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# **Interferon therapy**

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Report of a WHO  
Scientific Group

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Geneva, 1-4 March 1982

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# **INTERFERON THERAPY**

## **Report of a WHO Scientific Group**

A WHO Scientific Group on Interferon Therapy met in Geneva from 1 to 4 March 1982. The meeting was opened on behalf of the Director-General by Dr Lu Rushan, Assistant Director-General, who noted that modern technology had made it possible to produce interferons in much larger quantities than had been available in the past. This had meant that more patients could be given much more material than had been the case in earlier clinical trials, and many countries were asking for a review of the present situation, especially with respect to the clinical effects of interferons.

Dr Lu also noted that the World Health Organization is keeping a careful watch on the International Standards by which the biological activity of the different preparations is measured, to ensure that they continue to be useful, particularly for the interferons produced by recombinant DNA techniques.

### **1. INTRODUCTION**

Substances produced in cell cultures in response to viral infection and shown to have the property of transferring resistance to viral infection to other cells were discovered in 1957 and named interferons (IFN). Subsequently it was found that interferons are also produced under a variety of other circumstances, e.g., in response to certain bacterial products and to chemicals of low molecular weight. In particular, cells produce large amounts of interferon when stimulated with double-stranded RNA. The antiviral action of interferon involves the induction and/or activation of certain cellular proteins which block virus replication, notably the synthesis of viral components and/or their assembly into new virus particles.

Experiments on animals have established that endogenous interferons play an important role in natural recovery from virus infections: blocking the interferon system can increase mortality and morbidity from virulent infections and may convert avirulent virus infections into lethal ones. Interferon produced early during a virus infection may delay virus replication and systemic spread, thereby providing time for humoral and cellular immunity to develop.

Two approaches can be used to exploit the antiviral potential of interferon: (a) the administration of interferon produced outside the body, and (b) the administration of substances which induce the production of interferon (so-called "interferon inducers"). Experiments in animals have demonstrated that early treatment with interferon or interferon inducers can prevent the development of a virus disease, and that the later treatment is started, the less the effect. In general, treatment started when signs of the disease were already present has not been effective.

In addition to their antiviral action, interferons exert many kinds of action on cells, including inhibition of cell growth and immunomodulatory effects. The antitumour effect of interferon and interferon inducers may depend upon a combination of these different kinds of action. The applicability of interferon to human therapy could not be investigated before methods for producing human interferon in large quantities were developed.

It is the purpose of this report to inform national health authorities of the advances that have been made in the production of human interferon and in the assessment of the clinical effects of interferon preparations in man. This information is offered to help these authorities to judge the desirability of acquiring interferon for clinical studies in their countries and to establish the conditions under which authorization can be given to use interferons in humans.

## 2. PRODUCTION OF INTERFERONS

The abbreviation IFN has been adopted to designate interferons; so far, three types of human interferon (HuIFN) have been recognized, namely HuIFN- $\alpha$ , HuIFN- $\beta$ , and HuIFN- $\gamma$ . There are a number of subtypes of HuIFN- $\alpha$ , probably about 14 in all, each being the product of a particular gene. There are differences of up to 30% in their amino acid sequences, and some biological differences have also been observed *in vitro*. The clinical significance of these differences is not yet clear.

There appears to be only one HuIFN- $\beta$ , which shares some amino acid sequences with HuIFN- $\alpha$ , but in its chemical and biological properties it is readily distinguishable from the latter. Similarly there appears to be only one HuIFN- $\gamma$ , which differs from the other interferons in its structure.

Table 1. Interferon preparations produced in human cells

HuIFN preparation	Common production system	Constituents	Old nomenclature
leukocyte IFN	buffy coat leukocytes and virus <sup>a</sup>	HuIFN- $\alpha$ , different subtypes	Le(leukocyte) type I, pH2 stable, foreign-cell-induced
lymphoblastoid IFN	B lymphoblastoid cells and virus <sup>a</sup>	HuIFN- $\alpha$ , different subtypes	Ly(lymphoblastoid) type I, pH2 stable, foreign-cell-induced
fibroblast IFN	fibroblasts and double-stranded RNA	HuIFN- $\beta$	F (fibroblast), Fi, type I, pH2 stable
"immune" IFN	buffy coat leukocytes or T lymphoblastoid cells } and mitogen	HuIFN- $\gamma$	HF(immune) type II, T, pH2 labile, antigen-induced, mitogen-induced

<sup>a</sup> Sendai virus or Newcastle disease virus.

The designations IFN- $\alpha$ , - $\beta$ , and - $\gamma$  are used to classify the interferons according to their structure. In contrast, the commonly used terms leukocyte IFN, lymphoblastoid IFN, and fibroblast IFN refer to preparations made from the cells concerned (see Table 1). The term "immune", or "type II", IFN was formerly used to denote crude preparations of IFN- $\gamma$  which probably also contained other lymphokines. Both leukocyte and lymphoblastoid IFN contain several alpha-type human interferons and preparations of leukocyte IFN made and purified in different laboratories may differ in the number or proportion of alpha-type interferons present. Similarly, lymphoblastoid IFN preparations may differ according to the cells and purification process used.

A human interferon is formed when the information in an interferon gene is transcribed and translated into functional interferon protein. The gene may be one naturally present in a human cell and activated by an appropriate stimulus, or it may have been inserted into a bacterial, yeast, or other cell by recombinant DNA techniques.

## 2.1 Production using human cells

### 2.1.1 Preparations containing HuIFN- $\alpha$

(a) *Leukocyte IFN*. Until quite recently, human leukocytes (buffy coat) obtained from donated blood have been the source of most of

the interferon used (1). In brief, the blood is collected in an anticoagulant and is centrifuged. The so-called "buffy coat" cells, which collect at the interface between the plasma and the red blood cells, are separated and resuspended in fresh balanced salt solution containing human serum. They are stimulated to form interferons by the addition of an inducing virus, usually Sendai virus, grown in hen's eggs. Production of interferon is complete within 18 hours, and the crude HuIFN- $\alpha$  is separated from the white cells by centrifugation.

This crude interferon has usually been further purified by precipitation with acid potassium thiocyanate and extraction of the precipitate with alcohol with fractional reprecipitation at different pH values. The resulting preparations of purified interferon (P-IF) have a specific activity of about  $3 \times 10^6$  International Units (IU) per mg protein (they are about 1% pure HuIFN- $\alpha$  protein) and yields are about 5 million IU for each buffy coat, derived from a single donation of 0.45 l of blood. This technique for preparing leukocyte interferon is simple in concept and requires no elaborate equipment. However, experience has shown that considerable attention to detail is required. Leukocytes removed for therapeutic reasons from patients with leukaemia have similarly been used as a source of leukocyte interferons.

(b) *Lymphoblastoid IFN*. Many human transformed cells can yield interferons when appropriately stimulated. Cells of one particular B lymphoblastoid cell line, Namalwa, have been used as the source of large amounts of HuIFN- $\alpha$ .

The cells are stimulated with Sendai virus in the same way as the buffy coat cells; this has the advantage that the cells can be grown and interferon produced in suspension culture. Vessels with a capacity of up to 4000 l have been used on an industrial scale, with consequent economies in production. In one system (2), Namalwa cell interferons are routinely processed to 80–95% purity. The product contains at least 8 HuIFN- $\alpha$  subtypes, which differ in their chemical, physical, antigenic, and biological properties. Extensive clinical trials with this formulation of lymphoblastoid interferons are in progress.

#### 2.1.2 Preparations containing HuIFN- $\beta$

Fibroblast interferon, HuIFN- $\beta$ , is obtained from cultured fibroblasts, either diploid cell strains derived from newborn foreskin or embryonic tissue and certain continuous cell lines (3, 4, 5). The best

results to date have been obtained by treating these cells with the synthetic ribonucleic acid, polyriboinosinic-polyribocytidylic acid, in the presence of the metabolic inhibitors, dactinomycin and cycloheximide, added in a precise time-sequence. The IFN- $\beta$  so made can be purified in various ways and the preparations that have been used in clinical trials have contained  $10^6$  IU or more per mg protein.

Since fibroblasts will grow only when attached to a surface, special technical procedures have been devised to provide the large surface area needed for mass culture. Even so, production on an industrial scale continues to be relatively difficult.

### 2.1.3 Preparations containing HuIFN- $\gamma$

Human buffy coat cells yield HuIFN- $\gamma$  when stimulated with lectins, staphylococcal enterotoxins, or other mitogens. It is thought that the T lymphocytes are the source of interferon. Crude preparations frequently contain various lymphokines, as well as HuIFN- $\alpha$  and - $\beta$ , but purification is relatively difficult because the molecule is unstable. There have as yet been no reports of the use of this type of interferon in patients.

## 2.2 Production by recombinant DNA techniques

Production in bacteria and yeasts is a way of making a single IFN protein at what may be a relatively low cost.

### 2.2.1 HuIFN- $\alpha$

HuIFN- $\alpha$  genes have been cloned with bacterial *Escherichia coli* (prokaryotic) and yeast (eukaryotic) cells. One group has succeeded in obtaining the expression of 6 different interferon genes, and very high yields of interferon protein have been reported (6).

Preparations of particular subtypes, mainly HuIFN- $\alpha_2$  (recombinant A) and HuIFN- $\alpha_1$  (recombinant D), have been purified to 80–95% and have been used in clinical trials. The different cloned IFN- $\alpha$  subtypes can have different biological activities.

### 2.2.2 HuIFN- $\beta$ and HuIFN- $\gamma$

Human interferons - $\beta$  and - $\gamma$  can also be obtained from bacterial, yeast, and animal cells by recombinant DNA techniques. Whereas

the native HuIFN- $\beta$  and HuIFN- $\gamma$  are glycoproteins, those produced by bacterial cells do not contain carbohydrate. At present there is little published information about the biological characteristics of such interferons, and nothing is known about their clinical behaviour.

### 3. SAFETY OF INTERFERONS

The safety of interferon preparations of different types must be considered in relation to the cells used to produce each particular type, the production and purification techniques employed, and the possible long-term consequences of administering the interferons.

Although interferon preparations may have antiviral activity in the cells of some heterologous species, for example, calf and rabbit cells, not all their side-effects have been reproduced in such animals as rhesus monkeys, cynomolgus monkeys, or rabbits. Furthermore, interferons are antigenic in animals, which means that the results of chronic toxicity studies in rodents are of questionable significance for man. This emphasizes the need to monitor patients receiving interferons for possible long-term consequences.

Whether a particular HuIFN preparation is approved for clinical use is a matter for decision by the appropriate authority in the country concerned, but the following considerations provide some guidance.

#### 3.1 Cells

Where human blood cells are used to provide HuIFN- $\alpha$  or - $\gamma$  preparations the problems to be considered are the same as in the case of products made from pooled human plasma. It is recommended that the Requirements for the Collection, Processing and Quality Control of Human Blood and Blood Products, published by WHO (7), should be followed. Since many individual blood donations are pooled to provide the production cells, more extensive quality control checks, such as those for the detection of contaminating cytomegalovirus, are not practical.

Cultured human cells are also used in production. The extensive safety tests that have been developed to monitor the human fibroblasts used for making live virus vaccines can be applied to those used to make HuIFN- $\beta$ . If transformed human cells are used, such as B lymphoblasts or fibroblastoid cells, there are other considerations to bear in mind. These have been brought to the fore by the use, as a

source of HuIFN- $\alpha$ , of Namalwa cells, which originated from a child of that name who had a Burkitt lymphoma. The cells are polyploid and tumorigenic in immunosuppressed mice and contain half of the genome of the Epstein-Barr virus, but no infectious virus can be formed. No specific transmissible tumour-inducing agent has been identified in the cells, or in the crude HuIFN- $\alpha$  made from them, but the theoretical role of such an agent has to be considered. It follows that HuIFN- $\alpha$  derived from Namalwa cells (or other transformed or leukaemic cells) should be purified by a method shown to free the HuIFN- $\alpha$  from virus and cellular DNA. This should yield a product that is likely to be safe even if some as yet unidentified tumorigenic agent were to be present in the cells (8).

### **3.2 Production and purification**

The possibility must be considered that the substance used to induce interferon formation, or constituents of the cell culture medium, or chemicals used in the purification procedure might be present in the final interferon preparation; for example, calf serum has been used in the culture of fibroblasts and Namalwa cells. While the amounts present in the final product must be reduced to the lowest levels, there is no general agreement on what level should remain.

### **3.3 Possible long-term consequences of interferon therapy**

Although interferons are formed naturally in the body during virus infections, and perhaps in many other circumstances, it is necessary to consider what possible long-term adverse consequence might follow from the administration of interferon preparations made by various techniques and injected by several routes. For example, antibodies to interferons can be formed.

In newborn mice and rats, injections of homologous interferon preparations have produced severe toxic effects in the liver and kidneys, but such effects were not seen with human leukocyte interferon in newborn monkeys and rabbits.

Since interferons may have important regulatory functions (for example, in the bone marrow), it is conceivable that the continued administration of interferon preparations could have long-term consequences, and patients receiving such therapy should be kept under surveillance.

#### 4. INTERNATIONAL REFERENCE PREPARATIONS: STANDARDIZATION AND MEASUREMENT OF BIOLOGICAL ACTIVITY

Interferon preparations are usually complex mixtures of proteins, and even those intended for clinical use may contain contaminating proteins—such as interferon molecules inactivated during processing, purification, and storage—in addition to biologically active interferon. The term “specific activity” is used to relate the biological potency of a given preparation to the total amount of protein present. It is expressed as “units” per mg of protein. Potency is determined in a biological assay that measures the degree of resistance acquired by cells in culture against viral growth or as a result of treatment with dilutions of the interferon sample. The titre of the sample is the dilution that achieves an arbitrarily determined end-point indicating a significant degree of inhibition of virus effect or growth; the reciprocal of the titre expresses the potency as units per ml. A variety of virus-cell combinations with different types of end-point is currently used. No generally acceptable standard bioassay exists, and no reference bioassay with defined materials has yet been officially designated. However, the expression of titres can be standardized by the use of the appropriate International Reference Preparations of human interferons (see Annex).

The intended use of the current International Reference Preparations is for calibration in bioassays of interferon. An essential requirement in calibration is that the dose-response curves in assays done with the International Reference Preparation, the internal laboratory reference preparation, and the test specimen should be parallel; otherwise activity cannot be expressed in International Units. Since the dose-response curves of HuIFN- $\alpha$ , - $\beta$ , and - $\gamma$  differ, the standard for one type cannot be substituted for another. The sensitivity of a single cell type to the different types of human interferon may vary independently.

In addition to their antiviral activity, interferons have various other biological effects. There is at present no consensus on the need for or feasibility of standardizing tests for these non-antiviral effects, and the International Reference Preparations have not been calibrated for this purpose. Furthermore, the International Reference Preparations of interferon have not been analysed in terms of their antigenic content or of the proportion of subtypes present. They remain, none the less, extremely valuable reference preparations when correctly used.

Several reference polyclonal antisera are available from the US National Institutes of Health (NIH); they have been prepared against human leukocyte, lymphoblastoid, and fibroblast interferons (9). Antiserum against HuIFN- $\gamma$  should soon be available.

Radioimmunoassays or enzyme immunoassays that rely upon the use of either interferon purified to homogeneity or monoclonal antibody (reagents not now readily available) have recently been developed, but their general acceptability must await careful correlations with determinations of biological potency.

## 5. INTERFERON INDUCERS

A different approach to the therapeutic application of the interferon mechanism is the induction in patients of endogenous interferons. It should be noted, however, that inducers that are active in animals have in general failed to produce large amounts of interferon in man. In addition, the period of hyporesponsiveness or refractoriness to reinduction of interferon, which follows initial production, and the toxicities of the inducer compounds have hampered progress. Nevertheless, methods of augmenting the effectiveness of available inducers are being investigated.

The complex of polyinosinic-polycytidylic acid, poly(L-lysine), and carboxymethylcellulose induces significant amounts of circulating interferon in nonhuman primates and man, and some clinical studies with this material are in progress. New inducers of low and high molecular weights have been discovered and are undergoing toxicological and preclinical evaluation. There is better understanding of the mechanism of the hyporesponsive state, and in mice prostaglandins are effective in overcoming the period of refractoriness to reinduction of interferon.

An acceptable inducer might be effectively utilized for prophylaxis of viral or neoplastic diseases; such an inducer, especially if it could be given by mouth, would be very practical. Inducers might prove useful in combination chemotherapy with exogenous interferons, antivirals, or cytotoxic drugs.

## 6. CLINICAL STUDIES

Interferons were introduced into clinical trials over a decade ago, the original investigations relating to the treatment of viral disease

being subsequently extended to the treatment of malignant disease (10, 11, 12). Improvements in the techniques of preparation and purification referred to above have resulted in a dramatic increase in the quantities of the different types of interferon available. As a result it has now become possible to conduct formal Phase I and Phase II studies to determine the toxicity and clinical effects of increasing doses of interferon. Observations of side-effects have concentrated on toxicities occurring in immediate relation to the administration of the material. It should not be overlooked, however, that all current interferon preparations contain at least traces of extraneous protein and that interferon may itself be antigenic.

### 6.1 Side-effects of interferons

The side-effects of interferons are determined by the route of administration, the schedule, and the dose. As experience has been gained in the use of purified natural interferons as well as those produced by recombinant DNA techniques, it has become apparent that many of the toxicities do not result from impurities in the interferon preparations but are due to interferon itself. Most clinical experience has been with preparations of leukocyte interferons given intramuscularly on a daily basis.

- Fever with or without rigor has been an almost universal finding, but it usually abates with continued therapy. Doses of  $3-5 \times 10^6$  IU can usually be given indefinitely without serious symptoms or physiological side-effects. With doses greater than  $10 \times 10^6$  IU, anorexia and general fatigue, often so pronounced as to be incompatible with normal ambulatory status, may occur.
- Significant weight loss may occur.
- Granulocytopenia usually occurs within the first 4 weeks of therapy but it has rarely required discontinuation of treatment; the symptom usually reverses quickly when administration of interferon ceases.
- Mild to moderate abnormalities may be observed in biochemical liver function tests with continued administration. These are reversible and have not been associated with any recognized histopathological alterations.
- Other symptoms have been seen in some patients, including headaches, appearance of superficial herpetic lesions, hair shedding, dry mouth, and peripheral sensory neuropathy.
- At doses greater than  $150 \times 10^6$  IU given daily by continuous infusion, unacceptable but reversible central nervous system toxicity

(severe drowsiness and seizures) and marked ionic imbalance have occurred.

Because of the potential risks, only limited studies of intravenous bolus injections of interferons have been conducted to date.

Some, but not all, of the side-effects observed with leukocyte IFN have also occurred in patients treated with HuIFN- $\beta$ . It must be noted, however, that high doses have not been given. Since the serum levels obtained with intramuscular injections of fibroblast interferon are relatively low, it is often given as an intravenous infusion. Limitations in supply have prevented the increase of doses much above  $10 \times 10^6$  IU.

Both subjective and objective side-effects occur with pure interferons. Leukocyte interferons, purified by monoclonal antibody affinity chromatography to  $2.5 \times 10^8$  IU/mg protein, produced the same acute side-effects in normal volunteers as did impure leukocyte interferons at  $2 \times 10^6$  IU/mg protein. No such side-effects were observed with placebo injections (13). Human leukocyte interferon subspecies ( $\alpha_2$ ) prepared by recombinant DNA techniques and subsequently purified to  $2-4 \times 10^8$  IU/mg protein have been used in patients with cancer (14). Again, side-effects were noted similar to those observed with impure, buffy coat, leukocyte interferon preparations.

## 6.2 Antigenicity

Interferons are weakly antigenic in homologous species. In 20 patients receiving leukocyte interferon for 6-18 months, no evidence of interferon antibody was detected (15). Antibody to leukocyte interferons has been found in at least 2 patients who had never been treated with interferons.

Furthermore, 3 out of 16 patients receiving interferon- $\alpha_2$  produced by recombinant DNA techniques in a Phase I trial developed neutralizing IgG antibody (14). Whether antibodies result from the high doses of  $\alpha_2$  administered or from a physicochemical modification present in interferon synthesized in *E. coli* remains to be determined. Treatment with interferon- $\beta$  has also resulted in IgG antibody formation in at least one patient (16).

## 6.3 Activity of interferons against virus diseases

Many reports have accumulated on the clinical use of exogenous interferon for the prophylaxis and treatment of a number of viral

infections (17). The following findings are taken from some of these reports (18, 19, 20, 21, 22). The majority of the studies were performed with HuIFN- $\alpha$  interferon obtained from human leukocytes.

### 6.3.1 *Virus infections of the upper respiratory tract*

In 1973 it was found that it was possible to ameliorate the symptoms of rhinovirus infections in volunteers, when  $14 \times 10^6$  IU of interferon were administered intranasally in divided doses given before and after infection. The studies indicated that similar results could be expected with influenza virus. In one country, low doses of locally prepared interferon, using fewer than 2000 "units" per application, were considered as sufficient for prophylaxis against influenza. However, this is not generally accepted.

More recently,  $3 \times 10^6$  IU of HuIFN- $\alpha$  applied by aerosol intranasally every 4 hours, commencing 16 hours before inoculation with rhinovirus type 13 and continuing until 16 hours after inoculation, produced no significant change in the incidence of illness among volunteers as compared with controls. However, it has been reported that highly purified IFN- $\alpha$ , at a dose of  $9 \times 10^7$  IU given intranasally, offered substantial protection against inoculation with a rhinovirus. One of the major reasons why such large amounts of IFN are required is the rapid clearance of locally applied interferons by mucous and ciliary action. The value of interferon as a therapeutic agent for respiratory virus infections remains to be proven.

### 6.3.2 *Herpesvirus infections*

Although herpesviruses are not as sensitive as some other viruses to interferon *in vitro*, there is considerable clinical evidence that interferon treatment of herpesvirus infections is beneficial. It is estimated that about 150 patients have taken part in studies of herpes zoster. The results of two controlled trials indicate that there was a trend towards fewer complications. In one study, treatment was begun soon after the onset of the lesions and continued for 7–8 days, or until no new lesions appeared, with different doses ranging from  $3 \times 10^5$  IU/day, the highest being  $3.5 \times 10^7$  IU/day. With the highest dose, there was inhibition of new vesicle formation in the primary dermatome, dissemination was prevented, and complications (including post-herpetic neuralgia) were reduced.

In a study in children with neoplasia and varicella, it was noted that, although the length of the period of new vesicle formation was

not affected, there were fewer visceral complications in patients receiving interferon than in those receiving a placebo.

There is a report that HuIFN- $\alpha$  ( $5 \times 10^6$  IU/day), given 1 day before and for 5 days after surgery on the trigeminal ganglia for trigeminal neuralgia (tic douloureux), reduced the frequency and duration of the shedding of herpes simplex virus from the oropharynx. Herpes labialis also occurred less frequently in treated patients, i.e., in 41%, as compared with 83% in the placebo group.

The effects of interferon against cytomegalovirus were studied in renal transplant recipients,  $3 \times 10^6$  IU of HuIFN- $\alpha$  being administered preoperatively and then twice weekly by intramuscular injection for 6 weeks. The incidence of viraemia with cytomegalovirus was significantly less in this interferon-treated seropositive group than in the control group. However, there was no apparent effect on the incidence of clinical illness. In 30 patients with antibody to herpes simplex virus before the operation, no difference was found between the interferon-treated group and the control group regarding virus activation, rise in antibody titre, or the development of mucocutaneous herpetic lesions. Negative results were also obtained in a similar study with HuIFN- $\beta$ .

Chronic cytomegalovirus infection has also been treated with interferon. The virus titre fell temporarily in some patients, and the virus was cleared from the urine in a few patients.

The effect of interferon on acute initial genital herpes and the subsequent establishment of recurrence is also under study.

Both leukocyte and fibroblast interferon have been evaluated for the treatment of herpetic dendritic keratitis. The results with interferon alone have been discouraging even when the potency was high. However, excellent results were obtained in double-blind placebo-controlled studies when the interferon ( $30 \times 10^6$  IU/ml) was combined with trifluorothymidine or with debridement. The combination of trifluorothymidine and potent interferon offers the best currently known treatment for this infection, which is of considerable medical importance. There is also a report of improvement of adenovirus keratitis following treatment with interferon.

### 6.3.3 *Chronic liver disease associated with hepatitis B virus*

Chronic active hepatitis associated with infection with hepatitis B virus is a serious condition which may progress to cirrhosis of the liver

and primary hepatocellular carcinoma. It is a highly prevalent condition particularly in East and South-East Asia, the Western Pacific area, and Africa, where the carrier rate may be as high as 5–20%, or more, of the population. Patients with chronic hepatitis B, as well as asymptomatic carriers of hepatitis B virus, may be a source of infection to contacts. There have been a number of uncontrolled studies using high doses of HuIFN- $\alpha$  and HuIFN- $\beta$ ; these show that treatment for prolonged periods results in biochemical, histopathological, and clinical improvement. In a proportion of patients (and in several chimpanzee carriers of hepatitis B virus), the first (and sometimes the only) effect of interferon has been a rapid decrease in viral DNA polymerase activity, sometimes associated with a marked decrease in the number of circulating hepatitis B viruses and in *e* antigen, as well as a fall in titre of hepatitis B surface antigen. Frequently, on cessation of treatment, these hepatitis B markers returned to their initial levels with or without a "rebound" phenomenon. Complete remission has also been reported.

The beneficial effect of interferon could be mediated by an effect on the immune system as well as by direct antiviral activity. The former suggestion is supported by a marked fall in DNA polymerase levels in some patients following withdrawal of long-term immunosuppressive therapy with prednisone (with and without azathioprine). However, spontaneous remissions, clearing of hepatitis B antigen markers, and the appearance of anti-HBe and, at times, anti-HBs are also known to occur.

Because of the inconsistencies in the studies using interferon alone, combinations of interferon with other antivirals were attempted. Recently, one group of investigators reported that the best results were obtained when treatment was alternated every 28 days, using about 0.5 g/day adenine arabinoside monophosphate (ara-AMP) and interferon in a dose of  $5 \times 10^6$  IU/day. A placebo-controlled study is in progress comparing this schedule of treatment for 6 months with ara-AMP alternating with a placebo. Similar studies are being conducted in several countries, using either interferon alone, ara-AMP alone, or a combination.

The aims of the studies to date have mainly been to reduce infectivity and improve clinical symptoms and signs. If complete eradication of the viral genome is sought, a reliable indicator of therapeutic efficacy in future studies could well be the assay by hybridization of the serum levels of hepatitis B viral DNA and of viral DNA in liver biopsy tissue.

Combination treatment must not be overlooked. It appears that both herpetic keratitis and chronic hepatitis B infection respond best to interferon plus another antiviral agent. Perhaps this will prove to be the method of choice for the clinical use of interferon.

#### 6.3.4 *Virus-associated papillomas and warts*

The virus infection in which interferon appears to be most effective is warts; in many studies, local administration of interferon caused their complete disappearance. Local and systemic application of HuIFN- $\alpha$  and HuIFN- $\beta$  for several weeks has been successful in the treatment of verruca vulgaris, molluscum contagiosum, and condylomata acuminata (vulval warts), in both normal and immunosuppressed patients. Direct inoculation of HuIFN- $\beta$  into penile warts has also been reported to have a small but significant inhibitory effect on their growth.

Impressive results have been obtained in the treatment of about 30 patients with severe juvenile laryngeal papilloma, a condition associated with a virus. The frequent need for surgical removal of the papillomas may be avoided by the administration of interferon. Daily doses of approximately  $2 \times 10^6$  IU, or a minimum of  $3 \times 10^6$  IU 3 times a week, for prolonged periods appear necessary. As long as they receive interferon, most patients remain free from papillomas, but if maintenance therapy is stopped the tumours may reappear. The course and clinical manifestations of this condition are variable, and spontaneous remission and recovery occur. A properly controlled study must therefore be carried out before it can be established that interferon is the preferred method of treatment.

#### 6.3.5 *Other virus diseases*

The studies just mentioned are those from which most information is available about the effect of interferon on virus diseases. However, other studies have shown that interferon may also have a part to play in the treatment of rabies, Japanese B encephalitis, and epidemic keratoconjunctivitis, of such serious infections as those caused by Ebola virus and other haemorrhagic fever viruses, and of chronic illnesses, such as Creutzfeldt-Jakob encephalopathy and multiple sclerosis, in which viruses may be implicated. The recently reported association of Kaposi sarcoma and/or pneumocystis pneumonia with severe immunodeficiency in young homosexual men may also offer scope for inter-

feron treatment, and appropriate studies using interferons produced by recombinant DNA techniques have been initiated. Mononucleosis caused by Epstein-Barr virus and cytomegalovirus may also be candidates for interferon treatment, as may infections with arbovirus; for example, some studies have been carried out in patients with dengue, arenavirus infections, and certain exotic virus infections.

#### 6.3.6 *Prospects*

Although much has been learned about the role of interferon in virus diseases, much more information on the subject is required. If interferon or other antiviral drugs are to be used effectively, new and rapid techniques for the diagnosis of virus infections must be developed and applied. Unfortunately many of the studies to date have been uncoded or not placebo-controlled. It is now known that interferon has some beneficial effect in some viral infections, but in most instances the optimum dose and treatment schedule have not been established. There are still uncertainties concerning the results of local or topical versus systemic treatment where these alternatives are applicable. The merits of the various interferon preparations must be determined.

It cannot be overemphasized that placebo-controlled studies are necessary before any claim for the efficacy of interferon in treating virus infections can be made. Uncoded and uncontrolled preliminary studies are often misleading.

#### 6.4 **Interferons in the treatment of malignant disease**

Most of the information about the clinical efficacy of interferons in the treatment of malignant disease has been obtained from studies using HuIFN- $\alpha$  prepared in Helsinki from human leukocytes (11, 12, 21, 23, 24, 25). More limited data exist on the efficacy in this respect of HuIFN- $\alpha$  prepared from lymphoblastoid cells, of interferon-produced by recombinant DNA techniques, and of HuIFN- $\beta$ . Nothing is yet known about the antitumour effects in man of IFN- $\gamma$ . IFN has been administered both parenterally and locally to patients with haematological malignancies and solid tumours. The results reviewed below are mainly from Phase II studies and in part from Phase I studies (21, 23, 24).

#### 6.4.1 Systemic administration

The most widely utilized schedule of administration tested to date consists of daily intramuscular injections given for at least a month unless an increase in the tumour has occurred before a month has elapsed. (If the tumour has progressed or increased, interferon has been discontinued and the patient removed from the study.) The Phase II studies have commonly employed doses of between  $1 \times 10^6$  and  $10 \times 10^6$  IU/daily.

(a) *Haematological malignancies.* A marked decrease in objectively measurable parameters of myelomatosis has been reported. Both serum monoclonal protein concentration and Bence Jones protein excretion in the urine have been reduced markedly by treatment with interferon. Furthermore, a decrease in the size of measurable plasmacytomas, an increase in erythrocyte volume fraction (haematocrit), and an improvement in the general state of the patient have all been recorded. An overall objective response rate of 20 has occurred in approximately 100 patients treated with leukocyte interferon preparations. Both partial and complete responses have been observed. The data suggest that there is a higher response rate in the case of IgA myelomas, and in patients with light-chain immunoglobulins present only in urine, than in the case of IgG myelomas. The duration of the objective responses has ranged from several weeks to more than 3 years. Some of the more persistent responses have been maintained for extended periods after treatment has been discontinued.

Phase II trials with leukocyte interferon preparations suggest that follicular (nodular) lymphomas may respond to treatment. Published data indicate that, of 13 patients treated, 7 have had partial or complete remissions. In at least 2 instances, regressions have continued to occur after interferon was discontinued.

Higher doses of interferon may be required for the treatment of acute leukaemia. Results from Phase I trials, which have as a primary objective the determination of safety and side-effects, indicate that doses of  $50 \times 10^6$  IU/day for a week may be required. Significant decreases in peripheral blasts have occurred in roughly a third of 20 patients treated. In one or two patients, marrow improvement has also been observed.

(b) *Solid tumours.* The effects of leukocyte interferon preparations on metastatic breast carcinoma have been evaluated in 40 patients. In 12 of them, a partial response (defined as a reduction of at least 50%

in the diameters of the measurable lesions) was obtained. The duration of response was between 2 and 70 weeks. The best responses in patients with breast carcinoma have been obtained in cases of predominantly soft tissue disease, but regression has been noted in patients with bone and bone marrow infiltration. Individual soft tissue masses have regressed completely in some cases.

The results of systemic treatments of patients with melanoma and non-small-cell bronchogenic carcinoma have so far been unimpressive. Among 59 patients with melanoma treated in Phase I and II trials, there was evidence of an objective partial response in only 3. Fifteen patients with bronchogenic carcinoma treated at similar doses did not respond.

The primary management of osteosarcoma has been the subject of an adjuvant therapy trial (treatment with a leukocyte interferon preparation of patients clinically free from disease after tumour ablation). In this trial, interferon was initiated before surgery and continued for 30 days. Thereafter, interferon was continued 3 times weekly on an outpatient basis for an additional 17 months. The trial has not yet been completed, but 60% (18 out of 30) of the interferon-treated group were still free from metastatic disease 3 years after the original diagnosis. A concurrent control group of osteosarcoma patients, who received no adjuvant therapy, had a 3-year disease-free survival rate of 35% (15 out of 42). The effects of interferon in this trial are comparable to those reported for doxorubicin and high-dose methotrexate adjuvant therapy for osteosarcoma. Since some changes in the natural history of osteosarcoma may have occurred, a prospective randomized study will be required to substantiate the therapeutic benefit of interferons and compare their efficacy with that of cytotoxic chemotherapy.

#### 6.4.2 *Local administration*

Repeated local administration of interferons, sometimes supplemented by systemic administration for limited periods, has resulted in a significant regression of recurrent tumours that have sometimes proved refractory to conventional treatments. Preparations of leukocyte interferon have been administered intravesically to patients with low-grade bladder carcinomas. In 8 out of 14 patients, there was complete disappearance of tumours, and in 3 additional patients there was partial regression. In 10 patients with recurrent glioma, interferon- $\alpha$  preparations were administered by Rickham catheter

placed directly into the tumour. A computerized axial tomography (CAT) scan showed evidence of tumour regression in 6 of these patients. Two other patients with medulloblastoma, who had similar treatment, showed excellent responses. Subcutaneous tumour nodules in patients with malignant melanoma have been injected locally with preparations of fibroblast interferon; marked local tumour regression was observed in a number of instances. No lesion injected with control material regressed. Intratumour injections have suggested the possible sensitivity of other skin carcinomas to leukocyte interferon preparations. Their local administration, supplemented in some cases by application of an interferon ointment, to 30 patients with squamous and basal cell carcinomas resulted in complete regressions in 10 patients and partial responses in 15. Most patients who responded have not shown evidence of any recurrence.

#### 6.4.3 *Prospects*

The use of interferon for the treatment of malignant disease in man remains experimental and its clinical benefit is not proven. The optimum dose and schedule of treatment remain to be defined, and these may not be the same for different types of tumour. Since interferons are both antiproliferative proteins and immune-response modifiers, further trials are required to establish the maximum tolerated dose and the biologically effective dose. The correlation of basic interferon pharmacokinetics with desired biological responses may provide valuable information for modifying doses and schedules.

The currently available systemic treatments have only occasionally proved curative in patients with disseminated malignant disease. Consequently, on the basis of the results summarized above, continued investigation of interferons to define their potential therapeutic value is warranted. Many additional controlled trials will be required to confirm or refute the ultimate role of interferons as antitumour proteins. These studies are justified only in research hospitals and clinics.

## 7. SUMMARY

Twenty-five years after the first description of "the interferon", there is still much to be learnt about the role of the proteins known as interferons in the body and their value in human medicine.

Appropriate interferon preparations have produced antiviral and antitumour effects in animal studies, and it is likely that valuable

information can be gained from additional studies. Exactly what mechanisms are involved in such effects is not clear, and indeed they are probably multiple. For example, the antitumour effects may reflect a direct anticellular action of the interferon on the tumour cells and indirect effects mediated through host defence mechanisms.

Even though animal model systems, especially those involving rapidly growing tumours, are seldom directly relevant to human disease, the results obtained with interferon in animal studies have suggested that beneficial effects might also follow its use in human virus infections and cancer. At first, only very small amounts of human leukocyte interferon were available for clinical studies. More recently, larger amounts have become available from various sources, but the picture has become more complicated. It is now known that there are at least three main types of human IFN, classified as HuIFN- $\alpha$ , - $\beta$ , and - $\gamma$ , which can be made by treating particular human cells with appropriate inducing substances. Furthermore, there are many subtypes (possibly as many as 14) of HuIFN- $\alpha$ , and a mixture of at least 8 of these is obtained when, for example, the human B lymphoblastoid cell line, Namalwa, is treated with Sendai virus. The application of recombinant DNA techniques to interferon research has led to the insertion of individual HuIFN- $\alpha$ , - $\beta$ , and - $\gamma$  genes into bacterial, yeast, and animal cells. The proteins obtained by the expression of these genes have been found to differ in their chemical structure and biological properties. It is therefore necessary to consider carefully controlled and comparative clinical studies with these individual interferons and with the mixtures derived from human cells. While the former may be easier to produce in large quantities, it is possible that the mixtures may have clinical advantages because of interaction between the different types of interferons. Interferons derived from human cells or made by recombinant DNA techniques are now available in large amounts as almost pure preparations.

One problem that plagued much early work has been largely overcome: the availability of WHO International Reference Preparations now makes it possible to correlate results from different laboratories working with HuIFN- $\alpha$  and - $\beta$ , and to report the potency of preparations of these interferons in international units. An HuIFN- $\gamma$  reference preparation will shortly become available from the US National Institutes of Health.

The present position in the clinical evaluation of HuIFN can be summarized as follows:

Phase I studies with preparations of HuIFN- $\alpha$  have been carried

out and have given some information about the maximum tolerated dose for various routes of administration and about the side-effects to be expected: such side-effects will usually be encountered with the doses likely to be used in clinical trials.

Beneficial effects of HuIFN against herpetic keratitis, herpes zoster, and laryngeal papilloma have been reported. The use of interferon combined with antiviral drugs such as ara-AMP has given promising preliminary results in selected patients with chronic hepatitis B infections.

In malignant disease complete regression of tumours has rarely been achieved by interferon treatment, and no response has been observed in the majority of patients. However, objective improvements have been reported following the systemic use of interferon in patients with multiple myeloma, disseminated follicular lymphoma, and recurrent breast cancer. Occasional responses have also been seen in patients with other types of tumour. Nevertheless, the overall *clinical* benefit of HuIFN in the treatment of patients with malignant disease remains to be defined. In further trials, it is desirable that all laboratory investigations relevant to the use of a biological response modifier should be carried out and that attempts should be made to correlate the results of such investigations with the course of the disease and with any response to treatment.

Interferons are not a panacea for the cure of human virus infections or cancer, and there is no case for their use at present except in properly controlled clinical trials.

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## Annex

### CALIBRATION IN INTERFERON ASSAYS

The following standard preparations, when used correctly, permit the reporting of results of interferon bioassays in International Units: (1) preparation 69/19 (British Medical Research Council Research Standard B), which is the International Reference Preparation of Interferon, Human Leukocyte, having an assigned activity of 5000 (or  $3.7 \log_{10}$ ) International Units of HuIFN- $\alpha$  per ampoule;<sup>1</sup> (2) the US National Institutes of Health (NIH) preparation GO23-902-507, which is the International Reference Preparation of Interferon, Human Fibroblast, having an assigned activity of 10 000 (or  $4.0 \log_{10}$ ) International Units of HuIFN- $\beta$  per ampoule; and (3) the NIH preparation GO23-901-527, which is the International Working Reference Preparation of Interferon, Human Leukocyte, having an assigned activity of 20 000 (or  $4.3 \log_{10}$ ) International Units of HuIFN- $\alpha$  per ampoule.<sup>2</sup> An NIH Reference Preparation of HuIFN- $\gamma$  has been prepared and appropriate testing undertaken in order to assign a value for its potency. Until this preparation is released for distribution, HuIFN- $\gamma$  preparations must be reported in laboratory units with a full description of the assay used. Reference preparations of interferons obtained by recombinant DNA techniques are not yet available.

For calibration in interferon bioassays, more than 4 independent titrations of the International Reference Preparation should be made on separate days in each laboratory with the usual interferon bioassay technique employed in that laboratory. It has been recommended that the results should be summarized as the observed geometric mean titre, and the standard deviation stated as the logarithm. For publication or other official reports, this information, including the number of determinations performed as well as other technical details of the bioassay (i.e., virus, cells, end-point), should be reported along with the name, catalogue number, and stated assigned unitage of the appropriate standard. In addition, a suitable homologous-type laboratory reference preparation that gives dose-response curves parallel to the International Preparation should be calibrated simul-

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<sup>1</sup> Available from the National Institute for Biological Standards and Control, Holly Hill, Hampstead, London NW3 6RB, England.

<sup>2</sup> Available from the Research Resources Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20205, USA.

taneously in repeated titrations (more than 4) against the appropriate International Reference Preparation. Subsequently, this laboratory reference preparation should be titrated every time other, unknown, test samples are being measured for their interferon activity.

All results should be reported in International Units, if the International Reference Preparation of the homologous type exists. To do this, an appropriate adjustment can be made by the ratio method relating the observed geometric mean titre of the test sample to the geometric mean titre of the appropriate laboratory reference preparation that has previously been calibrated, as above, against the International Reference Preparation. If no international or national reference preparation exists, units may be expressed as the minimum amount of interferon producing an arbitrarily defined degree of activity in the given test system, essentially a laboratory unit (26).