN-α i.m. at a dose of 5×10^6 IU daily. A minimal response was obtained. After IFN therapy was stopped, partial remission was obtained through the use of chemotherapy. Ozer *et al.* (1983a) treated eight patients with lymphoproliferative malignancies (NHL, multiple myeloma, and CLL). The patients received six i.m. doses at weekly intervals starting with 1×10^6 IU, continued with 3, then 10, 30, 60, and finally 100×10^6 IU of recombinant IFN-α2. Four patients (three NHL patients and one myeloma patient) had partial remissions, and, in addition, there were three patients (one with CLL and two with NHL) who showed minor responses. The final patient who had myeloma showed progression. Toxicity was dose related and all side effects, which were those commonly seen after IFN injections, were of short duration. IFN-α2 could be detected in the serum of the patients. No anti-IFN antibodies were formed. B cell functional deficits and radioresistant T helper and radiosensitive T suppressor function were unaffected by the IFN administration. There was some minor augmentation of ADCC and NK cell activity at the lower IFN-α2 doses, but there were decreases at the higher doses. At the very high

doses, there was suppression of NK cell activity, ADCC, and blastogenic responses to T cell mitogens and recall antigens.

Synergism between natural human leukocyte IFN and chlorambucil in multiple myeloma patients (N. Dimitrov, personal communication) led to a pilot study in seven heavily pretreated patients with Hodgkin's and non-Hodgkin's lymphoma who had failed therapy either on IFN alone, on chlorambucil alone, or on other treatments. IFN was given at a dose of 6×10^6 IU i.m. per day 5 days per week for 8 weeks and was given together with chlorambucil at a dose of 16 mg/m^2 orally Days 5–9. The whole course was repeated every fourth week. Toxicity was moderate and there was a response in six of seven patients. There were two complete responders [one patient with Hodgkin's disease and one with diffuse lymphocytic poorly differentiated lymphoma (DLPL) and four partial responders [three Hodgkin's lymphoma patients and one diffuse histiocytic lymphoma (DHL)]. In addition, one Hodgkin's disease patient was stable for 4 months. This trial is ongoing and was reported on at the A.S.C.O. meeting in 1984 (Clark *et al.*). Rapson *et al.* (1984) treated 26 patients who had lymphoproliferative malignancies with natural human leukocyte IFN-α. All of the patients had received prior chemotherapy and half of them had received prior irradiation therapy. The dose given was 6×10^6 IU i.m. of the IFN 5 days per week for 4 weeks. If patients showed regression or remained stable after this time, a maintenance schedule of 6×10^6 IU three days per week was given until the tumor progressed. The results are clearly encouraging since, of four patients with Hodgkin's disease, two showed partial regression (50%) and two had stable disease. Of 10 patients with NHL, there were three partial responders (response rate, 30%) and, in addition, two patients were stable. The results in myeloma were worse, since, of nine patients treated, there was only one partial response (11%) and one patient with stable disease. Toxicity was considered tolerable. It was concluded that natural IFN-α has anti-tumor activity against lymphoproliferative malignancies.

The Stanford group (Merigan *et al.*, 1978a) presented in 1978 data on six patients with NHL who were treated with natural human leukocyte IFN-α. Three of the patients had previously received chemotherapy and radiotherapy, and one patient had received radiotherapy only. Three patients had DHL, and three of the patients had NLPDL. The patients received 2.5×10^6 reference IU of the IFN i.m., and this dose was increased to 5×10^6 IU twice daily on the second day. The treatment was continued for 30 days. This series of patients has been extended and a later report (Louie *et al.*, 1981) presented follow-up

data for 11 patients with NHL treated with semipurified human leukocyte IFN-α at Stanford University. After the initial group of patients had been treated, further therapy consisted of giving 5×10^6 IU i.m. twice daily. In total, 60 injections were given to the patients. Of seven evaluable patients with nodular NHL, there was one complete response, three partial responses (total remission rate, 57%), and three minimal responses. The response duration varied from 6 to 12 months. It was emphasized that three patients with histiocytic lymphomas did not respond to treatment. It was also found that patients who had been previously treated with chemotherapy could respond provided they belonged to the nodular lymphoma type.

Recombinant IFN has also been used on lymphomatous malignancies. IFN-α2 was used at a dose of 50×10^6 IU/m^2 per day i.v. every 2–3 weeks for 5 days (Leavitt *et al.*, 1983). The patients treated were 16 advanced disease patients with non-Hodgkin's lymphoma of unfavorable histology. They had received prior chemotherapy and radiotherapy. Some had previously even received IFN, and two patients had actually shown partial response on previous IFN treatment. Eleven evaluable patients were reported, and there were two partial responses (14%) It seems that the recombinant IFN-α2 can have some effect in this disease, but further studies are required in order to find out what subgroup of patients would show benefit from the treatment. Also, optimal schedules have to be worked out.

Siegert *et al.* (1983) treated 10 patients with advanced NHL of low malignancy with natural semipurified human IFN-β. All patients had been treated previously with chemotherapy and irradiation had also been given to some of the patients. Four of the 10 patients had stable disease on IFN when it was given as induction therapy with 4.5×10^6 IU i.v. daily for 4 weeks and thereafter at a dose of 9×10^6 IU i.v. daily for 2 weeks. The patients were then treated with the lower dose, 4.5×10^6 IU i.v., three times per week as consolidation therapy. At that time, there was one partial remission and one questionable complete remission (20%). It was concluded from this study that IFN seems to have activity on NHL. NHL and, in addition, Waldenström's macroglobulinemia patients were treated in a multicenter pilot trial with recombinant IFN-α by Ozer and collaborators (Ozer *et al.*, 1983b). Ten patients were treated. All but one had been previously treated by other means. They had well-differentiated lymphocytic lymphoma (three patients), NLPDL (four patients), nodular mixed lymphoma (two patients), and Waldenström's macroglobulinemia (one patient) and had received $10–25 \times 10^6$ IU/m^2 s.c. t.i.w. for up to 3 months. All patients showed toxicities similar to the ones reported in other trials.

Also, CNS changes could be documented. Two patients (one patient with NLPDL and one with nodular mixed lymphoma) responded, giving a partial response rate of 20%. There were, in addition, two patients with stable disease. Two patients had questionable responses since they were too recent to be evaluable, and therefore the response rate may have been greater. In most of the parameters studied, the IFN seemed to be immunosuppressive, but at lower doses, immunoaugmenting results were obtained. One drawback in lymphoma treatment should be mentioned in this connection. Warrell *et al.* (1983) reported on a case of acute nonlymphoblastic leukemia in a patient with nodular lymphoma whose only previous treatment consisted of 3×10^6 IU of IFN-α, the natural product, from human buffy coat leukocytes. This case is important in view of the rarity of the occurrence of this disease in untreated patients with nodular lymphomas.

The South Eastern Cancer Study Group conducted a Phase II trial of lymphoblastoid IFN given to patients with malignant lymphoma who had failed standard therapy (Gams *et al.*, 1984). The patients received 5×10^6 iu/m^2 three times a week for 8 weeks. The patients who did not respond to this regimen were treated after a 4-week rest period with 30×10^6 IU/m^2 daily for 10 days in order to evaluate the hypothesis that there might be a difference in the results achieved by low-dose and high-dose IFN administration. At the time of the ASCO meeting in 1984, 32 patients were evaluable. Responses were seen in all histologic categories. There was some toxicity, as presented in other studies with a similar preparation. Results were extremely hopeful, and this means that this type of IFN preparation can be considered for further Phase III trials in previously untreated patients. The response rates were as follows: On the low-dose schedule, there were 17 evaluable patients with NLPDL or nodular mixed lymphocytic–histiocytic (NM), and among these there were one complete and three partial responses (24%) and five cases of stable disease. The high-dose patients with similar histology showed one of three patients with a partial response and one stable patient. The next histologic category consisted of diffuse lymphocytic poorly differentiated (DLPD), diffuse mixed lymphocytic–histiocytic (DM), and nodular histiocytic (NH) patients, and here eight patients were evaluable on the low dose. Among these, there were one complete and one partial response (25%) and one patient with stable disease. On the high dose schedule, there were no responders among three patients. Among diffuse histiocytic lymphoma (DHL) patients there were seven evaluable on the

low dose and here there were two partial responses (29%). One of three evaluable patients showed a partial response with the higher dose. It is clear that the lymphoblastoid IFN preparation employed has an effect on malignant lymphomas.

The first Phase II efficacy trial of recombinant leukocyte IFN-αA in previously treated patients with NHL has been summarized by Foon *et al.* (1984b). Fifty × 10^6 units/m^2 of recombinant leukocyte IFN-αA was given by i.m. injections three times weekly for 3 months or longer to 45 patients with advanced NHL. Thirty-eight patients have so far been evaluable for response. Of the 24 patients with favorable-histology NHL, four had complete remissions and nine showed partial responses (response rate, 54%). Three of the 14 evaluable patients with the more unfavorable-histology NHL had partial responses (21%). The median duration of response was found to be longer than 8 months. It is interesting that all of the responding patients had been heavily pretreated with combination chemotherapy. Of special interest was the fact that many patients (nine) had received doxorubicin, which has been considered to be a dangerous drug to use in combination with IFN. These results could emphasize a therapeutic role for recombinant leukocyte IFN-αA, especially in patients with favorable-histology NHL. It is interesting that some patients, when restarted on IFN, showed a complete response (one patient) and partial regressions (two patients) (Stevenson *et al.*, 1984). Clearly, IFN therapy has interesting implications in NHL.

Bunn *et al.* (1984) treated 20 advanced cutaneous T cell lymphoma patients, who were refractory to other types of therapy, with 50 × 10^6 IU/m^2 of IFN-αA i.m. three times weekly. Of the treated patients, 10 had cutaneous tumors, 5 had generalized plaques, 5 had erythroderma, 7 had peripheral blood involvement, 8 had histologic node involvement, and 2 had visual involvement. Partial responses were observed in 9 of 17 evaluable patients (response rate, 53%). These responses lasted for a median of 5+ months, with five responses continuing for 5+ to 19+ months. The side effects of the treatment were the ones expected using such a regimen. The toxicity was of course rather severe at this high dose, and other schedules are now being considered for future treatment in order to reduce toxicity and perhaps also achieve complete responses that have not been seen so far. It would be of interest if these types of schedules could later be combined with monoclonal antibody therapy which has already been initiated on patients with cutaneous T cell lymphoma (Dillman *et al.*, 1984). For studies on T cell leukemias, see the preceding section.

IV. Systemic Treatment of Myelomatosis

Results obtained with chemotherapy for the treatment of multiple myeloma have been rather similar to the ones registered more than 10 years ago (see Alexanian *et al.*, 1972). For reviews on the IFN treatment of multiple myeloma patients, see Alexanian *et al.* (1982) and Priestman (1983c). Some results have already been mentioned in this book.

In the mid-1970s a woman with multiple myeloma, relapsing after 6 years of satisfactory tumor control with melphalan therapy, was given exogenous human leukocyte IFN-α at a dose of 3×10^6 IU twice daily i.m. (Ideström *et al.*, 1979). Side effects were as expected; there was especially some thrombocytopenia. An effect of the therapy was documented since there was improvement of her general health, there was more than a 50% reduction of the M component (an IgG-λ in the serum), and there was also partial remission on the basis of the number of plasma cells that were found in the bone marrow. After 5 months of therapy, there was again a progression. These observations on a chemotherapy-resistant case initiated the first pilot study on multiple myeloma patients with natural human leukocyte IFN therapy. In the first pilot study on previously untreated patients (Mellstedt *et al.*, 1979), four multiple myeloma patients (one with IgG, two with IgA, and one with Bence-Jones myeloma) were given human natural leukocyte IFN-α as the sole treatment by i.m. injections for 3–19 months. Remission was complete in two patients and partial in the other two. This study initiated the randomized studies later performed on myelomatosis patients in Sweden. The future myeloma trials in central Sweden have been conducted by the Myeloma Group of Central Sweden. The results obtained with IFN-α therapy have been updated and presented on many occasions (cf. Strander, 1977a,b, 1981–1982, 1982b, 1983a,b, 1984; Einhorn and Strander, 1978a; Mellstedt *et al.*, 1979, 1982a,b, 1983, 1984; Lönnquist *et al.*, 1981; Strander and Einhorn, 1982c; Einhorn *et al.*, 1982a,b; Björkholm *et al.*, 1983; Åhre *et al.*, 1984). For the most extensive information on results achieved, see Mellstedt *et al.* (1984) and Åhre *et al.* (1984).

In a randomized trial, intermittent high-dose melphalan and prednisolone treatment, which is the routine treatment in Sweden for multiple myeloma, were compared to human natural leukocyte IFN-α administration. The preparation was semipurified and used i.m. at $3–6 \times 10^6$ IU daily. Therapy continued until progression of the disease. There were 55 patients who were randomized to melphalan–prednisolone treatment and 75 were allotted to IFN therapy (due to deliber-

ately skewed randomization for a limited period). In 43% of the melphalan–prednisolone patients there was a response to therapy, as opposed to only 12% of the IFN patients. This difference was mainly due to a low response rate in IFN-treated IgG myelomas; the response rate for the other types did not differ significantly among the treatment groups. In an attempt to increase the therapeutic efficacy of sole IFN treatment, other schedules were then used on IgA and Bence-Jones multiple myeloma patients (Stage A). First, 15 patients were initially given 20–30 $\times$ 10^6 IU daily. The treatment had to be deferred after 3–36 days due to side effects. Five to 99 days after the IFN had been removed, it could be reinstituted, but later it had to be withdrawn again. Thus, in fact these patients were given IFN on an intermittent schedule up to limiting toxicity. Eight patients (53%) responded to this type of treatment. The median time to response was 27 days and the median response duration time was 25–512+ days. This study looked encouraging, and therefore another treatment schedule was formed to reduce the side effects. The patients then received 20 $\times$ 10^6 IU daily for 7 days every fourth week. There were 15 patients in this second study. This time, however, only two patients (13%) responded. It is clear that further studies are required before an optimal dose schedule for IFN can be found for these patients in order to later allow randomized Phase III trials comparing efficacy to conventional therapy (see references above).

Early results reported by American investigators on the treatment of multiple myeloma by natural human leukocyte IFN-α did not look as good as could be expected from these European pilot experiments (Osserman *et al.*, 1980). In a trial sponsored by the American Cancer Society, Osserman *et al.* (1980, 1981) treated multiple myeloma patients with human leukocyte IFN-α. A dose of 3 $\times$ 10^6 IU daily was given i.m. If granulocytopenia did not occur, the dose was increased to 6 $\times$ 10^6 IU daily. The treatment was to continue for half a year. By using this protocol, one was able to demonstrate objective evidence of disease regression in 4 of 21 cases. There was a 20–70% reduction in the myeloma serum protein and a significant decrease of skeletal symptoms in these four patients. In three of the cases, there was also an increase in blood hemoglobin. Transient pyrexia occurred in all cases; also, moderate leukopenia was registered. After completion of the IFN trial, the patients were given chemotherapy and there was then evidence of disease suppression in two of the IFN responders and four of the nonresponders. The same group also used another protocol in which the natural IFN-α was given at a dose of 3 $\times$ 10^6 IU daily for 3 months together with ongoing chemotherapy in patients in

partial remission. The results of these studies were clearly disappointing. It has to be remembered that in these studies on the treatment of myeloma patients, all of the patients had been heavily pretreated with chemotherapy. Alexanian *et al.* reported on multiple myeloma patients treated with doses of 3–9 × 10^6 IU daily for 3 months i.m. Of 12 patients treated, objective partial responses occurred in three patients and minimal responses occurred in two others (response rate, 25%) (Gutterman *et al.*, 1980; Alexanian *et al.*, 1982).

Billiau *et al.* were unable to demonstrate any effects on light-chain disease (1981). IFN-β was employed in these studies and 10 patients were treated. In 1981 Misset *et al.* reported on the treatment of 16 patients with multiple myeloma and two with Waldenström's disease who participated in a Phase II trial in which human natural IFN-β was given i.v. at a dose of 6 × 10^6 IU weekly i.v. or 3 × 10^6 twice weekly i.v. Three patients had to stop the treatment after a few infusions due to side effects. No patients showed partial remission, but there were minor responses in three patients. A Bence-Jones proteinurea disappeared in one patient. The same group also treated meningeal relapse of ALL and these patients were given intrathecal IFN, 1–3 × 10^6 IU daily or every other day, up to remission or failure. One of the patients responded with complete remission. Nine patients were treated with s.c. injections of semipurified natural human leukocyte IFN-α. Seven of the patients received intermittent 10-day courses of 1.5–6 × 10^6 IU. Four patients also received continuous daily administration of 1.5 × 10^6 IU for 3–9 months.

In these studies, tumor mass reduction was seen in three patients (details not given), but a decrease in peripheral lymphocytosis was observed in seven patients. The conclusion drawn from these studies was that IFN therapy had an effect on lymphoid malignancies. Eighteen patients with malignant gammapathies (16 myeloma patients and two with Waldenström's disease) were treated in Villejuif with human natural IFN-β at a dose of 3 × 10^6 IU twice weekly or 6 × 10^6 IU weekly i.v. during at least 3 months (Misset *et al.*, 1982). There were no partial regressions but three minimal responses. The conclusions drawn by the authors were that the IFN use appeared as efficient as single-drug chemotherapy advocated in other Phase II trials.

Constanzi *et al.* (1983) administered IFN-α2 to 19 patients with multiple myeloma. All had previously been treated with chemotherapy. Induction was started with 3 × 10^6 IU/m^2 i.v. This dose was increased every second day to a maximum of 100 × 10^6 IU/m^2 over a 2-week period. Maintenance therapy consisted of giving s.c. administra-

tion of 10×10^6 IU/m^2 of IFN three times weekly for a minimum of 3 months. According to international criteria, there was one complete remission. Ohno *et al.* (1983) treated 16 cases of plasma cell neoplasms with recombinant IFN-αA, and 14 cases were treated with human lymphoblastoid IFN produced by Namalva cells. The recombinant IFN was administered i.m. daily in escalating doses up to 5×10^6 IU every third day. The human lymphoblastoid IFN was administered daily i.m. at $3–6 \times 10^6$ IU. In the recombinant group, there were 14 multiple myeloma patients and one patient each with plasma cell leukemia and primary macroglobulinemia. In the lymphoblastoid group, there were two patients with primary macroglobulinemia in addition to 12 patients with multiple myeloma. In the recombinant group, 16 patients were evaluable: one complete and one partial responder (response rate, 12.5%), five minor responses, eight stable patients, and only one showing progressive disease. In the lymphoblastoid group, there were 12 evaluable patients: two showed partial response (response rate, 17%), four had minor responses, and five were stable, and only one showed progressive disease. Side effects were as expected and noted in more than two thirds of the patients. It is concluded that both types of IFN preparations had activity on plasma cell neoplasms, and clearly, myelomatosis is a disease that should interest IFN therapists.

V. Systemic Treatment of Kaposi's Sarcoma

The Kaposi's sarcoma seen in young homosexual men is a different disease from the earlier described Kaposi's sarcoma (see Finkbeiner *et al.*, 1982). It is characteristic for the Kaposi's sarcoma in young people that it consists of diffuse skin and lymph node involvement and takes a fulminant course. At the Memorial Sloan-Kettering Cancer Center, Krown *et al.* treated epidemic Kaposi's sarcoma with IFN. The idea behind this treatment is that the IFN should augment immune functions that are known to be impaired in this disease. The IFN should also act as an antiproliferative agent and an antiviral agent. There are also other reasons. Krown *et al.* reported their results on Kaposi's sarcoma and immunodeficiency in homosexual men treated with recombinant leukocyte IFN-αA in 1982 (1982b). It could already be concluded that this type of IFN had anti-tumor activity against Kaposi's sarcoma and that it was capable of improving immune functions in these patients. In one study (Krown *et al.*, 1983a; Krown,

1984), recombinant IFN-αA was injected i.m. at a dose of 36–54 × 10^6 IU daily for 28 days. This trial was started as a Phase I study but was extended to Phase II because of the encouraging results. The responders were maintained on a three-times-weekly schedule. Of 35 evaluable patients, eight showed complete response and six showed partial response (response rate, 40%). Thirty-one patients received a much lower dose in subsequent studies, 3 × 10^6 IU i.m. daily for 28 days and then three times weekly. Only a 3% response rate was seen (one partial response).

Clearly, the high-dose results are encouraging in this disease. Data are as yet preliminary, but it seems that some patients can experience extended remission periods. Some claims have been made that chemotherapy treatment should be tried in these patients (Kondlapoodi, 1983), but it can perhaps be argued that chemotherapy treatment of these patients will further destroy their immune functions. The patients might then more easily die from opportunistic infections, while an antiviral effect is reached in patients who achieve complete or partial responses on IFN (Krown *et al.*, 1983c). In one report the Memorial Sloan-Kettering Cancer Center IFN Group has concluded in the treatment of Kaposi's sarcoma with recombinant leukocyte IFN-αA that (1) this IFN-α subtype is active in the Kaposi's sarcoma occurring in AIDS patients, (2) that a low dose is ineffective, (3) that prior serum IFN activity correlates negatively with responses to IFN treatment, and (4) that the responses are associated with a low rate of opportunistic infections (Real *et al.*, 1984). It will be interesting to follow these studies in the future since they have a direct bearing on the important issue of IFN action.

At the ASCO meeting in 1983, Volberding *et al.* presented data on 28 patients with Kaposi's sarcoma who were treated in a randomized trial either with low-dose (1 × 10^6 IU/m^2 s.c.) or high-dose (50 × 10^6 IU/m^2 i.v.) leukocyte recombinant IFN-α2. The IFN was used daily for 5 days on alternate weeks during an induction period of 2 months. Eighteen of the patients had completed this schedule at the time of the meeting. The side effects were those reported previously by using this type of IFN at the high dose while the low dose caused no appreciable toxicity. Partial responses occurred in four of nine high-dose patients (response rate 44%), but only in one of nine patients (11%) treated with the low dose. Three patients in each group had stable disease on the schedules used. Maintenance IFN was continued on responding patients. It was concluded that the high-dose schedule using this type of recombinant IFN-α2 had an effect on Kaposi's sarcoma in eight patients and the studies are continuing. Rios *et al.*

(1984) treated 12 patients with AIDS and Kaposi's sarcoma with human lymphoblastoid IFN at a dose of 20×10^6 IU/m^2 i.m. daily for 60 days. Stable or responding patients were given maintenance doses of 20×10^6 IU/m^2 i.m. t.i.w. All patients had a good performance status before treatment. There were four complete responses and four partial remissions (response rate, 66%) in this group of patients. All of the complete responders were at the time of the report still on maintenance treatment. Toxicity was the same as what had been seen in other trials. Mitsyasu *et al.* (1984) treated 60 patients with epidemic Kaposi's sarcoma with 30×10^6 IU/m^2 s.c. of recombinant IFN-α2 administered three times per week. Fifty-six patients were evaluable. Fourteen patients (25%) demonstrated major objective responses (two complete remissions and 12 partial remissions). In addition, three minimal responses were observed, and 16 patients had stable disease and were still on therapy at the time of the report. Substantial subjective toxicity occurred when high doses were injected s.c., and it is considered that one has to try further studies to find optimal doses and schedules. In the future, it will be easier to evaluate Kaposi's sarcoma patients for treatment since prognostic factors are being analyzed (Volberding *et al.*, 1984). There are also proposals available for staging classification for Kaposi's sarcoma (Krigel *et al.*, 1984; Volberding *et al.*, 1984).

Odajnyk *et al.* (1984) treated seven males with the epidemic form of Kaposi's sarcoma with partially purified natural IFN-γ. The IFN was given at a dose of 5×10^5 IU i.m. daily as two 10-day induction courses with a 2-week interval. This was followed by maintenance therapy, which consisted of the same dose twice weekly. There were no responses. There were also no changes in the immune functions tested pre- and posttreatment. Some lymphotoxin was present in the preparation, but its significance is unknown.

When the treatment of AIDS patients with IFN is discussed, it has to be emphasized that better data have now been presented on the use of chemotherapy in Kaposi's sarcoma, as exemplified by the study of Markowitz *et al.* (1984) with etoposide and bleomycin. The patients tolerated their chemotherapy despite their poor immune status, and all seven patients so far treated responded to chemotherapy, with complete response in four patients and partial response in the remaining three. It would obviously be of interest to use such chemotherapeutic schedules and during periods between chemotherapeutic cycles give IFN therapy both as an anti-tumor agent and as an effort to prevent opportunistic infections, thereby presumably reducing the mortality.

VI. Systemic Treatment of Soft Tissue Sarcomas

Harris *et al.* (1984) treated 13 patients with soft tissue sarcomas (five with liposarcomas, four with leiomyosarcomas, three with fibrosarcomas, and one with malignant fibrous histiocytoma). They used human natural IFN-β and the dose was 5×10^6 IU i.v. over 10 minutes, then 5×10^6 IU i.v. over 3 hours daily for 10 days with the cycles repeated every tenth day. If the disease was stable or responsive, maintenance therapy was to be given after three therapy periods twice weekly. All patients had previously been treated with chemotherapy, radiotherapy, or both. Partial remission (response rate, 8%) was seen in a fibrosarcoma patient. Two other patients showed stable disease. Toxicity did not prevent therapy in any patient. The conclusion was that IFN-β, in the manner used, had low activity against soft tissue sarcomas.

VII. Systemic Treatment of Osteosarcomas

A clinical adjuvant trial employing human natural leukocyte IFN-α was initiated on classical osteosarcomas in central Sweden in 1971, and the results have since been reported on many occasions (Strander *et al.*, 1974, 1977a, 1979a, 1982a,b, 1984a,b; Strander, 1977a,b, 1981–1982, 1982b, 1983a,b, 1984; Cantell and Strander, 1977; Einhorn and Strander, 1978, 1984; Broström, 1979; Ingimarsson *et al.*, 1979, 1980a–c, 1981; Adamsson *et al.*, 1979a,b; Nilsonne and Strander, 1979, 1981; Broström *et al.*, 1980d, 1982; Aparisi *et al.*, 1981; Strander and Einhorn, 1982c; Broström and Nilsonne, 1983). For a more recent update, see Strander *et al.* (1984b). This series of patients has been much debated because of the small number of patients and the fact that it was run as a nonrandomized trial comparing an IFN-treated group with a historical group at the same hospital (Karolinska Hospital) and a concurrent control group consisting of all other classical osteosarcoma patients without signs of metastases at the time of admission to the university hospitals elsewhere in Sweden. The latter group of patients was, up to 1976, not treated with any adjuvant therapy and constituted then a nonadjuvant control group. After 1976 these patients received chemotherapy, with either high-dose doxorubicin therapy or high-dose methotrexate therapy, and they then constituted a concurrent chemotherapy control group. Thus, there are four groups to compare: an IFN-α-treated group consisting of 51 patients, a nonadjuvant concurrent group consisting of 30 patients, a

concurrent chemotherapy group consisting of 21 patients, and a local historical control group consisting of 35 patients. At the beginning of the trial, the IFN employed was concentrated natural human leukocyte IFN-α of low purity (10^4–10^5 IU/mg of protein). In the latter part of the trial, the preparation was semipurified, containing 5×10^5 to 5×10^6 IU/mg of protein. These preparations have been described in detail previously (Mogensen and Cantell, 1977; Cantell *et al.*, 1981). The IFN was given by the i.m. route. During the first month after the diagnostic biopsy, which was open in all cases, 3×10^6 IU of IFN was given i.m. daily and thereafter the preparation was given to the patients three times per week for an additional 17 months. All of the various variables in the trial, prognostic factors, and so on, have been analyzed in detail (see references above). After 3 years of follow-up, 47% of the patients in the IFN-treated group are metastasis free; the corresponding figure is 48% for the chemotherapy-treated group, 31% for the nonadjuvant-treated concurrent control group, and 14% for the historical control group. At 5 years, the figures in the four groups are 39, 36, 31, and 14%, respectively. The corresponding survival figures at 3 years are presently 57, 57, 34, and 17%. At 5 years, the corresponding survival figures are 50, 36, 34, and 14%. Thus, there is a tendency for the IFN-treated group to do better than the other groups, but there are only numerical differences. It has to be emphasized that the prognostic factors for the historical control groups are worse, meaning that this group (for the moment at least) cannot be used as a meaningful control group in the trial. As expected, on the basis of the prognostic factor analysis, the development of metastasis and survival in the historical group differed from that seen in the concurrent control group (see Broström *et al.*, 1980b,c).

Aspects on diagnosis, prognosis, and endocrinology of osteosarcoma pertinent for evaluation of IFN therapy of this disease have been discussed in a thesis (Broström, 1979). The Swedish osteosarcoma IFN trial has been rightly criticized for not being randomized. What was done was to compare the various prognostic factors in the treated groups in collaboration with an NIH site visit group. On the whole, the prognosis seems to be less favorable for the historical group, as stated above, and there seems to be a definite risk in using this group as a control group for recently treated patients. Similar conclusions have been reached independently in investigations at the Mayo Clinic (see Taylor *et al.*, 1978). In the osteosarcoma IFN trial, there was a boy with a chondroblastic Grade III osteosarcoma who received long-term IFN therapy and survived for more than 5 years (Broström

et al., 1980a). Multiple cerebral metastases constituted the first sign of tumor spread, perhaps arguing for an anti-tumor effect of the human leukocyte IFN in the periphery.

Several of the patients included in the osteosarcoma trial received bone grafts. In this connection, it is interesting to know that mouse IFN treatment of mice did not prevent induction of heterotopic new bone formation (Broström *et al.*, 1983). It is also interesting that glucose tolerance is decreased in the osteosarcoma patients, probably due to decreased peripheral sensitivity (Adamsson *et al.*, 1980). No obvious effects on insulin levels by IFN treatment were found in our patients (Broström and Strander, unpublished observations).

A preliminary report on acute infections in the IFN-treated patients with osteosarcoma has been published (Strander *et al.*, 1976a). The results here are interesting and discussed in Chapter 13. Over the last few years, we have tried to treat pulmonary metastasis in this group of patients with a combination therapy consisting of IFN-α treatment, 3×10^6 IU i.m. given daily five times a week, 2–4 hours before 1.5–2 Gy irradiation to both lungs (total dose, 20 Gy). The results are too premature to evaluate. It is interesting that two patients presenting at admission with pulmonary metastasis, not being participants then of the prophylactic trial, have shown stabilization of their disease for 10 months and 50+ months after IFN and irradiation treatment.

It is clear that our osteosarcoma trial has been rather encouraging, but no firm conclusions concerning therapeutic effects can as yet be drawn, although there is indirect evidence that there might be some effect of the IFN therapy (see the references above). It would be extremely important if a randomized prospective trial could be initiated on a similar group of patients elsewhere since results with various therapies on osteosarcoma patients have been so debated and questioned (see Lange and Levine, 1982). Extremely successful treatment of human osteosarcoma patients employing multichemotherapy has, however, been reported by one group (for a review, see Rosen and Nirenburg, 1982). In the Nordic countries, a trial is being conducted to see if results similar to those reported by Rosen and collaborators can be obtained. No results are as yet available.

Kishida *et al.* reported in 1975 that human leukocyte IFN preparations were able to cause measurable regression of pulmonary metastases of advanced osteosarcoma in a patient (Kishida *et al.*, 1975). In Kyoto this group of investigators treated four patients with osteosarcoma who had pulmonary metastases after the affected limb had been amputated (Ito *et al.*, 1980; Ban *et al.*, 1982). The IFN was semipuri-

fied and given at a dose of 3×10^6 IU 2–3 times per week (mainly i.v.) in the first patient and i.m. twice a week to the second and the third patients, while the fourth patient received 5×10^5 IU twice a week and then the dose was increased to 1×10^6 IU twice a week. The last two patients did not respond to treatment but the first two patients had partial remissions. It can be mentioned that Ban *et al.* also treated two patients with primary lung cancer using the same type of IFN preparation. In these cases, no suppressive effects were seen. The dose given to both patients was, however, small.

Eleven patients with metastatic osteosarcoma and one patient with chondrosarcoma were treated by Caparros *et al.* (1982) with human natural leukocyte IFN-α given i.m. daily for a minimum of 30 days. Doses were escalated from 3 to 10×10^6 IU/day after 1–3 weeks of treatment. Most patients started directly on 10×10^6 IU/day. All of the patients except one had had previous chemotherapy, and their metastases were localized either to the lungs or bone. No objective response was registered in the osteosarcoma patients. The chondrosarcoma patient continued to have stable disease for 1 year while on IFN. It would be interesting to learn more about that patient's previous history.

A large, multicenter, well-constructed, cooperative adjuvant chemotherapy study on osteosarcoma patients was run in West Germany between December 1979 and March 1982 (Winkler *et al.*, 1983). One hundred ninety-two patients were allocated, but 41 were excluded from the study for various reasons. One hundred fifty-two remaining patients were randomized to receive either a combination of bleomycin, cyclophosphamide, and dactinomycin or *cis*-platinum within a course of sequential multidrug chemotherapy, which included doxorubicin and high-dose methotrexate. A second part of the study consisted of 100 selected patients remaining after the exclusion of 51 patients, who were randomized, after preoperative chemotherapy and surgical removal of the primary tumor, once more to receive natural IFN-β or not. Some stratification was also included in the trial. The IFN preparation was prepared from fibroblasts, and it was a natural preparation that was semipurified. In the report of 1983, there was no difference between the combined groups receiving dactinomycin versus *cis*-platinum or IFN versus no IFN. It will be interesting to follow these patients and see at the next follow-up what happens with long-term survival. The IFN was applied at a dose of 10^6 IU/kg of body weight as a 30-minute i.v. infusion twice weekly, starting on Week 16. It was given daily from Week 19 when the surgery was done, through Week 22 and then again twice weekly through Week 27. Sixty-one

clinics participated in the study. In the trial, called COSS 80, there was no difference between the groups receiving or not receiving IFN-β, but it is much to early to draw any final conclusions (Treuner *et al.*, 1983a).

Combination therapy of osteosarcoma with IFN as one agent is an interesting concept, and it should be mentioned that a monoclonal antibody directed against a human osteosarcoma cell line has been developed by Embleton and co-workers (1981) and that such an antibody is already being tested clinically for various purposes (R. Baldwin, personal communication).

VIII. Systemic Treatment of Malignant Melanoma

Many of the melanoma trials have been discussed in Chapter 9, Section II, but sole systemic treatment has also been employed. In 1981, eight patients at Westminster Hospital with metastatic malignant melanoma were treated with human lymphoblastoid IFN (Retsas *et al.*). Treatment consisted of i.m. injections of a daily dose of 2.5×10^6 IU/m^2 for 30 days. Six of the seven patients had been previously extensively treated with chemotherapy. In one of the patients with lymph node metastases, there was stabilization of the disease for 5 weeks, but all other tumors in other patients progressed. In 1981, there were partial regressions in two of the Westminster patients included in their Phase I and Phase II studies. This corresponded to a response rate of ~10%. It is difficult to give a precise figure as patients were treated in different ways. Since two patients had partial remissions, since there was a stabilization in one patient, and since there was also a response in a patient given intralesional injections, the conclusion from this study in 1981 was that lymphoblastoid IFN-α had some activity on advanced melanoma (Priestman *et al.*, 1981). Compiled Westminster data on advanced malignant melanoma patients were presented in 1983 (Retsas *et al.*, 1983b). Seventeen patients with metastatic malignant melanoma were treated with human lymphoblastoid IFN. Fifteen of the patients received i.m. injections, one patient had i.m. injections followed later by i.v. infusions, and the last patient received the injections i.t. The intended dose in these patients was 2.5×10^6 IU/m^2 by i.m. injections for 30 consecutive days before evaluation. The i.v. infusions were given at a dose of 26×10^6 IU per 12 hours once a week. One patient showed tumor regression and had a partial response (6%). This response lasted for 6 months.

Data obtained at the Memorial Sloan-Kettering Cancer Center in the treatment of malignant melanoma with IFN were reviewed at an

NIH meeting in 1983 (Krown, 1984). In a previous multicenter study, 1, 3, or 9×10^6 IU of natural human IFN-α was injected i.m. daily for 42 days into patients with generalized malignant melanoma. Among 44 evaluable patients, there was one partial response (response rate, 2%). It became known at about the same time that another trial using natural IFN-α in malignant melanoma had failed. In that study, 0.3, 3, or 20×10^6 IU were given i.a. daily for 28 days, and there was no response in 16 patients (Hawkins *et al.*, 1982). Therefore, these trials were not promising. Since there were some positive hints from trials going on with recombinant IFN-αA and IFN-α2 in malignant melanoma, and since it had been reported at least from two sources that the combination of IFN and cimetidine could lead to complete responses in melanoma (see Chapter 9, Section II), it was decided to do a trial at the Memorial Sloan-Kettering Cancer Center to determine whether i.m. administration of natural IFN-α with cimetidine given orally would lead to regression of melanoma metastases. In that study, IFN was given at a dose of 3×10^6 units 5 days per week. In some patients, the dose was increased to 9×10^6. Also, i.t. injections were tried on some patients. Nineteen patients were evaluable at the time of the NIH meeting in the autumn of 1983. Only one patient had a partial response (5%) while six others showed some signs of anti-tumor activity. The experience so far with melanoma at the Memorial Sloan-Kettering Cancer Center has not been encouraging (see also Krown *et al.*, 1981a).

Ernstoff *et al.* (1983c) reported that in seven patients with metastatic melanome receiving i.v. recombinant IFN-α2 side effects were registered at 10 and 30×10^6 IU daily. The tumor response could be evaluated in six patients, and there was one complete response and one partial response, and two patients had stable disease at the time of the report. Creagan *et al.* at the Mayo Clinic reported in 1983 on the use of leukocyte IFN-αA given to 23 patients with disseminated malignant melanoma. The patients received 50×10^6 IU/m^2 three times weekly. Among the 20 evaluable patients, in 1983 they observed three objective regressions (15%). There was one complete responder and there were two partial responders. Seven more patients had stable disease from the time the study was initiated (3 months). Toxicities were extremely severe with heavy weight loss and fatigue. Six of the patients required hospitalization due to the toxic effects. All of the patients had high fever. The conclusion from that study was that one could achieve an anti-tumor effect in malignant melanoma, but that there were substantial complications connected with the treatment. This group then decided to use a reduced dose of 12×10^6 IU/m^2 three

times weekly to study in more detail the response–toxicity relationship.

Due to toxicity seen in the study by Creagan *et al.* (1983) on patients with malignant melanoma, Hawkins *et al.* (1984a) presented data in which IFN-α2 was given at a dose of 30×10^6 IU/m^2 i.v. from Days 1–5 every twenty-second day until anti-tumor progression. Of 29 patients treated in this manner, the dose did not have to be modified in 25. Four patients had to terminate therapy due to hypotension, serum glutamic pyruvic transaminase (SGPT) elevation, or fatigue. One partial response was seen when employing this schedule (response rate, 4%). In addition, three patients had stable disease. In nine of the patients with progressive disease, cimetidine was added to the treatment regimen, but no response occurred in eight of these patients. The remaining patient developed complete regression of lymph node metastases after one such cycle. It is suggested on the basis of this study that one has to be careful when developing cyclic IFN schedules for treatment, as was the case also for myelomatosis (see Chapter 10, Section IV).

Thomson and McLeod (1984) in Australia injected human recombinant leukocyte IFN-αA into advanced melanoma patients. IFN was given at a dose of 10×10^6 IU/m^2 i.m. twice weekly for 12 weeks. Twelve patients have been treated so far, and six of these had previously been treated by other means. There was one partial response (8%) lasting for 14 weeks and two stable responses for 4 and 8 weeks, respectively. Toxicity was mild to moderate and of the same magnitude as that seen in other similar studies. The authors suggested that one should try IFN therapy in combination with chemotherapy or together with other biological response modifiers. Goldberg *et al.* at Georgetown University treated generalized malignant melanoma patients with lymphoblastoid IFN in Phase II trials (Goldberg *et al.*, 1984). Seventeen patients received four cycles of 15×10^6 IU/m^2 per day on Days 1, 3, 5, 8, 10 and 12 with 1-week rest periods. There was one complete remission and one partial remission (response rate, 12%). In addition, they saw minimal response in one patient and one patient had stable disease. Patients then received 0.5×10^6 IU/m^2 per day once weekly for 12 weeks, and here they did not see any responses. Eight patients had been treated with 0.5×10^6 IU/m^2 per day weekdays for 6 weeks, and here no responses were seen either. Toxicity was what could be expected on the basis of previous studies. This group now believes that one should probably give human lymphoblastoid IFN in malignant melanoma at doses near the maximum tolerated dose and that one should use prolonged treatment periods.

In Chapter 9, Section II, I have discussed results obtained by the combination of IFN and cimetidine. In some of the studies employing this combination, the IFN therapy was given systemically. Hill *et al.* presented their first data on combination treatment of natural IFN-α and cimetidine in malignant melanoma in 1982, and the results seemed to confirm what other investigators had found in Lund, Sweden (Hill *et al.*, 1982). In 1983 the group in Dallas treated 19 patients with all stages of recurrent malignant melanoma, who also had percutaneously injectable lesions, with intralesional natural IFN-α therapy. IFN-α was given at a dose of 6×10^6 units 5 days weekly and also cimetidine 300 mg p.o. q.i.d was given at the same time. Of 16 evaluable patients, five showed a clear response—two complete and three partial (total, 31%). They also saw one minor response and four patients with stable disease during the treatment period. The authors concluded that their data supported the findings of Borgström *et al.* (1982) (see Chapter 9, Section II). In the study by Hill's group, both injected and uninjected lesions (also lung metastases) responded to treatment (Hill *et al.*, 1983a–c), which argues for a systemic effect. A multicenter study is now being performed in Sweden, where patients are treated with either cimetidine, 1000 mg daily, or i.m. human natural leukocyte IFN-α at a dose of 3×10^6 IU daily 5 days per week. After 4 months of treatment, if there is a failure on this therapy or if the disease is stable, combination therapy is initiated with both cimetidine and i.m. IFN. If regression is seen, the treatment is continued until complete regression or progression is established. The trial was recently initiated, and no results are as yet available.

The Yale University group failed to see any response in 14 of 30 patients with metastatic melanoma treated in their two IFN trials [see also Chapter 10, Section I, and Krown (1984)]. Eight patients then received IFN induction of $10–100 \times 10^6$ IU daily i.v. for 5 days per week for a 4-week treatment period. Seven of eight patients had stable disease and continued on maintenance at the same dose 5 days per week every third week until failure. The eighth patient failed therapy at completion of induction and received another drug. IFN and cimetidine were both given and there were no responses. Six patients received induction IFN therapy at 10×10^6 IU/m^2 s.c. t.i.w., and on subsequent combined IFN and cimetidine therapy, no response was noted in this group. It can be rather firmly concluded in this trial that with the schedules and preparations used, cimetidine does not enhance the anti-tumor effects of IFN in the patients with metastatic melanoma who have not responded to sole IFN treatment (Ernstoff *et al.*, 1984).

Lipton *et al.* (1984) used a combination of recombinant IFN-α2 and

cimetidine on malignant melanoma patients. The patients first received 30 × 10^6 IU/m^2 i.v. for 5 consecutive days every third week. The patients who did not respond with regression on that schedule were continued on this IFN dose, but with the addition of cimetidine 1200 mg/day orally. Seven of the patients had cimetidine added to their IFN-α2 therapy. Of these, three had stable disease and four had progressive growth at the time of this addition. On cimetidine, all of these patients continued to have progression of their malignant melanomas. So, in this study employing recombinant IFN, there was no sign of any synergistic action of cimetidine and IFN therapy. At the ASCO meeting in 1984, Slater *et al.* reported on 23 evaluable patients with malignant melanoma who were treated with 300 mg of cimetidine orally q.i.d. and 3 × 10^6 IU of natural IFN-α 5 days per week. Of the 23 evaluable patients, there were two partial (8%) and three minor responses. Again, it was emphasized that this combination had only marginal efficacy in this disease.

In a multicenter study, metastatic malignant melanoma patients were treated with recombinant human IFN-αA (Robinson *et al.*, 1984). In one protocol, 30 × 10^6 IU/m^2 was given i.v. for 5 consecutive days every third week. There were 31 patients treated, and one patient with subcutaneous disease showed partial response after the eighth cycle while another patient responded completely when cimetidine was added. The response rate was 6.5% (2 of 31 patients) when the IFN–cimetidine combination was used. In a second protocol, 32 patients received 10 × 10^6 IU/m^2 of the same IFN preparation s.c. three times per week for a minimum of 3 months. Six of 32 patients (19%) responded. Of these, two patients showed complete response. The reported side effects consisted mostly of flulike symptoms, dry mouth, and weight loss. It is interesting that it looks as if the s.c. route is perhaps associated with a better efficacy, although too few patients have so far been treated. This would suggest that one has to elucidate optimal doses, routes, and schedules prior to doing more extensive trials in malignant melanoma with various IFN preparations. An important question to answer is also whether natural IFN-α now gives better response rates than recombinant IFN-α in this disease and especially in combination with cimetidine.

IX. Systemic Treatment of Renal Cell Carcinoma

The development of IFN therapy for renal cell carcinoma has been exciting. Metastatic renal cell carcinoma can sometimes be difficult to evaluate clinically. Objective regressions of tumors are, on the other

hand, difficult to visualize spontaneously (Holland, 1973; De Kernion *et al.*, 1978). Quesada and Gutterman reported in 1982 on their studies on Stage IV renal cell carcinoma patients injected by partially purified human natural leukocyte IFN-α. There were 36 patients and all had measurable metastatic disease. Twenty patients had lung metastases alone, 13 had lung and bone metastases, and three patients had other sites involved. Twenty-one of the patients had received other therapies. The IFN was given at a dose of 3×10^6 IU/day. Tumor responses were documented in 10 of the patients with partial remission (response rate, 28%), and five additional patients had minimal responses. In 47% of the patients, there was progressive disease during treatment. In one of these latter patients, antibodies to human IFN-α were detected. There were two parameters that correlated with a response; namely, a performance status above 90 and the ability of the human IFN-α preparation given i.m. to induce leukopenia and granulocytopenia. A higher response rate was observed in patients with lung metastases, but this impression could not be statistically documented. Toxicity was as expected with his type of IFN. In 1983, Quesada *et al.* (1983b) published an important paper on a study in which semipurified natural human leukocyte IFN-α had been administered i.m. at a dose of 3×10^6 IU/day to 19 patients with generalized renal cell carcinoma. A partial remission rate of 26% (five patients) was achieved. Several other patients also showed minor responses or disease stabilization, and only seven patients (37%) showed progressive disease during the time of the study. The responses were seen in patients having lung or mediastinal metastases. As found in some other studies, antibodies in these patients did not seem to affect the anti-tumor effect. Again, an important correlation was found between anti-tumor response and the ability of the IFN preparation to cause leukopenia and granulocytopenia. This was also found on patients with metastatic breast carcinoma. If this is true on a more general basis, it might indicate that leukocyte and granulocyte count suppressions have predictive value. This could mean that it is more important to determine how the patient reacts than how the tumor reacts to IFN treatment to be able to predict clinical responses.

In Denmark, seven patients with recurrent renal cell carcinoma who had previously been treated with chemotherapy were treated with human natural leukocyte IFN-α (Magnusson *et al.*, 1983). Five of the patients received daily escalating i.m. doses 4 to 16×10^6 IU during a minimum of 4 weeks. The two remaining patients received intralesional IFN therapy at a dose of 20×10^6 IU. There were no serious side effects reported but also no tumor responses in any of

these patients. Kirkwood *et al.* (1983b) presented results from the American Cancer Society collaborative trial on metastatic renal cell carcinoma in which two doses of naturally produced leukocyte IFN-α were used in a randomized manner. Thirty patients entered the study and the doses employed were 1 and 10×10^6 IU daily. The IFN-α was given i.m. for 28 days. In case of response, maintenance IFN therapy was given, employing the same dose at which the response was seen, but this time the IFN was given three times weekly. IFN-α toxicity was similar to what had been reported previously. An anti-tumor response was observed in 6 of 27 evaluable patients, but there was only one complete and one partial response (response rate, 7.4%). Both of these responses were seen at the higher IFN doses. A patient in Switzerland with generalized renal cell carcinoma showed a complete response on human natural leukocyte IFN-α therapy at a dose of 4×10^6 IU/m^2 (Medenica, 1984).

A current analysis was made of the UCLA data from patients with metastatic renal cell carcinoma treated with natural human leukocyte IFN-α. It was found that the median survival of responders was not statistically different from that of nonresponders (Figlin *et al.*, 1984). The UCLA group has now treated 24 patients with metastatic renal cell carcinoma with a combination of human semipurified natural leukocyte IFN-α at a dose of 3×10^6 IU daily i.m. 5 days per week and vinblastine, which was administered i.v. at a dose of 0.15 mg/kg of body weight to the first treated five patients while in all other patients 0.1 mg/kg of body weight per week was given. Treatment was ambulatory and 23 patients are evaluable for response. There were two partial responses (8%). In addition, there were four minimal responses and seven patients with stable disease, while 10 patients showed progression. Seventeen of the 24 patients (71%) developed myelotoxicity. Many patients (42%) required vinblastine dosage modifications. So far, this study does not indicate that better results are to be achieved with a combination regimen than with human leukocyte IFN-α used on its own.

At the moment, a randomized study is performed at the Karolinska Hospital in which patients with radically operated primary renal cell carcinoma, but with pulmonary metastases, either receive human leukocyte natural IFN-α i.m. at a dose of 3×10^6 IU daily or the standard treatment at the hospital, which consists of a combination of vincristine and bleomycin together with irradiation of the lungs.

Lymphoblastoid IFN has also been used on patients with renal cell carcinoma. At the ASCO meeting in 1983, Retsas *et al.* (1983a) presented their data on metastatic renal cell carcinoma patients treated

with human lymphoblastoid IFN. The trial was initiated in January 1981, and 14 patients were treated. Thirteen of the patients were evaluable. The IFN was given by i.m. injections, 5×10^6 IU daily for 5 consecutive days per week, or 4×10^6 IU daily for 28 consecutive days and thereafter three times per week. The median duration of the treatment was 12 weeks. Toxicity was as recorded previously. The main toxic effects were anorexia, malaise, and lethargy. Mental confusion also occurred. One partial response was seen (response rate, 7%). There were also two patients with stable disease. The study showed that human lymphoblastoid IFN had some activity in renal cell carcinoma. Tazaki *et al.* (1983) used human lymphoblastoid IFN to treat 18 patients with advanced renal cell carcinoma. The treatment was given by the i.m. route with daily injections of 3×10^6 IU and the treatment was continued for 1–3 months. Toxicity was as expected and similar to the one reported in other studies using human lymphoblastoid or natural IFN-α. There was one complete response (6%), two minor responses, and eight patients with stable disease. When adjuvant therapy with a streptococcal preparation was added, the results were one complete response (9%), two minor responses (18%), and six patients with stable disease (55%). It was shown that the streptococcal preparation was able to induce the production of IFN-γ.

In 1983, Kisner *et al.* presented data on their Phase II trial with lymphoblastoid IFN on metastatic renal adenocarcinoma. This was a Southwest Oncology Group study in which the IFN was administered i.m. at a dose of 5×10^6 IU/m^2 t.i.w. to 33 patients with measurable metastatic disease. Twenty-three patients had not received any other therapy previously, three had been treated with chemotherapy, and seven had received irradiation. Dose-limiting toxicities found, as by other groups, were fatigue, chills, fever, and anorexia. Leukopenia occurred in 11 patients (33%). Hepatopathy occurred in 12 patients and constituted an important side effect (36%). In this study, 30 patients were available in the autumn of 1983 for response evaluation and of these, five had an objective partial remission (17%). Eleven of the other patients had, after at least eight weeks of treatment, stable disease, which could mean that the response rate might increase in the future. Again, it was concluded that an IFN preparation had activity in renal cell carcinoma and also that the effects were achieved with tolerable toxicity.

Neidhardt *et al.* (1984b) reported on 33 patients with renal carcinoma treated with human lymphoblastoid IFN, given by i.m. injection at a dose of 5×10^6 IU/m^2 three times per week. The treatments continued for at least 6 months. Five of the patients demonstrated

partial response. In two of these patients, the responses continued for up to 239+ and 300+ days. It was found that prolonged therapy was required on most occasions in order to see a response. Toxicity was rather severe. The types of side effects were similar to the ones reported previously. It is of interest that no correlation was found between the IFN level found in the blood, the clinical toxicity, and the therapeutic response in these patients. The achievement was considered significant since reports on trials in renal carcinoma usually show rare responses, if any, when strict response criteria are employed. It is important that in the treatment of this particular disease there exist cases that show a stable picture for longer periods. Long-term follow-ups are more reliable since less than 10% of these patients are expected to survive for 5 years (Hendry, 1983). Neidhardt *et al.* (1984a) have continued to treat patients with renal cell carcinoma with human lymphoblastoid IFN. Twenty-three patients received a 10-day escalating regimen of 2.5 and 10×10^6 IU i.m. followed by 20×10^6 IU i.m. for 7 days. This regimen was repeated every 21 days, and seven courses were given. There was a 38% response rate with one complete remission and four partial remissions. The complete remission persisted for 273 days and the partial remissions lasted for 164+ days. It is interesting that 10 patients who had previously been exposed to human lymphoblastoid IFN failed to respond. These latter patients had shown stabilization or response on a previous low-dose regimen. The responding patients had never been exposed to IFN. The same group has also treated 11 additional patients first with 5, then with 10×10^6 IU/m^2 i.v. on Days 1–2 followed by 50×10^6 IU/m^2 i.v. for 3 days, and this was repeated every 21 days. Of these patients, two of nine evaluable patients had a partial response (response rate, 22%). The studies contained responses of intraabdominal, renal, and pulmonary lesions. This group suggested that one should further define the optimal dosage.

An ECOG pilot study was made on 40 patients with renal cell carcinoma who were injected with lymphoblastoid IFN (Trump *et al.*, 1984). In this study, 23 of 40 patients included in the trial were treated within 2 months after the first diagnosis was made of their renal cell carcinoma. Seven patients in the study were to receive 30×10^6 IU/m^2 per day for 10 days, but toxicity made it impossible to continue on that schedule. Instead, the 33 following patients received lymphoblastoid IFN at doses of $3–20 \times 10^6$ IU/m^2. The first day, they were given 3×10^6 IU, the second day 5×10^6 IU, the third day 10×10^6 IU, and on the following 6 days 20×10^6 IU/m^2. Repetition was made on every twenty-first day. Twenty-eight patients were evaluable for response,

and partial responses were seen in five of these (18%). Three additional patients showed stable disease. These studies aimed at defining optimal schedules and routes of administration. In May 1984, 224 patients with renal cell carcinoma had been injected with human lymphoblastoid IFN in several studies all over the world (J. Whisnant, personal communication). In these patients, there were six complete responses and 21 partial responses (response rate, 12%). It will be interesting to find out how different doses and schedules will affect these response rates and if it will be possible in these large series of patients to find any parameters that would be important to use as monitoring variables when patients with renal cell carcinoma are selected for IFN treatment. Let us now see how successful the recombinant IFNs have been in the treatment of this rather common malignancy.

Quesada *et al.* reported in 1983 on the effect of recombinant leukocyte IFN-αA on renal cell carcinoma. Thirty patients with metastatic renal cell carcinoma, 21 males and nine females, received the IFN by the i.m. route on a daily schedule basis for 8 weeks. The patients were randomized to receive either 2×10^6 or 2×10^7 IU/m^2. Prognostic factors were similar for these two groups of patients. With the highest dose, there were two partial remissions among eight evaluable patients at 8 weeks (response rate, 25%), and there were three minor responses. At the low dose, there were two minor responses but no partial remissions. Among the minor responses, there was relapse of the disease within 4 weeks. The conclusion was that this type of purified IFN was able to achieve an anti-tumor effect on renal cell carcinoma, but that it is possible that substantially higher doses have to be employed when using this IFN than the natural IFN-α to accomplish optimal effects (Quesada *et al.*, 1983c). In 1983 Krown *et al.* (1983b) reported on 27 patients with advanced renal cell carcinoma given recombinant IFN-αA at a dose of 50×10^6 IU/m^2 i.m. three times weekly. All patients had metastatic disease. Responses were documented in 6 of 19 evaluable patients, with two partial remissions (11%) and four minor responses. The responding patients were continued on a similar treatment schedule. The high dose was rather toxic. Yoshida *et al.* (1983) treated 10 patients with advanced renal cell carcinoma with escalating single doses of recombinant IFN-αA. Doses were escalated every third day. The treatment was given i.m. daily, and the dose went up to $18–36 \times 10^6$ IU and was reduced if side effects were too severe. Two of the 10 patients achieved minor responses and six patients were stable. There were no complete or partial responses, however. Also, there was no regression in nude mouse

experiments in which these types of tumors were tested for IFN sensitivity. Also, *in vitro* work in clonogenic assays seemed to indicate the same thing. So the conclusion from these data is that the recombinant IFN employed in these studies was probably not very effective against renal cell carcinoma cells, at least not at the doses and schedules investigated. The studies are continuing and it will be interesting to follow them, especially in order to see whether correlations can be found between various laboratory tests and clinical results.

In 1983, De Kernion *et al.* reported on a series of 43 evaluable patients who had been included in a Phase II trial employing partially purified human leukocyte IFN-α for metastatic renal cell carcinoma. One patient (2.5%) had a complete response, and six patients (14%) had partial responses. It is interesting that an additional 23% of the patients had a minimal response or stable disease after having had growing metastases prior to treatment. So far, the results obtained with this treatment, which consisted of 3×10^6 IU of IFN i.m. daily from Monday to Friday weekly, was considered to be superior to other forms of therapy used at the same institution.

When Kempf *et al.* (1984) found that recombinant IFN-α2 produced a partial response in two of five patients with renal cell carcinoma they decided to initiate a Phase II study with pure IFN-α2. Such a preparation was administered to 26 patients with advanced renal cell carcinoma. The patients were randomized to receive either high-dose IFN therapy, which consisted of 30×10^6 IU/m^2 i.v. daily five times per week every 2–3 weeks, or low-dose therapy, which consisted of 2×10^6 IU/m^2 given s.c. three times a week. After 3 months, the patients were evaluated, and stable or responding patients were continued on IFN. A crossover was then made, so low-dose patients were given high-dose IFN. At the time of the ASCO meeting in 1984, 24 patients were evaluable for response. Side effects were those expected using this type of IFN. Two patients were reported to show responses, but it is difficult to say whether they were partial. Seven additional patients continued on the maintenance schedule. This group concluded that the IFN-α2 preparations had activity on renal cell carcinoma. The recombinant preparations have not been compared to other types of IFNs in this disease. In order to avoid, as far as possible, the high-dose toxicity of recombinant IFN-α given to renal cell carcinoma patients, Einzig *et al.* (1984) treated patients with objectively measurable disease by injecting daily recombinant IFN-αA in gradually escalating doses from 3 to 36×10^6 IU for a 10-day period. The patients were then maintained at the higher dose daily i.m. for 9 weeks. Thereafter,

the responding and stable patients continued on a three-times-weekly schedule. Thirty-one patients have so far been evaluated, and of these, 14 patients had previously not been treated by other drugs. In 24 evaluable patients, there were two partial responses (response rate, 8%) and four minor responses. It will be interesting to see in the future whether the recombinant IFNs are as effective as the other IFNs in this particular disease.

X. Systemic Treatment of Lung Cancer

IFN results in non-small-cell lung cancer have been negative. Stoopler *et al.* reported their negative results in Phase II trials of non-small-cell lung cancer with natural human leukocyte IFN-α in 1980 (see also Krown *et al.*, 1980). No responses were seen in this disease when treatment was given by i.m. injections at a dose of 3×10^6 IU for 30 days. In 52 patients with bronchogenic carcinoma treated with IFN by Krown *et al.* (1982a) and Figlin and Sarna (1983), there has only been one partial response (2%). From these studies, it can be concluded that the non-small-cell lung carcinomas do not seem to be tumors of choice for sole IFN treatment. Figlin and Sarna treated non-small-cell lung cancer and adenocarcinoma of the colon/rectum with semipurified human leukocyte IFN-α. This was a Phase II trial. The dose was 3×10^6 IU daily i.m. 5 days per week. One patient with adenocarcinoma of the lung had a partial response (response rate, 2.6%). A minimal response was also seen, and five patients had stable disease among the lung cancer patients. There were no objective responses in the 19 patients with adenocarcinoma of the colon who had been treated. The conclusion was that in these two diseases this type of schedule and this type of IFN used, according to these authors, did not seem to have any beneficial effect. Leavitt *et al.* (1984) treated eight patients with non-small-cell carcinoma of the lung with recombinant IFN Type A, 20×10^6 IU/m^2 per day i.m. for 12 weeks. No complete or partial responses were seen. Two patients had minor reductions in their chest masses. The authors concluded that IFN would have little role in the treatment of these types of malignant tumors. In a study on non-small-cell lung cancer employing the maximum tolerated dose of recombinant IFN-αA (Grunberg *et al.*, 1984), it was concluded that no patients achieved complete or partial responses. Eleven of 15 treated patients were evaluable for response. The recombinant IFN-αA was given at a dose of 50×10^6 IU/m^2 three times weekly i.m. and when toxicity was too severe, reduction was

performed, even to 10% of the initial dose. Severe fatigue was the most prominent dose-limiting toxicity. Using such an approach, it seems that in treating advanced non-small-cell lung cancer IFN-αA is probably of no value, at least at the very high doses. In a Danish study, recombinant IFN-α was given at a dose of 50×10^6 IU/m^2 three times per week until progression in a series of 17 patients with refractory small- and large-cell lung cancer (Ernst *et al.*, 1984). HLA- BC-antigens and β_2-microglobulin concentrations on lymphocytes were increased and so was peripheral NK cell activity. The clinical results were disappointing, as only 1 of 17 patients responded with a partial remission (response rate, 6%).

Some results on small-cell lung cancer have been slightly more encouraging. Mattson *et al.* (1983) treated eight patients with lung cancer by giving human natural leukocyte IFN-α. Six patients received partially purified preparations and two patients received highly purified IFN preparations. The patients all had small bronchogenic carcinomas confined to the hemithorax, had a good initial performance status, and had all been previously untreated. Four patients received a dose of 8×10^8 IU for 5 days. This could not be achieved in the other patients, who received 4.25, 5, and 7×10^8 IU respectively, due to hematologic parameter changes, while one patient received 6.8×10^8 IU due to hypovolemic shock. Maintenance therapy consisting of 6×10^6 IU was given i.m. three times weekly, commencing on Day 8, while the induction treatment was given by the i.v. route. Fever, severe shaking, malaise, and muscular pain occurred in all patients after the infusions and lasted through the i.v. infusion period. The patients showed excitement on Day 2 and thereafter, a progressive slowing in mental and motor functions. The characteristic findings consisted of fatigue, somnolence, and lack of initiative. Speech was affected and the handwriting of the patients changed. Perseveration occurred, and loss of smell and taste was typical. EEG changes were found with diffuse δ-waves predominantly in the frontal lobe. There was also a change in tendon reflexes. The various abnormalities described subsided 2–4 weeks after the high-dose treatment was terminated, while the low-dose IFN treatment was continued. The highly purified IFN preparations caused the same type of symptoms as the partially purified, and it was suggested that the neurologic effects were caused by the IFN itself. These are interesting findings also when one considers the role substantial local IFN production must play in the pathogenesis of encephalopathy caused by viruses.

In the Finnish studies (Mattson *et al.*, 1984b), patients with previ-

ously untreated small-cell lung cancer of all stages who responded with complete or partial remission on induction chemotherapy and consolidation radiotherapy were then randomly assigned to one of three arms. A control arm was instituted without maintenance, one arm received chemotherapy, and one arm consisted of naturally produced human leukocyte IFN-α at a dose of 3×10^6 IU i.m. daily for 1 month and 6×10^6 i.m. three times per week for an additional 5 months. In the report, 63 patients had been included in the study and 20 patients had already been randomized: 9 patients to the IFN arm, 10 patients to the cyclophosphamide–Adriamycin–cis-platinum (CAP) arm, and 9 patients to the nonmaintenance arm. The overall objective response rate after induction chemotherapy and radiotherapy was 84%. At the time of the report, it seemed that there was a trend for a shorter survival in the nontreated arm. This study is to be continued.

Jones *et al.* (1983) treated 10 patients with small-cell lung cancer with human lymphoblastoid IFN at a dose of $50–100 \times 10^6$ IU/m^2 for 5 days followed by a low-dose IFN treatment at 3×10^6 IU/m^2 for 3 weeks. No partial regressions were seen. Treatment caused rather heavy side effects, as had been reported earlier for patients receiving high-dose lymphoblastoid IFN, and there was clinical deterioration of three patients, who had hyponatremia. It was concluded that, despite considerable toxicity, there was no anti-tumor effect evident in these patients. Jackson *et al.* (1984) treated three patients with advanced small-cell carcinoma of the lung with recombinant IFN-α2. They had previously been treated with irradiation and chemotherapy. The dose was 50×10^6 IU/m^2 daily for 5 days, given by i.v. infusion in two patients, and the third patient received a bolus infusion with the same dose schedule. Every second week, the courses were repeated. One patient had stable disease but expired with pneumonia after a second course. The other two patients showed progression. There were profound CNS side effects.

Four previously untreated patients with small-cell lung cancer were treated with radiotherapy (40 Gy, 20 fractions) followed by i.m. injections of 3×10^6 IU of human natural leukocyte IFN-α given three times weekly in Stockholm (Wiman, Bringel, Strander, and Ringborg, unpublished results). One patient died after 1 year in local relapse and with a demonstrable brain metastasis on autopsy. Two patients showed progressive generalized disease after 4 and 5 months, respectively, and one patient had to discontinue the therapy because of fever and intolerable malaise. Clearly, it is too early to conclude much about IFN efficacy in small-cell lung cancer.

XI. Systemic Treatment of Gastric Cancer

A gastric cancer series with Phase II design, headed by Giles in Leeds, had to be terminated due to lack of efficacy on 14 patients in whom there were no complete or partial responses. The patients were given lymphoblastoid IFN at a dose of 4×10^6 IU daily by i.m. injection for 28 days and thereafter 4×10^6 IU three times each week for locally recurrent or metastatic adenocarcinoma (T. J. Priestman, personal communication).

XII. Systemic Treatment of Colorectal Carcinoma

Neefe (1983) has reviewed the results obtained by using IFN treatment of patients with gastrointestinal cancer. The results presented at the time of his review were not very positive. Neefe *et al.* (1984) have also studied immunological parameters in colorectal patients receiving IFN-αA injections and could not correlate effects on these to clinical results. Figlin and co-workers (1983) treated 19 patients with metastatic adenocarcinoma of the colon and rectum with semipurified IFN-α. This was given at a dose of 3×10^6 IU 5 days per week. No objective responses were seen. The toxicity level seemed to be similar to what had been found by others. It was concluded that this type of regimen, which could be administered safely, did not have any activity against adenocarcinoma of the colon or rectum (see also Chapter 10, Section X).

Lymphoblastoid IFN has also been used on these types of tumors. Chaplinski *et al.* (1983a,b) reported their results on metastatic colon cancer patients treated with human lymphoblastoid IFN at the ASCO meeting in 1983. The IFN was given i.m. three times per week for 6 weeks, and the dose was 3×10^6 IU/m^2. Five of the 15 patients treated experienced heavy toxicity. Seven of the 15 patients had objective stability of disease at the end of 6 weeks. The remaining patients showed progression. There were no partial or complete regressions.

Twenty-one consecutive patients with advanced adenocarcinoma of the colon or rectum were treated at Georgetown University (Neefe, 1983). Four of these patients had received adjuvant treatment previously. None had received therapy for advanced disease. Eighteen of these patients had liver metastases. IFN-αA was administered at a dose of 50×10^6 IU/m^2 three times weekly i.m. All but one of the 18 patients evaluable for response showed progressive disease. Circulating levels of IFN could be detected usually 2–8 hours following i.m. administration.

Several groups have employed IFN-α2 therapy. Weimar *et al.*

(1983b) treated 20 patients with advanced colorectal carcinoma with high-dose IFN-α2 therapy. The dose was 20×10^6 IU/m^2. Five patients received the injections twice weekly for 12 consecutive weeks, and the other 10 received 1–4 cycles of eight daily injections with the same dose. The cycles were divided by intervals of 3 weeks. One patient treated twice weekly showed a partial response of a liver metastasis (response rate, 5%). The other patients did not respond, and although one patient showed partial remission of a liver metastasis, he was classed among the nonresponders depending on a rapidly expanding brain tumor growing simultaneously. A short-lived augmentation of NK cell activity in injected patients was registered, but it was followed by a decrease. Silgals *et al.* reported at the AACR meeting in 1983 their results after also injecting recombinant leukocyte IFN-α2 into patients with advanced adenocarcinoma of the colon. At first 11 patients were treated with a daily dose of 50×10^6 IU/m^2 by i.v. infusion for 5 consecutive days. Toxicity was very severe, and subsequently the starting daily dose of the IFN was decreased to 30×10^6 IU/m^2. At this dose, eight patients were evaluable for response, and of these one had stable disease, while the others showed progression. These results were certainly not very encouraging. Lundell *et al.* (1984) treated 18 patients with advanced adenocarcinoma of the colon/rectum. These patients received recombinant IFN-α2 s.c. at a dose of 20×10^6 IU/m^2 three times weekly for 3 months, or, alternatively, pulse treatments of 50×10^6 IU/m^2 daily i.v. for 5 consecutive days every fourth week. There were considerable side effects reported on these doses, and there were no objective tumor regressions seen in the patients.

Also combination therapy has been studied. Wrigley *et al.* treated, in a multicenter trial, 18 patients with colorectal cancer with IFN-α2 with only one partial response. Therefore, 20 patients were entered into a trial employing the combination of recombinant IFN-α2 and 5-fluorouracil (1984). Patients were randomized either to receive IFN 20×10^6 IU/m^2 i.v. followed in 2 hours by 250–500 mg 5-fluorouracil i.v. daily for 5 days (11 patients) or IFN 5×10^6 IU/m^2 s.c. three times per week with 250–500 mg 5-fluorouracil administered daily for 5 days (nine patients). The patients experienced moderate toxicity. Two patients showed partial responses (10%). Eight patients with advanced inoperable colorectal carcinoma of the adenocarcinoma type were treated with a combination of human IFN-α2 and 5-fluorouracil in Stockholm (G. Lundell, personal communication). The doses of IFN given to a group receiving s.c. treatment was 5×10^6 IU/m^2 and for a group receiving i.v. treatment 2×10^7 IU/m^2. Two hours after the

IFN treatment and for 5 days once per month, 5-fluorouracil was given at a dose of 250–500 mg/m^2. No tumor responses were observed.

XIII. Systemic and Intraarterial IFN Treatment of Liver Cancer

Preliminary results on the treatment of primary hepatic cancer with human natural IFN-β were presented by Kato *et al.* in 1982. The same year 11 patients with hepatocellular carcinoma had been treated with human natural IFN-β by Okai *et al.* (1983). No patient had received other types of treatments. The IFN was given i.v. in nine patients and i.a. in two. The i.v. patients were given 5–10 $\times$ 10^6 IU daily or 5 $\times$ 10^7 IU twice weekly. Intraarterial injections were given by continuous treatment with 5–10 $\times$ 10^6 IU of IFN-β daily. Two patients had severe side effects, one during i.a. and one during i.v. treatment and had to stop the therapy. One minor response was registered on i.v. treatment. Otherwise, there were no responders. For sole intraarterial treatment of this disease entity, see Chapter 9, Section IX.

XIV. Systemic Treatment of Carcinoids

Preliminary data on IFN-α treatment of patients with midgut carcinoid tumors were presented in 1982 by Öberg *et al.* In 1983, Öberg and co-workers presented more extensive data on the treatment of midgut carcinoid tumors with semipurified natural human leukocyte IFN-α (Öberg *et al.*, 1983). In the initial study, nine patients with carcinoid tumors of the small intestine were treated with daily i.m. injections of 3 $\times$ 10^6 IU daily for 1 month and 6 $\times$ 10^6 IU daily for another 2 months i.m. Seven of these nine patients had previously been treated with a combination of 5-fluorouracil and streptozotocin without benefit. Six of the patients were suffering from the carcinoid syndrome. The IFN treatment ameliorated the manifestations, and one could see effects on laboratory parameters like urinary levels of 5-hydroxyindoleacetic acid, serum levels of chorionic gonadotropin subunits, and pancreatic polypeptide. Effects were excellent on patients with liver metastases but not so good on patients with lymph node involvement. When treatment was stopped, it was seen that five of six responders in the study developed relapses. Response rates were difficult to evaluate, since one looked for effects on laboratory parameters and clinical symptoms, but later on it has been shown that there were decreases in solid tumor masses (K. Öberg, personal communication). A prospective randomized controlled study comparing IFN to a combination of 5-fluorouracil and streptozotocin has been

discussed. In this system also, the NK cell system of the patients has been evaluated (Funa *et al.*, 1983). These results were the subject of a recent thesis (Funa, 1984). Clearly, this tumor group deserves further studies in connection with IFN therapy.

XV. Systemic Treatment of Nasopharyngeal Carcinoma

A disease that would be interesting to treat with a combination of various IFNs is generalized nasopharyngeal carcinoma (NPC). Here, there is the possibility of studying viral serology during the course of the disease (see Ringborg *et al.*, 1983). In 1982 an update of the West German clinical trials was made by Niethammer *et al.* (1982). They especially summarized their results of treatment of NPC children with and IFN (see also Treuner *et al.*, 1980, 1981a). All patients were between 9 and 14 years of age. Five of the six children treated had involvement of cervical lymph nodes at the time of initiation of treatment. Four of the patients had previously received extensive radio- and chemotherapy. One patient had to stop treatment after only 9 days because of severe side effects and rapid tumor progression despite therapy. One case was especially interesting, since this child had complete remission of his tumor, which had actually already penetrated the sinuses and the orbit of the right side and had even grown into the brain. It is of importance that this is the patient who later developed antibodies to IFN-β. The dose given to the children was 10^6 IU/10 kg of body weight by i.v. infusion for 30 minutes. Studies of the IFN treatment of NPC patients have also been initiated in China (G. DeThé, personal communication).

XVI. Systemic Treatment of Brain Tumors

Here the reader should also study the results reported on in Chapter 9, Section V. Ueda *et al.* (1982) described nine cases with recurrent metastatic brain tumors that were treated with human natural IFN-α given i.m. Two of the patients had finished surgical removal and cobalt irradiation more than 6 months before. Small doses of 5×10^4 IU weekly and a large dose of 3×10^6 IU every other day were compared. Side effects were not noticed to any extent at these doses. In two cases there were signs of tumor regression on serial CTs, but IFN was not effective on the tumors that had already shown active regrowth before IFN administration. Sawada *et al.* (1982) treated six cases with brain tumors and five with abdominal tumors with human natural IFN-α. When the six patients with brain tumors were seen to have recurrent

masses on CT scan and at the same time had symptoms, human IFN-α was injected i.m. into the patients. Three cases received a single i.m. injection of 5×10^4 IU weekly for 9–10 months. The other three cases received i.m. injections of 3×10^6 IU of IFN every other day for 4–9 months. In the first two cases (a glioblastoma and a medulloblastoma) in the low-dose group, there was an arrest of tumor growth and reduction of tumor size. In the remaining patient there was progression. Among the cases receiving the higher dose, there was no change of masses in Cases 4 and 5, and in Case 6 (a medulloblastoma), evaluation was not possible. Ten patients with malignant brain tumor were treated in 1983 by Hirakawa *et al.* (1983a) with natural semipurified human leukocyte IFN-α. In eight cases in which primary tumors were treated, IFN was given at the time recurrences were revealed. In the high-dose group, $1–3 \times 10^6$ IU of IFN was injected i.m. two or three times a week, while in the low-dose group, 5×10^4 IU was given once a week. It was revealed that partial remissions were achieved in two patients in the low-dose group and that these remissions lasted for 3–6 months.

It is difficult to know how relevant the CT pictures are for these patients, as discussed elsewhere (Boëthius *et al.*, 1983). It is also difficult to know whether the previous treatment might have affected efficacy, but it is possible that the clinical situation where other forms of treatment had been given to the primary tumor and IFN is given later on could have some advantage.

Twelve patients with histopathological diagnosis of glioblastoma multiforme were treated in Stockholm by daily i.m. injections with 3 or 9×10^6 IU of human natural IFN-α (Boëthius *et al.*, 1983). Most of the patients had been treated by surgical removal of the tumor followed by conventional chloroethyl-cyclohexyl-nitrosourea (CCNU) therapy. When recurrences occurred, IFN therapy was initiated. One patient had complete remission, but there were no partial remissions (response rate, 8%). The complete responder has now been on IFN therapy for more than 3 years without having a recurrence. Side effects were as expected on the basis of previous experience. An interesting finding in these studies was that there was no correlation between radiological findings as revealed by CT scans and intraoperative findings during IFN therapy. This means that assessment of regression of a tumor during IFN therapy by using CT scans should be interpreted with great caution, and this has to be taken into consideration in all future trials with glioblastomas and IFN therapy. It is interesting that glioblastomas are known for induction of marked

proliferation of endothelial cells, and it is important that the tumor cells themselves produce a growth factor for endothelial cells (Sudditth *et al.*, 1975; Kelly *et al.*, 1976). This finding would tally very well with the anti-growth concept of IFN as advocated by investigators like Inglot and Taylor-Papadimitriou (see Chapter 2, Section IV, and Chapter 3). Another pitfall in studies on IFN effects on brain tumors consists of cortisone effects on the CT investigations.

In an update in 1983, Hirakawa *et al.* (1983b) had treated 24 patients with primary malignant brain tumors; there were 17 adults and 7 children; 14 glioblastomas, 4 astrocytomas, 3 medulloblastomas, 1 ependymoma, 1 ependymoblastoma, and 1 pontine glioma. All patients had recurrences at the initiation of treatment and all had previously, at least 6 months before, received irradiation. There were seven patients treated by systemic administration and 17 patients treated locally. A partial response was obtained for four of 24 cases (17%) regardless of the route of IFN administration and whether natural human IFN-α or lymphoblastoid IFN was used. It is once more concluded by this group that IFN therapy might be useful as an adjuvant therapeutic agent for recurring gliomas and that further investigations should be carried out. The human lymphoblastoid IFN used was the one prepared in hamsters (Imanishi *et al.*, 1980, 1982).

Human lymphoblastoid IFN was given to 19 patients with recurrent gliomas who had failed either surgery, irradiation therapy, and/or chemotherapy (Mahaley *et al.*, 1984a). There were 10 patients with glioblastoma multiforme, 7 patients with anaplastic astrocytomas, 1 patient with medulloblastoma, and 1 patient with oligodendroglioma. Human lymphoblastoid IFN was given i.v. to 10 patients and i.m. to 9 patients. Total dose and schedule were similar in both groups. The doses were escalated from 10×10^6 IU/m^2 per day for 3 consecutive days per week up to 30×10^6 IU/m^2 per day 5 days per week for 4 weeks. Toxicity in the form of fatigue and lethargy was registered, but five of the patients were able to receive all the 33 scheduled doses. Some CNS toxicity and secondary infections and some other side effects were reported, but they were similar in the groups receiving IFN i.v. and i.m. In six patients, there were significant reductions, making a partial regression rate of 32%. There were as many responders on the i.m. as on the i.v. program. Five of these patients had glioblastoma multiforme, and the remaining one had oligodendroglioma. Two other patients showed minimal responses.

The study design of IFN treatment of gliomas by the Pittsburgh Group working on brain tumors was presented already at an interna-

tional IFN conference in 1982 (Mahaley *et al.*). An update was presented at the Southern Surgical Society meeting in New Orleans in 1983 (Mahaley *et al*, 1984b). The Phase I study has been published in more detail (Mahaley *et al.*, 1984c). The results of this group suggest that IFN can be used for patients with anaplastic gliomas (see Mahaley *et al.*, 1984a). Further studies are warranted. The IFN has now also been given as adjuvant therapy in combination with radiotherapy for patients with anaplastic gliomas (Mahaley *et al.*, 1984d).

Nagai and Arai updated their results on the treatment of malignant brain tumors with IFNs in 1983. Three types of IFN preparations were used: natural human IFN-β, human lymphoblastoid IFN, and recombinant leukocyte IFN-αA. The IFN-β was administered intratumorally (i.t.) or intrathecally daily via the Ommaya's reservoir. Dose levels of $0.3–1 \times 10^6$ IU to maintenance dose levels of $3–9 \times 10^6$ IU were given. In a few cases, the IFN-β was given as systemic administration by i.v. drip instillation. Human lymphoblastoid IFN was given systemically by the i.m. route at a dose of $3–6 \times 10^6$ IU. The recombinant leukocyte IFN-αA was injected i.m. in escalating doses up to 54×10^6 IU. Maintenance doses in most cases were $3–9 \times 10^6$ IU because of toxic effects. Human IFN-β was given to 20 cases of glioblastoma, four cases of medulloblastoma, two cases of malignant lymphoma, and one case each of pontine glioma, pinealoma, and nasopharyngeal carcinoma. The human lymphoblastoid IFN was given to three cases with glioblastoma, one case with a medulloblastoma, three cases with malignant lymphoma, and one case having a metastasis of squamous cell carcinoma. The recombinant IFN-αA was given to nine patients with glioblastoma and one patient with malignant lymphoma. Altogether, this study included 47 patients with brain tumors. The following results were obtained. With human fibroblast IFN-β there was one complete response and seven partial responses, giving a response rate of 40%. In addition, there were five patients with stable disease. With human lymphoblastoid IFN there was one partial response out of three evaluable patients (response rate, 33%). The two remaining cases were stable. With recombinant IFN-αA, there were two partial responses of nine evaluable patients (response rate, 22%). One of the remaining seven cases had stable disease. Altogether, the authors reported that the response rate for the α-type was 25% and for the β-type, 40%. It is difficult, however, depending on the various ways of administration, to exactly compare the various IFN treatments. Side effects were the ones to be expected, and the most common symptoms were fever, chills, and lassitude. These authors

want to use a combination of IFN therapy and radiation therapy and/or anti-cancer chemotherapy in the future. Their results seem promising.

In a pilot study, Miyoshi *et al.* (1983) treated five children (four with medulloblastoma and one with ependimoblastoma) with malignant brain tumors with irradiation of the brain and spine in combination with treatment by human natural IFN-α. The IFN was given twice or once a week after irradiation and the dose of IFN was $2.5–5 \times 10^6$ IU. The IFN was injected also during irradiation. The total amount of IFN administered to these patients varied from 3.75×10^7 to 1.75×10^8 IU. Recurrences occurred in three patients after remissions. The survival rate at 1 and 2 years was 100%. These pilot studies were therefore considered to be promising, and further studies on combined therapies are being pursued.

XVII. Systemic Treatment of Neuroblastoma

An interesting case report was presented by Sawada *et al.* in 1979 when a 21-year-old female patient with Stage IV neuroblastoma was injected with semipurified natural human leukocyte IFN-α. When the patient was injected with 3×10^5 IU every second day i.t. or around the tumor tissue, there was a reduction in tumor size. Several tumors were also measured in the supraclavicular area. When systemic administration of 3×10^6 IU of IFN by i.m. injections was used, then no effects were seen on tumor growth. This might indicate that in order to bring about regression of large solid tumors, a high IFN dose has to be employed. Sawada *et al.* (1983) reported also later on patients with neuroblastoma treated with human natural IFN-α. A dose of 10^6 IU was given i.m. twice a week in combination with cyclophosphamide and/or vincristine. Four Stage IV patients were treated without any effects documented of the therapy. The evaluation of the treatment of Stage II patients was considered premature. It should be emphasized, however, that three of four Stage III patients at the moment are doing well, and the study also shows that the combination of the doses of IFN and chemotherapy employed can be given to these patients (Sawada *et al.*, 1983).

Two trials in pediatric oncology were updated in 1983 (Treuner *et al.*, 1983a). One of them was the neuroblastoma Stage IV trial, which had been described earlier (see Lampert *et al.*, 1982; Niethammer and Treuner, 1982). This trial was started in 1979 and closed in 1982. Thirty-four patients received IFN-β, and 39 children did not receive IFN. There was no difference between the two groups.

XVIII. Systemic Treatment of Prostate Carcinoma

Studies going on at Radiumhemmet, Karolinska Institute, comparing effects of pepleomycin and IFN on generalized prostate carcinoma of low differentiation have not given any promising results (Edsmyr, Esposti, Andersson, and Strander, unpublished results). The dose of human natural IFN-α employed was 3×10^6 IU i.m. per day. So far, there are no responders on IFN in the study.

XIX. Systemic Treatment of Carcinoma of the Uterine Cervix

Ikić *et al.* (1981) and Seto *et al.* (1983) treated patients with precancerosis and intraepithelial neoplasia of the uterine cervix by IFN preparations. Intraepithelial neoplasia of the uterine cervix was considered to be a good model, and in one study (Seto *et al.*, 1983) 11 patients with intraepithelial neoplasia of the uterine cervix were treated with natural leukocyte IFN-α, lymphoblastoid IFN, and natural IFN-β. All of the 11 patients in that study showed partial response following treatments with either IFN-α or IFN-β. Similar results were reported by Ikić *et al.* Since only partial regression was obtained and continued treatment did not seem to give rise to any complete remissions, it is difficult to imagine, however, that these types of treatments can be used in practical medicine at the moment. Conventional treatment gives rise to satisfactory results at this stage of the disease.

N. Einhorn *et al.* (1983b) conducted a study on the use of recombinant leukocyte IFN-αA in metastatic or recurrent cervical cancer. Eighteen patients with histologically proved recurrent cervical cancer and/or patients with distant metastases were included in this study, which was comprised of 11 patients with local recurrence, six patients with local recurrence and distant metastases, and one patient with distant metastases only. All patients were treated with 5×10^7 IU of the IFN per m^2 i.m. three times per week during 12 weeks. Nine patients have completed the study, and improvement was seen in only one patient in whom it was difficult, however, to be absolutely certain whether the registered effects depended on difficulties in local assessments. Otherwise, there were no responses in the nine patients who received the full course. Adverse reactions consisted mainly of flulike symptoms, gastrointestinal problems, and fatigue. Only six patients completed 12 weeks of therapy at a full dose. The dose, together with the poor response, indicates that this kind of treatment cannot be advocated for metastatic or recurrent cervical carcinoma (N. Einhorn *et al.*, 1983b). For further discussions on this disease, see Chapter 9.

XX. Systemic Treatment of Ovarian Carcinoma

N. Einhorn *et al.* (1981, 1982) treated five patients with advanced ovarian carcinoma with daily i.m. injections of 3×10^6 IU of human leukocyte IFN-β. All patients were refractory to other treatments. Two of the patients had ascitic fluid production, and this ceased on the IFN therapy. A partial response was observed in one patient (response rate 20%), and in two of the other patients the disease was stable for more than 1 year. Continued studies on the treatment of advanced ovarian carcinoma at the Karolinska Hospital have confirmed the beneficial effects on ascitic production. Partial responses were observed occasionally, but so far no complete responses have been achieved. At the moment 28 patients with advanced ovarian carcinoma have been treated with $3–27 \times 10^6$ IU daily of natural human IFN-α i.m. (P. Ling, personal communication). Fifteen patients with epithelial ovarian carcinoma were treated at the M. D. Anderson Hospital after previous chemotherapy or irradiation (Freedman *et al.*, 1983). One patient showed a partial response. Four other patients showed stable disease. The findings were very similar to the ones reported above from Stockholm.

Human lymphoblastoid IFN was given as 10-day infusions to 19 patients previously treated with chemotherapy for ovarian cancer (Willson *et al.*, 1984). All patients had chemotherapy resistant tumors. The dose given was 30×10^6 IU/m^2 per day for 10 days, and this was repeated every twenty-second day. The 10-day course was difficult to complete on account of toxicity. Hepatotoxicity occurred in five of seven patients, and in one case there was a fatal hepatic necrosis. One partial response was observed (response rate, 5%). On the basis of the toxicities observed, it was decided that 10×10^6 IU/m^2 per day was optimal as far as toxicity was concerned, but on that dose there was no response. Abdulhay and collaborators (1984) treated patients with advanced epithelial ovarian cancer with low doses of human lymphoblastoid IFN. All of the patients had received previous chemotherapy or irradiation. The IFN was administered at a dose of 5×10^6 IU/m^2 i.m. for 5 days per week for 6 consecutive weeks. If there was some sign of stabilization or response at 6 weeks the patients were placed on IFN therapy, but only for 2 days per week up to 12 months or until they progressed. No major organ toxicities were reported in this multicenter study. One complete response, three partial responses (response rate, 19%), and 13 patients with stable disease (62%) were reported. It was concluded that lymphoblastoid IFN has activity on ovarian carcinoma.

XXI. Systemic Treatment of Breast Cancer

In a Phase II trial, four American institutions treated disseminated human breast carcinoma patients with leukocyte-derived IFN-α (Borden *et al.*, 1982b). It was administered at a dose of 3×10^6 IU daily i.m. for a period of 28 days. Five of 23 patients (22%) had an objective partial response of 92 days' mean duration. It was found that responding patients were significantly older than nonresponders and that dose escalation did not lead to any additional evidence of response. Major toxicities were the ones previously reported for natural IFN-α therapy. ADCC and NK cell cytotoxicity were enhanced 48 hours after IFN administration. It was concluded also that serum β_2-microglobulin concentrations could be used as a parameter for the IFN injections. Peak serum IFN titers were higher after injections of higher doses. There were some long-term responses. In the long-term setting a fixed schedule was not used on these patients. Further data were presented in 1983 when 40 patients had been treated. A partial response was achieved in 11 (27.5%) of these patients. An additional five patients improved, while the remaining 24 patients either had stable disease during the time of IFN treatment or showed progression during treatment (Borden *et al.*, 1983a). It was emphasized that there was weight loss and fever. Escalation in these trials of doses from $3–9 \times 10^6$ IU did not give rise to any additional increase in response rate. These studies were especially important in the sense that they showed definitely that human leukocyte IFN prepared from buffy coats has an anti-tumor effect on generalized solid human tumors. The responses were noted at four American institutions. It was concluded from these trials that additional studies should be made on dose and schedule, and only later on should IFNs be employed in Phase III studies in which they could be used either on their own or combined with other modalities for the treatment of breast carcinoma.

A Phase II trial was conducted with human lymphoblastoid IFN in metastatic breast carcinoma (Goodwin *et al.*, 1984). Thirty-two patients with generalized breast carcinoma were randomized to receive either 0.5×10^6 IU i.m. three times weekly or 3.0×10^6 IU i.m. three times weekly for 12 weeks. The idea behind the rather low dose was the desire to suppress the NK cell activity caused by the high doses as had been found in previous studies. Sixteen of the patients had received previous chemotherapy and hormone therapy. It could be seen that one of 22 evaluable patients showed a partial response (4.5%) while four showed a stable situation. The conclusion from this study

was that a low response rate is achieved by using human lymphoblastoid IFN therapy at such low doses.

In 1982, Quesada *et al.* reported that partial remission was achieved in one of six patients with metastatic breast carcinoma injected by the i.m. route at a dose of 3–6 × 10^6 IU with semipurified human IFN-β. In addition, there were two minor responses registered. Systemic side effects were similar to the ones seen when employing natural IFN-α. There were augmenting effects on cell-mediated immunity and also effects on NK cell activity and ADCC in *in vitro* experiments. This shows that IFN-β has a biological impact irrespective of the difficulty in finding it in the serum. Again, two of the three responding patients exhibited lymphopenia and again this argues for a relation between anti-tumor effects and effects on leukocyte counts in injected patients. Pouillart *et al.* reported also in 1982 on the treatment of 11 patients with generalized breast cancer who received eight i.m. injections of 6 × 10^6 IU of human natural IFN-β over a period of 40 days. The therapeutic effect could not be determined, but there were some changes in skin nodules in 10 of 11 patients suggesting anti-tumor activity. In the patients tested, the receptors for estrogens and progestogens were increased on the tumor cells in patients receiving IFN.

Also recombinant IFNs have been employed on breast carcinoma patients. Smedley *et al.* (1983) treated 10 women with locally recurrent breast cancer who had failed on irradiation, hormone treatment, and cytotoxic therapy. They were given recombinant leukocyte IFN-αA at a dose of 2 × 10^7 IU/m^2 daily or 5 × 10^7 IU/m^2 three times weekly for up to 3 months. Side effects consisted particularly of lethargy, anorexia, nausea, and weight loss. Also there were signs of somnolence, confusion, paresthesia, and upper motor neuron lesions. These effects, as well as slow wave activity in EEGs disappeared, when the IFN was withdrawn. Two patients had partial responses (response rate, 20%), three patients had minimal responses, and five patients showed no response. Fifteen patients with advanced breast cancer were treated in a Phase II trial with recombinant IFN-α2 by Muss *et al.* (1983). Fourteen of the patients were evaluable at the time of the ASCO meeting in 1983. All patients had been previously treated. The IFN was given at a dose of 50 × 10^6 IU/m^2 i.v. per day for 5 consecutive days every 2–3 weeks. There were four courses given. Later on subcutaneous maintenance was initiated. No responses were noted in evaluable patients, but there were four patients with stable disease. Toxicity was the same as had been reported in other studies. It was concluded that breast cancer patients receiving maximally tol-

erated doses of i.v. recombinant IFN-α2 are unlikely to respond to such therapy, at least when previously treated with chemotherapy and hormone.

In 1983, Sherwin *et al.* reported the first Phase II efficacy trial of recombinant leukocyte IFN-α in the treatment of malignant disease. They had selected advanced metastatic breast cancer patients, and all 19 had progressive disease when entering the trial. All patients had metastases and were no longer responsive to chemotherapy. The patients in this trial received the maximum tolerated dose, 50×10^6 IU/m^2 by i.m. injections three times weekly. The toxicity observed was that reported for natural IFN-α; fatigue was the most limiting factor. All patients required dose reductions. With this heavy dosage 16 of 17 evaluable patients had evidence of tumor progression, and only one patient had stable disease. No responders were registered, and therefore it was concluded that refractory breast cancer does not respond well to this type of recombinant IFN.

Already in 1979, IFN was used together with chemotherapy in the treatment of breast cancer. Kolarić *et al.* (1979) added crude leukocyte IFN to their chemotherapeutic regimen for recurrent metastatic breast carcinoma. The chemotherapeutic regimen consisted of cyclophosphamide, methotrexate, 5-fluorouracil, and prednisone given as 5-day treatments with 3-week intervals. Human leukocyte IFN was given i.m. at a dose of 2×10^6 IU daily 3 days before the chemotherapy cycle, during the cycle, and also 5 days after its completion. Toxicity levels were similar in both groups except that some IFN-treated patients experienced additional vomiting and fever. At the time of the report a higher response rate was actually seen in the group not receiving IFN as opposed to the other group. This study clearly emphasizes the need for caution when combining chemotherapeutic agents with IFN in the treatment of cancer patients.

CHAPTER 11

INDUCERS

It is known that double-stranded RNAs can induce IFN in humans, but their toxicity is still an obstacle for their systemic use (Borecky *et al.*, 1981–1982). Effects have, however, been reported by using IFN inducers. It is very important that various IFN inducers are tested for therapeutic efficacy in animals (Levy, 1977; Storch *et al.*, 1983) and that in such studies comparisons are made to effects achieved by treatment with exogenous IFN. There are also studies where mismatched double-stranded RNAs are being screened for various activities for prospective use in clinical situations (Strayer *et al.*, 1981–1982). This is going to be an interesting research area in the future.

Two patients with recurrent respiratory papillomatosis with pulmonary lesions were treated by Leventhal *et al.* (1981) with the IFN-inducer poly(IC·LC) (poly(I)·poly(C) stabilized with poly-L-lysine in carboxymethyl cellulose). Some beneficial effect of the therapy was seen, but there was no regression of pulmonary lesions. Doses of poly(I)·poly(C) ranging from 0.5–24 mg/m^2 were administered to terminal cancer patients (Levy and Riley, 1981). The schedule was such that one injection was given first, and then after one week's period of observation the patient received another injection. This second injection was followed on 13 consecutive days by new injections. The aim was to treat the patients at least three times before going to a higher dose level. The mean optimal concentrations of IFN found in the serum were very high, 5820 IU/ml of serum, in the group receiving the highest dose. There were toxic manifestations by the higher doses as expected on the basis of the IFN titers detected. Children who had juvenile laryngeal papillomatosis, otherwise in good health, could tolerate 12 or even up to 15 mg/m^2. There were indications that clinical effects could be achieved on patients with juvenile laryngeal papillomatosis, multiple myeloma, and perhaps also in dysimmune neurological disease. In 1981, however, no details had been given on these effects.

An extensive review on the preliminary clinical studies performed with poly(I)·poly(C) to that date was presented in 1982 (Levy *et al.*). In the early studies, although they were not designed to test efficacy, it was seen that in one case of acute lymphoblastic leukemia there was

a complete temporary remission. High serum levels of IFN were detected as mentioned previously. In juvenile laryngeal papillomatosis there was marked improvement in the clinical condition in 7 of 7 patients. Also, in seven patients with multiple myeloma there was evidence of biological effects and at least two partial remissions. Other studies were performed on viral diseases and neurologic disease. In summary, it can be said that in the treatment of tumors biological effects were clearly demonstrable in some patients in whose serum IFN was detected.

Levy *et al.* (1983) have also used poly(IC·LC) in patients with neurological diseases. From these studies, the conclusion was drawn that the drug is safe to use in clinical pilot experiments (Levy *et al.*, 1983). An extensive review of the clinical trials made thus far with IFN inducers appeared in 1983 (Levine and Sherwin, 1983). The most significant toxicities seen in Phase I trials were hypotension, polyarthralgia, and polymyalgia. These effects were dose dependent. High serum levels of IFN could be seen in patients treated with the polynucleotides employed. One partial remission was seen in a patient with AML; otherwise, the clinical results were not at that time very promising. It is important that this area is pursued, and it is possible that in time better inducers will be found that can be used on a more long-term basis. It is also possible that one can use the inducers for induction therapy and then use exogenous IFN therapy for maintenance. However, all of this is speculation, and it has to be taken into consideration that any large substance, like some of the inducers, might cause antibody formation in patients. Studies with IFN inducers should continue, and it will be exciting in the future to see whether good such inducers can be found.

Injection of patients with recurrent herpes simplex infections with double-stranded ribonucleic acid (phage 2-RNA) were reported to give rise to a significant therapeutic effect (Borecký *et al.*, 1977). To my knowledge, this approach has not been pursued elsewhere.

CHAPTER 12

OTHER FORMS OF IFN THERAPY

I. IFN as an Antiviral Agent in Tumor Patients

The development of antiviral agents has expanded over the last years (see Galasso, 1981b). Several new antiviral drugs are being tested against clinical viral infections, and some of them are going to be used or are being used in combination with IFNs (cf. Luby, 1979; Chang and Snydman, 1979; Stringfellow, 1981). For general reviews on new trends in antiviral chemotherapy, see De Clercq (1979) and Liu (1982). Already in 1963, Old *et al.* reported their results on increased resistance to Mengo virus following infection with BCG. If this effect were due partly to induced IFN production, there is a rationale for using IFN therapy as an antiviral agent on some tumor patients.

It has been postulated that there exists a definite host-mediated antiviral effect exerted by IFNs in *in vivo* situations (Bolhuis *et al.*, 1981). There is also evidence, however, from experimental systems, that IFN might protect directly against viral infections rather than through activation of NK cells or exertion of other indirect actions (see Chong *et al.*, 1983).

Potent inhibitors of the replication of EBV *in vitro* have been found (Lin *et al.*, 1983), and it would be interesting to combine these inhibitors with IFN treatment in experimental systems for further possible use in *in vivo* trials. Viral latency (see Tovey, 1980), in general, is probably of utmost importance for human disease. How the IFN system affects such viral persistance is unknown.

IFNs have been used both in the treatment and the prophylaxis of human viral infections (see Merigan, 1982a,b). In the antiviral area it was discussed more extensively around 1981 whether IFN preferably should be used in combination therapy against virus infections (see Myers and Galasso, 1981–1982). It is of interest that it has been reported in the treatment of viral disease that synergistic effects can be achieved with the combination of antiviral drugs and human leukocyte IFN (Mecs *et al.*, 1979).

It is difficult to evaluate future possibilities for the use of IFNs in treating clinical infections by studying animal model systems. An important point is that the virus replication has gone on for quite some

time at the time the patients become symptomatic, and it can sometimes by difficult to find corresponding models in animals (see Kern and Glasgow, 1981). Actually, the antiviral effects obtained by IFN *in vivo* might differ much from what has been seen *in vitro* (see Maheshwari *et al.*, 1983). This has implications, of course, also for the anti-tumor therapy, and it will also be important for determining the dose and schedule employed for IFN therapy.

It should be mentioned that IFN-γ has been claimed to be involved in the lymphokine components responsible for the restriction of chlamydia replication (Byrne and Krueger, 1983) and that IFN preparations have direct effects on the growth of chlamydiae (Rothermel *et al.*, 1983).

Falcoff *et al.* (1966) were the first to treat viral infections with natural IFN-α, in this case cytomegalovirus (CMV) infections, with systemic low-dose IFN therapy. Another historical finding was the observation that concentrated human leukocyte IFN-α preparations containing 100 units of IFN injected intradermally had an effect on vaccinia lesions in monkeys (Scientific Committee on Interferon, 1970). Finally, monkey IFN was shown to reduce the infectivity of vaccinia in monkey eyes in 1960 (Cantell and Tomilla, 1960), and during the next year it was shown to be active against this virus also in monkey skin (Andrews, 1961). This type of IFN was then used in the first successful experiment with IFN in humans, where it afforded complete protection against vaccinia after local injections in 24 volunteers (Scientific Committee on IFN, 1962). It has been shown after that time in many diseases, employing many different preparations, that it can affect various virus infections (see Scott, 1983b).

It has definitely been demonstrated that IFNs can have strong effects on ocular viral diseases (see Sundmacher *et al.*, 1982), and IFN is probably of value for the treatment of ocular viral disease (Sundmacher *et al.*, 1981; De Koning *et al.*, 1981). For the effect of IFN on herpes simplex virus infections, cf. Ho *et al.* (1984). In this connection the experience in Israel on the treatment of life-threatening viral infections with IFN should also be mentioned (Levin *et al.*, 1982).

When the antiviral drug acyclovir is employed on herpes virus infections, it must be remembered that virus production can resume when the drug is removed, and it has therefore already been suggested that such therapy should be combined with IFN (Hanto *et al.*, 1982). Acyclovir seems to constitute an effective treatment for mucocutaneous herpes simplex virus infections in immunocompromised patients (Mitchell *et al.*, 1981) and thus would be interesting to combine with IFN therapy in various situations. In fact, IFN treatment has

already been used in combination with acyclovir in dendritic keratitis in a double-blind study. The combination of acyclovir and semipurified human leukocyte IFN-α in such treatment seems to be excellent (Colin *et al.*, 1983). The benefits of acyclovir in immunocompromised patients against herpes virus infections could perhaps be combined with various antiviral strategies employing the IFN system (Hann *et al.*, 1983).

Merigan *et al.* (1978b) demonstrated that semipurified human natural IFN-α has an effect on zoster progression, eliminates the distal cutaneous spread of this herpes virus, hastens pain resolution, and hinders visceral complications. In some patients with cancer it is obviously important to achieve antiviral effects and perhaps especially on zoster. In this context it is interesting to mention the convincing studies by the Stanford group with human leukocyte IFN-α as treatment for varicella in children. They made a randomized double-blind placebo controlled study in two phases. Forty-four children were treated within 72 hours after the exanthema appeared. All patients had malignant disease. It was seen that new lesion formation was delayed in the IFN-treated recipients, and by Day 7 few of the IFN-treated patients had developed new lesions. Also, it was found that among survivors treatment with IFN reduced the number of patients who had experienced life-threatening dissemination. The conclusion reached by using this type of therapy, which consisted of giving human natural leukocyte IFN-α at doses of 4.2×10^4 IU/kg of body weight or higher per day (i.m. every 12 hours), was that the therapy had an antiviral effect in this immunocompromised patient group (Arvin *et al.*, 1982). The Stanford University group has since concentrated on the treatment of hepatitis (see Scott, 1983b).

The clinical experience up to 1983 in the treatment of viral infections and malignant diseases with natural IFN-β in West Germany was reviewed by Obert (1982). The anti-tumor results obtained by the principal investigators in these studies are presented in several sections, especially in Chapter 13 in this volume. It should be emphasized, however, that a study by Heidemann *et al.* (1982), in the treatment of herpes zoster, led to the first registration of any IFN, in this case natural IFN-β, as a drug for the treatment of a disease in any country. Also, in Cuba, IFN therapy has been tried on viral disease. Positive results in the treatment of hemorrhagic dengue fever with natural IFN-α were presented in 1982 (Limonta *et al.*, 1982, 1983a).

In an important series of double-blind placebo controlled trials Hirsch and collaborators showed that semipurified human leukocyte IFN-α is a useful agent for the prophylaxis of CMV infections in hu-

man renal transplant recipients (Cheeseman *et al.*, 1979; Hirsch *et al.*, 1981, 1982, 1983). In their latest study they treated patients with 3×10^6 IU of IFN or placebo i.m. before transplant surgery was performed. After surgery the doses were reduced and given three times per week for 6 weeks and then twice a week for 8 weeks. It could be seen that the frequency of clinical CMV infections were reduced in the IFN recipients, that opportunistic superinfections only occurred in patients given placebo, and it was also concluded that minimal toxicity was observed with this type of IFN treatment. This is an interesting use of IFN since prophylactic treatment also in other studies seems to give rise to an effect on common virus infections (see Ingimarsson, 1980).

Sixteen patients entered a study in Holland in which recombinant DNA leukocyte IFN-αA was given in a double-blind study started on renal transplant recipients (Kramer *et al.*, 1984). There were eight patients in the IFN-treated group and eight in the placebo group. Acute rejection episodes were diagnosed in all patients between the second and seventeenth day after surgery in all eight IFN-treated patients, and in one of the eight placebo-treated patients. The rejection was of the acute vascular type. In addition, three IFN-treated patients had transient nephrotic syndromes. The IFN used had a specific activity of $2–4 \times 10^8$ IU/mg of protein, and it was given at a dose of 36×10^6 IU i.m. three times per week for 6 consecutive weeks followed by i.m. injections twice a week for another 6 weeks. The doses used in the studies were selected from rhesus monkey experiments in which similar doses per kg of body weight were prophylactic against intradermal vaccinia infections. The study indicates that large doses of IFN can probably have strong immunological effects, and, at least when this type of IFN is employed, one has to consider the side effects that can be obtained in patients at immunological risk.

It has been suggested by Åhström *et al.* (1974) that viral infections in leukemic children perhaps can be controlled by i.m. injections of human leukocyte IFN-α. Actually, this type of therapy has been used on many cases of childhood leukemia over the years at the Karolinska Hospital. The antiviral effects of various IFNs should now also be compared to other antiviral agents (see De Clercq *et al.*, 1981). An effect of human leukocyte IFN-α purified by affinity chromatography using monoclonal antibody on human rhinovirus 9 in volunteers given by repeated nasal sprays was reported by Scott *et al.* (1982), but it is difficult at the moment to advocate IFN therapy for respiratory viral infections in humans (see Greenberg, 1984). Actually, it is hard to know at the moment where IFN stands among all the other antiviral

drugs that have been developed and are now being tested for infectious diseases in humans (see Galasso, 1981a).

II. Additional Uses of IFN Therapy

IFN preparations are now being used for the experimental treatment of various other types of diseases than viral and tumoral. One of the most interesting ones, concerning which a positive report has appeared, is multiple sclerosis (Jacobs *et al.*, 1981). Jacobs *et al.* wrote a follow-up report (1982) on their multiple sclerosis patients and decided to start a large randomized study on multiple sclerosis patients. Six patients with multiple sclerosis were treated in Scandinavian studies (Osther *et al.*, 1981) with systemic IFN therapy—natural IFN-α at a dose of 4×10^6 IU of IFN daily for 5–16 months. The patients had a prehistory of 6–24 years of multiple sclerosis. There were no signs of any effect. The possible *role* played by IFN in multiple sclerosis will be exciting to follow, although it remains to be proved whether it is of importance at all in that disease (Vervliet *et al.*, 1983a). It is of some interest for the tumor–IFN research area that multiple sclerosis patients given IFN-α seem to respond normally by preaugmentation (Rice *et al.*, 1983). IFN is now being used also for the treatment of amyotrophic lateral sclerosis (A. Salazar, personal communication; W. Jablecki, personal communication; M. Färkkilä, personal communication; R. Smith, personal communication). For a discussion of the effects of IFN on neurological diseases, see Abb *et al.* (1982b) and Johnson (1984).

It is now clear that there are three clinically distinct disease syndromes in homosexually active men: (1) severe cellular immunodeficiency, (2) chronic benign lymphadenopathy, and, (3) Kaposi's sarcoma (Schroff *et al.*, 1983). In the acquired immunodeficiency syndrome patient materials the case fatality rate may exceed 90% after 2 years (see Gottlieb *et al.*, 1983). These patients also develop a whole area of infectious complications, many of which are viral. In AIDS patients, the herpes viruses causing simplex, genitalis, and zoster are common (see Gilmore *et al.*, 1983). Murray *et. al.* (1984) tested T lymphocytes from 16 patients with AIDS for their capacity to secrete macrophage-activating products including IFN-γ. They found that mononuclear cells from 10 of 11 patients tested did not generate an effective lymphokine in response to mitogens, and 11 of 16 produced subnormal levels of IFN-γ. Upon stimulation with antigens, cells from none of 40 tested patients generated any active lymphokines, and

cells from 13 of 14 completely failed to secrete any IFN-γ. The antimicrobial function of monocytes from the patients was intact and once stimulated with normal lymphokines the patients' monocytes responded with enhanced and effective intracellular antimicrobial activity. So the conclusion made by these authors was that impaired lymphokine production may predispose patients with AIDS to opportunistic infections. This would provide a rational for using IFN-γ as immunotherapy for this particular disease. Another argument for the use of IFN therapy in AIDS patients is the association of Hodgkin's and non-Hodgkin's lymphomas with this disease (Dancis *et al.*, 1984). It has by now been clearly established that IFN can exert effects on patients with lymphoma (see Chapter 10, Section III). It is interesting that also interleukin 2 has been given to patients with cancer and with AIDS (Lotze *et al.*, 1984). Perhaps it could be combined with IFNs in future studies.

A small investigation was made on five patients with Crohn's disease by giving semipurified human IFN-β i.m., four injections per week, each dose containing 2.4×10^6 IU (Vantrappen *et al.*, 1979). It was concluded that two cases showed objective improvement. In two other cases, there was marked clinical improvement but the endoscopic findings did not confirm this, and in the fifth patient there was no response to treatment. More and more diseases are being considered for IFN therapy, and IFN has even been tried for the treatment of severe psychiatric diseases (Cantell *et al.*, 1980a).

CHAPTER 13

CONCLUSIONS

I. General Discussion and Future Prospects

It is important that the reporting of results in the treatment of tumors in humans follows certain rules and guidelines (see Miller *et al.*, 1981). At the moment, there is an overwhelming number of reports of pilot IFN studies. I completely agree with Steven Carter, who stated that the oncology literature "needs more publication of final results" (1982). This is particularly true in the whole area of clinical IFN antitumor research. It would be especially important, of course, if the negative results seen in clinical trials, even though disappointing, were to be reported. So far, very few trials employing IFN therapy have been randomized, although the number of randomized trials all over the world in using different treatments of cancer has increased extensively in the last 12-year period (Reizenstein *et al.*, 1983). Large screening tests of patients with malignancies have now been undertaken using various recombinant IFN clones. Some of the results have been encouraging while others have not. It is still agreed on a general basis that Phase I clinical trials of new therapies against human malignancies are necessary and ethically justifiable (Lipsett, 1982). The toxicity and the response criteria of the WHO or other accepted staging systems should be employed whenever clinical trials are made with IFN preparations. If everybody were to agree on the applicability of such criteria it would be beneficial. Also, it would reveal the IFN effects achieved much better, if comparisons were made with radiotherapeutic, chemotherapeutic, immunological, or other approaches. When there are patients who respond less well, but in whom there is still some kind of response, they should be mentioned "off the record." Stringent criteria for use have been amply summarized (Oken *et al.*, 1982).

A list of review articles and reports from conferences relevant to the content of this book can be found in a shorter review (Strander, 1983a). Some of the earlier work on IFN therapy was treated by some people with overenthusiasm. The IFN system constitutes an interesting concept, and easily attracts publicity. There is even a space experiment program called "Interferon" (Tàlas, 1983a,b). In 1982, we actually felt that we had to write an article about our personal assessment

of the value of IFN therapy in tumor disease to that date, since it had at that time been overemphasized that IFNs soon might become the main cure for cancer, as stated by newspapers and magazines (Strander and Einhorn, 1982b). For a condensed summary of results obtained by modern cancer therapy, see De Vita and Kershner (1980).

It has been claimed that interfering with the promoting phase of the carcinogenic process would be the best way to combat cancer, especially since in humans that time period probably is very long (Berenblum, 1981). The role played here by IFNs within such a context is unknown. Woodruff has stated many times that both the prevention and the treatment of malignancies will depend on our understanding of the interaction of the malignancy with its host (Woodruff, 1980, 1982). The more we study the IFN system the better are our chances of reaching an understanding of at least part of such interactions. An example constitutes the IFN induction, since it has already been possible to use the inhibition of such induction as a screening test for the carcinogenicity of various chemicals (Sonnenfeld *et al.*, 1980).

It has been suggested by Oldham (1984) that biologicals and biological response modifiers constitute the fourth modality of cancer treatment. In order to draw such a conclusion one has to include several treatment modalities in this concept, and Oldham included immunomodulators, immunostimulating agents, IFNs, IFN inducers, lymphotoxins, other lymphokines, cytokines, monoclonal antibodies, antigens, effector cells, and various other approaches. He emphasized that the biological therapies are at an early stage but that already highly purified biologicals can cause regression of tumors in patients. It was also emphasized by Oldham (1983a) that the treatments concerned might have to be developed much more individually than for the other types of therapy so far developed for malignant diseases. Also, the *combination* of IFN with other modulators and antigens would be an interesting area for future developments. It might be interesting, for example, to use IFN combined with malignant cell vaccines, and one such model system that could be advocated is probably the melanoma system. However, so far it seems that serological responses to melanoma cell surface antigen can only be induced in very exceptional cases (Livingston *et al.*, 1982).

What kind of effects have been achieved with the use of IFN therapy on tumor patients? In the beginning of the clinical testing of antitumor effects in man receiving exogenous IFN therapy it was impossible to do Phase I, II, and III studies that are so important in order to evaluate new agents in the tumor area. This has been emphasized,

among others, by Malpas who discussed this in detail (1983). It was also shown in his article that it is important to use common criteria for response when evaluating effects, especially on solid tumors as stated in the beginning of this chapter. A World Health Organization (WHO) scientific group consisting of 14 scientific members and two members of the secretariate presented a report on IFN therapy in Geneva during a session in March 1982 (WHO Scientific Group, 1982). In this report, which can be obtained from the WHO, various facts are stated, which are important whenever the initiation of therapeutic trials with IFNs is under consideration. Nomenclature, induction systems, recombination requirements, safety requirements, international reference preparations, inducers, and the results obtained so far in clinical studies are all reviewed. In the section dealing with therapy of malignant diseases it was stated in that report that "investigation of IFNs to define their potential therapeutic value is warranted. Many additional control trials will be required to confirm or refute the ultimate role of IFNs as anti-tumor proteins. These studies are justified only in research hospitals and clinics." This is an important point. Future trials should be performed under properly controlled conditions.

An interesting compilation of data was made at the Vienna International Chemotherapy Congress (Borden, 1983b) where Borden had collected the various results obtained with systemic administration of natural human IFN-α in Phase I/II trials. In the series with various malignancies that he had compiled, there were 381 patients, and there were nine complete and 43 partial responders (response rate, 14%). It has on many occasions been emphasized that such percentages do not look impressive in the treatment for various types of tumors, but they do look promising in view of the fact that they have been obtained in Phase I/II trials and compete favorably with any conventional treatments used routinely today for malignancies (R. K. Oldham, personal communication). When Sherwin (1984) summarized the results of the Phase II trials of recombinant IFN-α, which had been performed to May 1984, he found signs of activity in various diseases such as NHL, mycosis fungoides, Kaposi's sarcoma, myelomatosis, melanoma, renal cancer, and bladder cancer. Rather disappointing results had been obtained in breast cancer, colon cancer, non-small-cell lung cancer, ovarian cancer, and acute myelogenous leukemia. He also concluded that higher doses of the materials employed and continuous treatment regimens were more effective in renal cell carcinoma, Kaposi's sarcoma, melanoma, possibly in lymphomas than short-term low-dose treatments. He also mentioned that Phase III trials already had been sponsored by the National Cancer Institute at the NIH. As a choice for

combination therapy NHL has been selected and for adjuvant therapy NHL, melanoma, colon cancer, and possibly other types of malignant tumors.

Different countries have concentrated on studies with different types of IFN preparations. In 1979 it was announced that the American Cancer Society was allocating 2 million dollars for the purchase of natural IFN-α for clinical trials in the United States (cf. Marx, 1979a; Culliton and Waterfall, 1979). The trials sponsored by the American Cancer Society were described by Borden and Hawkins (1980).

In 1967, the production of natural human leukocyte IFN on a large scale was initiated in the Soviet Union (see Kuznetsov and Soloviev, 1983). In 1969, this type of preparation was used for the treatment of influenza. During later years, the group at the Gamaleya Institute in Moscow has concentrated especially on the treatment of acute lymphoblast leukemia (Kuznetsov *et al.*, 1980; Orlova *et al.*, 1980). At present, large-scale natural human leukocyte IFN production is being performed in at least three centers in the Soviet Union (A. A. Smorodintsev, personal communication).

As early as 1970, work on the production and purification of natural human IFN-α was initiated in Japan (see Matsuo *et al.*, 1982). Natural IFN-α preparations are now being prepared in Hungary but as yet there are no reports on clinical results (see Beladi *et al.*, 1982). The mass production of human natural IFN-β for clinical trials has been achieved in Japan (see Kobayashi *et al.*, 1982). This preparation has been used in extensive clinical trials. The clinical trials employing exogenous IFN therapy in Japan to 1982 were summarized by Imanishi and Kishida (1981–1982). In most European countries IFN-α has been most commonly used. Reviews on the treatment with IFN of cancer patients in Europe have been presented (Strander, 1981a; Billiau *et al.*, 1984), and earlier work was reviewed in 1977 (Strander, 1977b). Human natural leukocyte IFN-α has now also been applied to various patients with benign and malignant tumors in Cuba (Limonta *et al.*, 1983b), and the results look encouraging especially in the treatment of JLP and breast carcinoma.

In England most studies have been performed with lymphoblastoid IFN. Toy reviewed clinical experiences with human lymphoblastoid IFN and the strategies of the further Phase II studies at the IFN meeting in Rotterdam in 1983 (Toy, 1983b). The planned clinical trials by the Wellcome Foundation at that time were extensive, and some results from these trials were presented in 1984.

Clinical trials with human natural IFN-β on malignant diseases had already been done in Belgium in 1977 (De Somer, 1977). Initial anti-

tumor studies in Germany were mainly performed on children (Niethammer and Treuner, 1981). The West German experience using IFN-β as an anti-tumor agent to 1981 was summarized by Niethammer and Treuner (1982). At that time, the pharmacokinetic behavior was rather well known (Treuner *et al.*, 1981), and the German group had already presented data on the successful treatment of a patient with nasopharyngeal carcinoma with IFN-β (Treuner *et al.*, 1980). It is interesting that the latter patient is the one that was later reported to have produced antibodies to IFN-β (Vallbracht *et al.*, 1981). A therapeutic trial for neuroblastoma Stage IV was constructed (Lampert *et al.*, 1982). The results of that trial have, so far, however, been negative (D. Niethammer, personal communication). Studies were also initiated in West Germany in 1981 on gastric carcinoma (Herfart *et al.*, 1982) and on non-Hodgkin's lymphoma of low malignancy (Huhn *et al.*, 1982; Fink *et al.*, 1982). The results of these studies were initially not very positive. A summary of 5 years work in West Germany with the administration of IFN-β to patients with malignant disease was reviewed by Obert (1982). The patients in Germany have generally received systemic therapy, but this type of preparation has also been given intrathecally to patients with viral diseases (Prange and Wismann, 1981).

Really extensive studies are now being undertaken on various malignant tumors, especially in the United States, using recombinant IFN-α, IFN-β, and IFN-γ (see Borden *et al.*, 1983b). The Biological Response Modifiers (BRM) program of the National Cancer Institute instituted Phase I and Phase II trials of various recombinant and non-recombinant IFNs in cancer patients. The philosophy behind the various schedules employed and the planning of future trials were presented at the Third Congress for Interferon Research in Miami (Sherwin and Oldham, 1982). The IFN trials within this program at the Frederick Cancer Research Facility have continued since then. Today, several countries have developed programs for the use of recombinant IFNs on an experimental basis.

To be able to construct meaningful trials in the future it would be important to elucidate the anti-tumor effects exerted by IFNs. Clearly it is difficult to determine how IFN inhibits tumor growth. Probably, several mechanisms are involved. Some of them have been listed by Gresser (1982). Not all of the IFN effects are advantageous for the host, however. It has been emphasized that the IFN-induced diseases in experimental animals do not constitute an argument against the use of IFNs in patients with specific diseases (Tovey and Gresser, 1982). Instead we should use the experimental system to be *aware* of possi-

ble problems in connection with the clinical use of IFNs. We are dealing with potent substances. For a discussion on the reports concerning toxic effects of IFNs, see Oldham (1983b). Particularly, there are suggestions that one should be cautious with regard to possible cardiotoxic effects.

The importance of immunological IFN effects have been discussed in Chapter 4. It will be interesting to see whether IFNs have any practical implications for the graft versus host reaction (see Seemayer *et al.*, 1983). Maybe IFNs should be employed in anti-cancer immunization programs, if active immunization fails according to the criteria of Berken (1982), i.e., when suppressor cells are generated due to low tumor antigen concentrations, when MHC gene products are absent, and when ADCC is required in solid tumors for them to be affected in the extravascular space. It should also be considered that IFN could be used in other therapeutic efforts to restore the *in vivo* immune system as in the experiments that have been undertaken with thymosine (see Dillman *et al.*, 1983).

IFN effects on the differentiation process are probably important. It seems, for example, that B cell maturation is blocked in patients with multiple myeloma. It will be interesting to see if this block, together with the reduced synthesis of polyclonal immunoglobulin, can be corrected in IFN-treated and responding myeloma patients. It has been predicted that immunoregulatory T cells, and particularly suppressor cells, have been responsible for the block in B cell maturation (Pilarski *et al.*, 1984).

The role played by combined prostaglandin and IFN effects in connection with tumor growth *in vivo* will be important to elucidate (for a review on prostaglandin effects, see Karmali, 1980). Actually, a more thorough understanding of the carcinogenic process is of importance for the development of IFN therapy. It must be emphasized, however, that chemical carcinogenesis consists of an extremely complex area of events; particularly, an understanding of the later phases of the process has been lacking (see Farber, 1981).

Should IFNs be used for therapy or prophylaxis? It is not clear from animal work whether IFN could be used in the adjuvant setting. It should certainly not be considered as an immunological adjuvant (for a definition, see Allison, 1979). Large-scale, randomized trials in various prophylactic settings should probably be undertaken. An interesting finding in this context can be mentioned. Blood samples from 26 patients receiving semipurified natural human leukocyte IFN as adjuvant treatment for osteosarcoma were tested for antibodies toward various microorganisms (Ingimarsson *et al.*, 1980a). There were 17 microbial antigens used in these tests. Fourteen of the patients devel-

oped metastases and 12 did not. When the metastasis group and the nonmetastasis group were compared, it was seen that clinical manifestations of infections and seroconversion during IFN therapy was confined to patients in the metastasis group. There was also a tendency for a chronologic link to the early incidence of metastases. If these findings imply that some patients are more resistant to the antiviral effects of natural human IFN-α than others, perhaps the same patients do not respond to the anti-tumor effects of IFNs either, while other patients do. Theoretical and practical important implications of such interpretations, if correct, are obvious. Recently, in Stockholm, a 15-year-old boy with osteosarcoma of the tibia in the IFN-α trial developed pulmonary metastases on 2 separate occasions during IFN therapy (Ingimarsson, Broström, and Strander, unpublished results). Simultaneously with the detection of the metastases, the patient on both occasions had clinical, and serologically verified, virus infections, herpes zoster and rubella, respectively. This emphasizes the presence of viral infections in these patients as a possible prognostic factor.

Several tumor diseases have reacted to IFN therapy (see above). Which disease should be most extensively studied in the near future? Two of the diseases about which we have some basic molecular information and in which also serological systems have been worked out extensively are Burkitt's lymphomas and NPC (see G. Klein, 1982). Burkitt's lymphoma is one of the diseases in which, thanks to what is know about the disease and the possibilities for a close follow-up and determination of various clinical parameters, it would be extremely interesting to do clinical trials employing various types of IFN. For present-day treatment of Burkitt's lymphoma, cf. Ziegler (1981). It is amazing that this disease has not yet been the subject of IFN trials.

The effects of various IFN preparations in tissue culture and clinically on lymphoma cells were reviewed by Horning (1983). She concluded that the lymphomas constitute disease entities that deserve further studies employing IFN treatment. She also concluded that there are going to be years of investigation before answers are obtained as to whether IFNs should be part of treatment schedules of choice for this group of diseases. The effects of IFNs on leukemias in animals and in humans were reviewed in 1983 by Rohatiner. The studies presented can be summarized by saying that it is too early to say anything about the role played by IFN in the treatment of patients with these diseases, especially so on the basis of new data reported later on hairy cell leukemia and CML. It is important to emphasize that the "biological activity" response concept does not suffice, since

it also has to be shown that IFN can exert meaningful effects leading to long-term positive results. On the other hand, however, this might be a disease group in which IFN might play a role depending also on its antiviral effects.

The solid tumors are more difficult to evaluate in connection with IFN therapy. Perhaps also the regional concept should be taken into consideration when patients are selected for IFN treatment, since the behavior of tumor development may vary depending on the region (see Auerbach and Auerbach, 1981). The treatment of HPV associated human tumors, finally, will be interesting for future etiological work on some of the malignancies. It is now seriously considered that HPV might play an active role in the development of several important human neoplasias (Anonymous, 1983).

Which IFN should be used in each instance? This is as yet unknown (cf. Bocci, 1981c). Over the last years most investigators have used recombinant IFNs, because they can be obtained in such highly purified forms (Sikora and Smedley, 1983). It has to be remembered, though, that IFN combinations might be better, since different, cloned IFNs exert different effects. For example, both leukocyte IFN clones A and D can stimulate NK cell activity, but some cloned IFN subtypes show differential effects on the proliferation of various leukemic cell lines (see Lee *et al.*, 1982b). Lymphoblastoid IFN has been advocated especially by English, Japanese, and Austrian groups for use in the treatment of malignancies (Finter and Fantes, 1980), and extensive trials are underway.

Borden (1984a) has stated what the main problems are in the clinical application of IFN to cancer patients. He has especially tried to discuss five variables: (1) the type of IFN used in any one disease, (2) what dosage and schedule should be employed, (3) what mechanism of action could be expected, (4) which tumor should be treated, and, finally, (5) how the IFN should be used. One could emphasize, of course, that some other variables also have to be taken into consideration and could be equally or even more important. One such variable is the very patient being treated with IFN, *i.e.*, irrespective of disease type and stage being treated. Some of the problems involved in doing clinical application work with IFN on patients with virus diseases or tumors was presented by Merigan in a review in 1981. It was emphasized that the IFN system is complex and that one has to analyze in detail, also on the basis of laboratory work, which clinical studies would be the most relevant and should be undertaken in the near future. It is interesting to see how the recombinant IFN concept

changed the whole IFN area as revealed by an article on the use of human IFN in the *New England Journal of Medicine* (Merigan, 1983).

The problems, possibilities, and results already achieved with the use of IFN in the treatment of cancer have been compiled by Oldham and Smalley (1984). In general, their summary is optimistic and states that it is quite possible that "biologicals in general, and IFN in particular, are to be real additions to our therapeutic armamentarium in the treatment of cancer." It has been considered by Borden *et al.* (1984b) that for the treatment of breast, bladder, and colorectal cancer, determination of the dose schedule at which IFN should be employed needs further assessment. They have also made suggestions for further Phase II and Phase III trials based on information obtained from *in vitro* and *in vivo* work. Ryd *et al.* (1979) made the observation that in an animal tumor system the same IFN dose that depresses the growth of ascitic tumors could enhance and in one case even increased the ability of the corresponding solid tumor to metastasize. This emphasizes once more the complexity of the clinical application of IFNs to patients with disseminated malignant disease. It is still not known in the treatment of various types of malignant diseases whether it would be preferable to use the same drugs for the induction period as for the maintenance period. It is known that, although clinically evident disease can respond very well to some drugs, the same drugs may not affect the relapse rate (Alexander, 1982). This dilemma is also affecting the IFN field, since it is not known whether it would be preferable to use IFN on a maintenance basis instead of using it in the same way as chemotherapeutic agents employed for induction treatments.

It has been argued that a preclinical rationale should be developed prior to extensive trials in the treatment of human malignancies (Borden *et al.*, 1982c; Balkwill *et al.*, 1983b; Borden and Balkwill, 1984). It would be extremely important to be able to monitor clinical trials on the basis of various parameters and experimental studies in animals in tissue culture (Strander and Einhorn, 1982a), but so far this has not been possible because of the lack of understanding of the IFN effects in patients with tumors reacting to IFN therapy. For example, it is known that patients with malignancies usually exhibit a decreased NK function in their peripheral blood when tested on chromium-labeled targets. Some investigators claim that variable levels of NK cytotoxicity can be observed in connection with the development of metastases and that monitoring of NK cell activity might be important in selecting patients for IFN treatment (Ching *et al.*, 1983). But no correlations

between NK effects and clinical response have been noted (see Chapter 4, Section II).

In a thoughtful article Hahn and Levin discussed in 1982 the use of the IFN system in patients with malignant disease. Their studies indicated that cancer patients had the ability to produce IFN, and they questioned whether a deficient *response* of their cells to IFN might play a role in the development of their malignant disease. The fact that many of the patients produced IFN spontaneously suggested to the authors the presence of an intracellular inducer such as a persistent virus infection. A main point, which is also important, is that if there is a high incidence of elevated blood levels of IFN it is questionable whether it is wise to give additional exogenous IFN to such cancer patients. Spontaneous IFN production was most frequent in lymphoreticular tumor patients. It is obvious from such reasoning that it would be important to find out what kind of IFNs are produced in various patients having different malignant diseases. Borden and Balkwill (1984) have written an interesting article in which they summarized preclinical and clinical studies that suggest that IFN and IFN inducers have an anti-tumor activity on breast carcinoma. In their paper they tried to combine the preclinical and the clinical approach that would allow them to compare animal and human data. The stem cell assay also was employed in these studies. Their attitude was optimistic in the sense that they concluded that the continued preclinic and clinical research along those lines will finally reveal how useful IFN may prove to be in this disease. In their hands, the IFNs are still considered experimental drugs that should only be used in the treatment of breast carcinoma within the frame of clinical trials.

It has been suggested by many investigators that the IFN system should be used in combination regimens. In this context, it must be mentioned that the development of such principles have been facilitated by "the biological response modifiers program" of the NIH (see Smalley and Oldham, 1983). A volume of progress in cancer research and therapy was devoted to papers on the mediation of cellular immunity in cancer by immune modifiers (Chirigos *et al.*, 1981). Clearly, this whole concept of studying mediators of biological response modification is going to be expanded (Herberman, 1981a). In a combined immunological approach to treating human malignancies, the immunotoxins will probably also play a part (Jansen *et al.*, 1983), and it will be interesting to see whether the various immunotoxins are going to be used in the near future in combination with various IFNs (see Vitetta *et al.*, 1983). In studies on IFN, lymphotoxin, and sodium butyrate, Khan *et al.* (1983) suggested that various immune response modifiers can have additive effects when given in combinations. Im-

munotherapy in general has been much debated, however, and it has as yet had no substantial effect on patients with acute myelogenous leukemia treated with chemotherapy (Foon *et al.*, 1983b).

Some completely new approaches deserve mention. Tumor necrosis factor (Carswell *et al.*, 1975) would be especially interesting to use in combination with various IFNs, since Williamson *et al.* (1983) have done comparative studies suggesting that the sensitivity to the human tumor necrosis factor and the IFN can be distinguished. Therefore, combined treatment with this factor and IFNs would be of interest due to the fact that one then might achieve synergistic effects (Williamson *et al.*, 1983). Perhaps, IFNs should also be used together with various cytotoxic agents that can be linked to immunoglobulins in order to direct these agents toward the surface of the cancer cells without disturbing the immune system (cf. Blair and Ghose, 1983). It will be interesting to follow the development of tumor therapy with monoclonal antibodies (see Levy and Miller, 1983) and to see if such therapy can be combined with IFN treatment. For a discussion on the use of hybridomas and monoclonal antibodies in oncology, see Zalcberg and McKenzie (1982), and for a discussion on monoclonal antibodies in connection with human tumors, see Phillips and Sikora (1982). A new approach to the treatment of tumors with IFN in this area consists of the system advocated by Baldwin's group in England (Pelham *et al.*, 1983). Human lymphoblastoid IFN was coupled to a murine monoclonal antibody directed to antigens expressed on human osteosarcoma cells. The purified conjugates retain antibody activity and also retain the capacity to activate NK cells among human peripheral blood lymphocytes. They localize specifically in human osteosarcomas xenographed in immunodeprived mice. These antibodies have a potential for target immunotherapy and have been shown by γ-studies following infusion of ^{131}I-labeled antibody to localize in primary osteosarcomas.

Various other combinations have also been suggested. It will be interesting to see if the liposome system can be combined with IFN treatment (see Schroit *et al.*, 1983). IFNs are also already used with chemotherapeutic agents (see Chapter 10). Jermy *et al.* (1983) investigated whether various patients having hematologic diseases receiving irradiation might respond to IFN production, as it had been shown by Kohan *et al.* (1975) that tumor DNA in a homologous culture system can induce IFN production. The total irradiation dose on seven patients varied between 2 to 40 Gy. There was no IFN detectable. Neither have we been able to detect any IFN induced in patients receiving chemotherapy (Strander and Einhorn, unpublished observation).

The clinical use of various anti-cancer agents and irradiation have led to specific problems that sometimes can lead to false conclusions (cf. Berry, 1982). It is important that international strict terminologies are used for the description of interactions between various IFNs, and between IFN and other treatment modalities. A glossary for such interactions has actually been proposed in another connection and it would be wise to follow this type of terminology so that we all will know what is meant by sensitization, inhibition, enhancement, antagonism, etc. (Steel, 1979).

The future of IFN therapy as part of the armamentarium against human tumor diseases should be viewed with cautious optimism. Some applications and results obtained to date have been presented earlier. Some of the knowledge acquired will no doubt lead to further developments. IFN therapy is beginning to be a factor to consider among the treatment rationales for tumors, but still it, perhaps with a few exceptions, should be considered experimental, and be controlled by specialized centers well-equipped for combining treatment with clinical research on treated patients. Within such a framework, this area of research now needs more biological knowledge and imagination on the part of investigators to make future achievements possible.

II. Addendum

Most of the references up to 1979 pertinent to subjects dealt with in this review can be found in the book by Stewart (1979a). For more detailed information, for reviews and overviews and symposia published over the last few years, and for special appraisement articles, a suggested reference list would read as follows (with only a few articles published before 1979): Anonymous, 1979; Attallah *et al.*, 1980; Baglioni and Nilsen, 1981; Balkwill, 1979; Baron, 1979, 1982, 1984; Baron *et al.*, 1981–1982; Basham and Merigan, 1982b; Berman and Frankfort, 1982; Billiau, 1981a, b; Bloom, 1980b; Bloom *et al.*, 1982; Bocci, 1980b, 1981b; Borden, 1981–1982, 1983a, b; 1984a, b; Borden and Balkwill, 1984; Borden and Ball, 1981; Borden and Krown, 1984; Borden *et al.*, 1984b, e; Borecký, 1979; Borecký and Fuchsberger, 1983; Burke, 1981; Burke and Morris, 1983; Cantell, 1977, 1978, 1979, 1981, 1983; Cantell and Strander, 1977; Chirigos *et al.*, 1982; Clemens, 1979; E. DeMaeyer, 1978; 1983; E. DeMaeyer and J. DeMaeyer, 1979; DeMaeyer and Schellekens, 1983; J. DeMaeyer-Guignard, 1979; DeMaeyer *et al.*, 1981; 1982; Djeu, 1984; Dunnick and Galasso, 1979; Einhorn and Strander, 1978, 1984; L. B. Epstein, 1979; Friedman, 1977, 1978a, b, 1981b, 1982; Friedman and Vogel,

1983; Galasso, 1983; Golub *et al.*, 1982b; Gordon and Minks, 1981; Gresser, 1972, 1977a, 1980, 1981, 1981–1982, 1982; Gresser and Tovey, 1978; Grossberg, 1981; Gutterman, 1981; Gutterman and Quesada 1981–1982; Gutterman *et al.*, 1982a; Hager, 1983; Herberman, 1980, 1981b; Ho, 1979; Ho and Armstrong, 1975; Horoszewicz and Mirand, 1983; Horowitz, 1981; Houglum, 1983; Ito and Buffett, 1980; Johnson, 1980; Khan *et al.*, 1980a; Kirchner and Beck, 1980; Kirkwood and Ernstoff, 1984; Kishida, 1983; Kono and Vilček, 1982; Krim, 1980a, b, 1981, 1981–1982; Levy and Levine, 1981-1982; Malpas, 1983; Marx, 1979b; Merigan, 1981a, b, 1982a, b, 1983; Mohr, 1983; Munk and Kirchner, 1982; Niethammer and Treuner, 1981, 1981–1982; Oldham, 1983a, 1984; Oldham and Smalley, 1983, 1984; Panem, 1982; Pestka, 1983b; Pestka *et al.*, 1982b, 1984; Pohl *et al.*, 1981; Pollard, 1980, 1981, 1982; Preble and Friedman, 1983b; Presber and Waschke, 1981; Priestman, 1979, 1983a, b; Scott and Tyrrell, 1980; Selbitz *et al.*, 1980; Sikora, 1980, 1983; Sonnenfeld, 1980; Stebbing, 1983b; Stebbing *et al.*, 1981; Stewart, 1979b, 1981, 1983; Stiehm *et al.*, 1982; Strander, 1977a, b, 1980a, 1981a, b, 1981–1982, 1982a–c, 1983a, 1984; Strander and Cantell, 1974; Strander and Einhorn, 1982a, b; Stringfellow, 1980; Sundmacher, 1982; Tamm and Sehgal, 1979; Taylor-Papadimitriou and Balkwill, 1982; Taylor-Papadimitriou *et al.*, 1983; Toy, 1983a, b; Treuner and Dannecker, 1981; Tyrrell and Burke, 1982; VanHelden, 1983; Vilćek, 1979, 1980, 1982a, b, 1984; Vilćek *et al.*, 1980, 1983; Warren, 1980; WHO Scientific Group on Interferon Therapy, 1982; Wilkinson and Morris, 1983b; Woodrow, 1983; Yabrov, 1979; Yamazaki, 1983; Zoon *et al.*, 1984; Zschieschke, 1980.

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