Drugs 42 (5): 749-765, 1991
0012-6667/91/0011-0749/$08.50/0

DRU1 81

The Use of Interferon-α in Virus Infections

N.B. Finter,[1] *S. Chapman,*[2] *P. Dowd,*[1] *J.M. Johnston,*[2] *V. Manna,*[1] *N. Sarantis,*[1] *N. Sheron,*[4] *G. Scott,*[3] *S. Phua*[5] and *P.B. Tatum*[2]

1 Wellcome Research Laboratories, Beckenham, Kent, England
2 Division of Clinical Research, Burroughs Wellcome Company, Research Triangle Park, North Carolina, USA
3 Department of Clinical Microbiology, University College Hospital, London, England
4 Liver Unit, King's College Hospital, London, England
5 Wellcome Singapore Pte, Singapore

Contents

Summary

The interferons (IFN) act too slowly to arrest acute viral infections, but interferon-α (IFNα) preparations have proved useful in some chronic infections and will clearly be used increasingly in these in the future.

In the preparations derived from human leucocytes or cultured B lymphoblastoid cells, which are in routine clinical use, mixtures of a number of distinct subtypes of human IFNα have been identified. There are also 3 slightly different versions of the same single subtype, IFNα-2, made by recombinant DNA procedures in bacteria.

IFNα preparations are injected intramuscularly or subcutaneously. Dose-related side effects are common but usually tolerable, but prolonged treatment may cause increasing fatigue and depression. Some patients form neutralising antibodies which block the effects of the IFN; these appear to be relatively more common after recombinant IFNα-2 than after IFN derived from human cells.

Given intranasally, IFNα can prevent a subsequent experimental rhinovirus infection, or the

spread of natural colds within a family. Repeated administration progressively damages the nasal mucosa, so that long term prophylaxis is not possible.

IFNα has proved useful in patients with papillomavirus warts of the larynx, ano-genital region (condyloma acuminata) and skin (common warts). Treatment regimens remain to be optimised and are likely to include surgery or other treatments.

IFNα and zidovudine (azidothymidine) synergistically inhibit the growth of HIV *in vitro*, and combination are on trial in patients with early AIDS. Very large doses of IFNα are effective against Kaposi's sarcoma in some AIDS patients.

In chronic hepatitis B, continuing virus replication may lead to cirrhosis or primary liver cancer. Earlier clinical trials with IFNα gave inconclusive results, but recent large studies have confirmed that 25 to 40% of patients obtain benefit; this probably results from both the antiviral and the immunomodulatory effects of IFNα.

In patients with chronic hepatitis C, the biochemical markers usually improve rapidly during IFNα administration, but relapse if treatment is stopped after only a few months; to increase the chances of sustained cure, the treatment period is now being prolonged.

1. Introduction

The interferons (IFNs) are antiviral proteins that play an important role in the natural control of viral infections (Gresser et al. 1976a,b). They are classified into α, β, ω, and γ types, and chemically-related but antigenically distinct variants of these are formed by the cells of each animal species. The human interferons (HuIFN) are formed by human cells naturally during life or in culture in response to various stimuli, especially a viral infection. There are at least 22 subtypes of HuIFNα, which have 70% of their 166 (or 165) amino acids in common, but differ in at least some biological properties (Finter 1991). The single HuIFNβ has many of the same properties as IFNα, and shares about 30% of the amino acids (the cytokine once termed IFNβ-2 is now classified as interleukin-6). There is one HuIFNω with 172 amino acids, which is chemically closely related to IFNα but antigenically quite distinct; it has not yet been separately tested in patients. IFNγ (formerly termed type 2 or 'immune' IFN) is a T cell lymphokine which is very different from other IFN in its chemical structure and most of its properties, and has thus far been little used in viral infections. This review, however, will mainly consider results obtained with preparations of IFNα.

The development of IFNs for clinical use has been reviewed by Billiau (1984). Because of 22 years of previous research on the virus interference phenomenon (Henle 1950), the potential value of IFNs as antiviral agents for use in patients was realised as soon as they were discovered by Isaacs and Lindenmann (1957). However, sufficient amounts of IFNs to allow clinical evaluation became available only in 1970, and were then nearly all used in trials in cancer patients. Indeed, IFNs have so far been used mainly in the management of some forms of leukaemia and other types of cancer, but they are now increasingly being used to treat the chronic viral infections discussed in this review.

IFNα and -β bind to the same specific receptors on the cell surface. As a result of intracellular processes that are still being elucidated, IFN-stimulated response elements (ISRE) in the cell nucleus are activated and a number of proteins are synthesised. These include the Mx protein and its human analogue (Weitz et al. 1989), which have specific antiviral functions, and 2 enzymes, 2′-5′-oligoadenylate synthetase (2-5 A synthetase) and a protein kinase, which probably produce the general resistance of treated cells to viral infection (Hovanessian 1989; Pestka et al. 1987). This antiviral state reaches its peak some 6 or more hours after a cell is first exposed to an IFN, but once established may persist, though slowly waning, for many hours (table I).

1.1 Sources of Interferon-α for Clinical Use

To make IFN in amounts sufficient for clinical uses required human cells to act as the source in numbers that initially seemed almost impossibly large. The first solution was to use white blood cells obtained as a by-product from freshly donated transfusion blood (Strander & Cantell 1966). When these were treated with a mouse parainfluenza virus, Sendai, they formed what was termed leucocyte IFN (fig. 1). After partial purification, this product was used in almost all clinical studies until about 1979. Leucocyte IFN is relatively expensive and difficult to make and control, and even the cells from many thousands of individual blood donations yield only sufficient IFN to treat a few patients at a time.

Another system, devised at the Wellcome Research Laboratories at Beckenham in 1975, is used for commercial scale production from human cells (Johnston 1985). Cells of an immortalised human lymphoblastoid line, Namalwa, are grown in large stainless steel tanks. When these are stimulated with Sendai virus, they secrete many different IFNα subtypes which are separated from the medium and highly purified. The final product, 'Wellferon', is an essentially pure mixture of at least 22 subtypes (Zoon et al. 1989). Because Namalwa cells originated from a Burkitt tumour biopsy, rigorous safety tests of a new type were devised and applied (Finter & Fantes 1980; Finter et al. 1985). This lymphoblastoid IFN was licensed for clinical trial use by the national control authorities in the UK, USA and Japan in 1980-1981, and subsequently for sale for the treatment of specified diseases in several countries. It was the first medicinal product to be manufactured from a transformed cell line, and its example has greatly facilitated the acceptance of other such cells for the manufacture of important therapeutic proteins such as Factor VIII and granulocyte macrophage colony stimulating factor (GM-CSF).

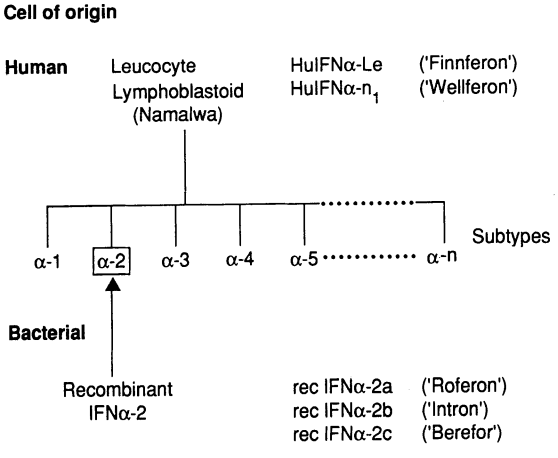

Fig. 1. Sources of human interferon-α (HuIFNα) for clinical use.

Table I. Antiviral effects of interferons

A natural antiviral defence mechanism, activated in a few hours (contrast onset of active immunity over several days in nonimmune host)
Not specific to particular viruses
No effect on extracellular virus particles
Mechanism involves active response by the cell after interferon molecules bind to specific surface receptors. Several proteins are synthesised, including antiviral proteins (Mx and its analogues) and enzymes (a protein kinase and 2′-5′ oligoadenylate synthetase) which degrade newly formed viral components
Antiviral effects are established over several hours (thus administration of interferons is unlikely to be useful in an acute viral infection but can be valuable in chronic infections)

In 1980, 2 groups independently used recombinant DNA procedures to obtain expression of human IFNα genes in transfected *Escherichia coli* (Goeddel et al. 1980; Nagata et al. 1980). This route is now used by 3 manufacturers for the commercial production of particular versions of the α-2 subtype of human IFN; these are IFNα-2a ('Roferon', Roche), IFNα-2b ('Intron', Schering) and IFNα-2c ('Berefor', Boehringer-Ingelheim) [fig. 1], each of which differs from the other 2 by a single amino acid. These 3 recombinant IFNα-2 preparations are also now widely licensed for use.

There are different WHO International Standards for leucocyte, lymphoblastoid and recombinant IFNα-2 IFN, which emphasises the fact that

these different IFN preparations are not completely interchangeable. At least 3 of the IFNα subtypes in leucocyte and lymphoblastoid IFN, including the subtype corresponding to recombinant IFNα-2, contain sugar molecules (Kojima et al. 1989; Kojima, personal communication; Zoon, personal communication), whereas the recombinant IFNα-2 expressed in *E. coli* is not glycosylated. Lack of glycosylation does not influence the activity of the recombinant IFNs *in vitro*, but may affect their pharmacokinetic behaviour and 3-dimensional shape. Also, the recombinant preparations contain only a single subtype, α-2, whereas induced human cells yield mixtures of many subtypes, which have interacting and sometimes synergistic effects on cells. Some subtypes have properties that differ markedly from those of other subtypes (Finter 1991). Whether any of these differences affect clinical utility is not at present clear, in spite of hints from the only published direct comparison of 2 different IFN preparations so far (Idéo et al. 1990).

1.2 Antigenicity

If patients are treated with IFNα preparations for periods of many months a proportion develop neutralising antibodies which, if present in high concentrations, may block the effect of the IFN with loss of any clinical benefit from the treatment. Antibodies seem to be more frequent with some IFNα preparations than with others, and have caused more clinical problems in the treatment of various cancers than in viral infections, probably because in the latter treatment courses are usually relatively much shorter.

Antonelli et al. (1991) examined sera from a relatively homogeneous group of chronic hepatitis patients. They found antibodies neutralising IFN in sera from 20.2% (15/74) of those treated with recIFNα-2a; in 6.9% (10/144) of those treated with recIFNα-2b; and in 1.2% (1/78) of those treated with lymphoblastoid IFN. Neutralising antibodies were also observed in 39% (21/54) of Chinese children with chronic hepatitis B treated with recombinant IFNα-2a, and those with higher titres were less likely to respond to treatment; with IFNα-2b, the incidence was 9.4% (5/54) and the titres were all low (Lok et al. 1990; Lok & Lai 1991).

Oberg used IFNs to treat patients with malignant carcinoid tumours (presented at Hannover Interferon workshop, February 1991). Neutralising antibodies developed and were associated with clinical relapse in 27% (7/26) of those treated with recIFNα-2a; in 4% (6/151) of those treated with recombinant IFNα-2b; but in none of 81 treated with leucocyte IFN. When relapsing patients were switched to treatment with leucocyte IFN, about half achieved a response. Similar problems with recombinant IFNα-2a or IFNα-2b have been seen in chronic myelogenous leukaemia (Freund et al. 1989; von Wussow et al. 1988, 1989); hairy cell leukaemia (Steis et al. 1988); essential mixed cryoglobulinaemia (Casato et al. 1990), and essential thrombocythaemia (Gugliotta et al. 1989): again, many patients were successfully retreated with a human cell-derived IFN preparation.

1.3 The Uses of Interferon-α in Virus Infections

IFNα preparations are given by intramuscular or subcutaneous injection; the intravenous route seems to have no advantages. IFNα is very potent: even a large dose such as 10 megaunits (MU, 10^6 International Units) of human IFNα only requires about 50μg IFN protein, and 3mg provides the complete 3-month course for a patient with chronic hepatitis. Treatment with an IFNα is accompanied by influenza-like side effects. Fever is almost invariable after the first dose, but usually diminishes after subsequent doses. Other relatively common side effects include lassitude, fatigue, depression, leucopenia, thrombocytopenia and elevation of liver enzymes. These effects are dose related at least over the range from about 1 to about 10MU, but vary greatly in severity from one individual to another, some patients being intolerant of even a very small dose, whereas others accept relatively large amounts without difficulty. If injections are given in the evening, side effects occur mainly during the hours of sleep. However, with a prolonged course

Table II. Chronic viral infections responsive to IFNα therapy

Papillomavirus infections
Laryngeal papillomatosis
Condylomata acuminata (genital warts)
Skin warts
HIV infections
Kaposi's sarcoma
AIDS?
Chronic active hepatitis B
Chronic hepatitis C (non-A, non-B hepatitis)

there may be progressively increasing fatigue and depression, which sometimes limit the duration of IFN treatment.

IFNs have not proved effective in acute viral infections, such as the common cold. In part this may be because the antiviral response is slow to come into effect, but a much more important factor is that treatment is likely to be late: a patient with an acute viral infection such as influenza does not feel ill until many millions of cells have already been infected and accordingly formed and released their quota of IFN. To inject still more is then unlikely to be helpful. In contrast, IFNs have been used successfully for prophylaxis and the treatment of certain chronic viral infections: in the latter, the number of cells infected and forming IFN at any one time is likely to be small so that to supply additional amounts can be beneficial (table II).

Since earlier information on the uses of IFN in viral infections has been considered in some detail elsewhere (Scott & Tyrrell 1985), this review concentrates on more recent results.

2. *Interferons for Respiratory Virus Infections*

Well-controlled studies in healthy volunteers have shown that intranasally administered IFNα can suppress the symptoms that follow experimental infection with a rhinovirus, as judged by objective criteria (reviewed by Scott & Tyrell 1985). About a quarter of volunteers given IFN nevertheless shed virus in the nasal secretions, even though they had minimal symptoms, if any. Human IFNα preparations of all types have approximately the same activity against rhinoviruses; IFNβ is slightly less active on a weight-for-weight basis (Higgins et al. 1986) and IFNγ is completely inactive (Higgins et al. 1988). IFNα also protects volunteers against experimental infections with a coronavirus (Higgins et al. 1983) or, though much less efficiently, with an influenza virus (Phillpotts et al. 1984).

Little has been published recently about methods of delivering IFN to the respiratory tract, although it has usually been applied in the form of an intranasal IFN spray with a mixed particle size, delivered through a fine nozzle from a self-actuated pump. Nevertheless, a comparison showed that the administration of nasal drops resulted in a more favourable distribution of radiolabelled albumin above the hard palate (Aoki & Crawley 1976).

The timing of the dose of IFNα in relation to the rhinovirus challenge seems to be critical to the outcome (Phillpotts et al. 1983); unless the IFN is given within a few hours before challenge, no protection results, irrespective of how much has been given during the previous few days. As IFN induces a long-lived antiviral state in nasal epithelial cells in culture, this is surprising and suggests that mechanisms other than straightforward protection of the cells against rhinovirus may operate *in vivo*. The minimum effective dose is 2MU given once (Samo et al. 1984) or preferably 3 times (Phillpotts et al. 1983) per day, but a single larger daily dose of 5MU or 10MU is slightly more effective in reducing symptoms and virus shedding (Hayden & Gwaltney 1983; Samo et al. 1983; Scott et al. 1982). In most protection studies against rhinoviruses, IFNα administration was continued for 3 days after virus challenge. If IFNα was given after the symptoms of a cold had started, it had a minor effect on virus shedding, but the course of the cold was not changed (Hayden & Gwaltney 1984).

Intranasal IFN does not cause systemic symptoms, but leads to local inflammation after 5 to 10 days of regular use. Histologically, the mucosa is ulcerated and filled with T lymphocytes of CD4

(helper) phenotype (Hayden et al. 1983, 1987). In one study, half of the volunteers had stopped taking IFN by the fourteenth day of dosing because of symptoms which were similar to, though in retrospect distinguishable from, those of the proven rhinovirus infections suffered by subjects in the placebo group (Scott et al. 1985). The mild leucopenia observed in some of the patients taking intranasal IFNα is to some extent dose related and associated with the occurrence of nasal symptoms.

Because of the local adverse effects, the long term administration of IFNα is not a practical way of providing prophylaxis against colds during a particular season. An alternative strategy would be for healthy subjects to treat themselves for limited periods when at high risk for contracting a rhinovirus infection. Such exposure is common with family groups, where children bring colds home from school, and opportunities for transmission from one member of the family to another are high. Two large studies (Douglas et al. 1986; Hayden et al. 1986) have shown that in families in which one member had already developed a cold, intranasal IFNα-2b (2MU sprayed 3 times per day) was very effective in preventing rhinovirus colds; colds due to other viruses, including some coronavirus and parainfluenza virus infections, were not inhibited. As yet, no IFNα preparation has been marketed for use in colds, perhaps because the benefits do not seem to match the costs.

It was suggested many years ago that failure to produce IFN during an acute episode of influenza may contribute to death from overwhelming pneumonia (Baron & Isaacs 1962). More recently, it has been shown that production of IFNα in response to influenza virus infections may be genetically determined (Haller et al. 1980). The therapeutic use of IFN during influenza epidemics therefore merits study in the future.

3. Interferons in Chronic Human Papillomavirus Infections

Because of their antiviral, antiproliferative and immunomodulatory effects, IFNs were a good choice for trials in patients with warts resulting from a chronic infection with one of the many serotypes of human papillomavirus; indeed IFNs have proved clinically active against these viral types.

Papillomavirus warts are of various types and appearance. They are found very commonly on the skin, occasionally in the genital region (condyloma acuminata), and very rarely on the larynx (laryngeal papillomatosis). It is estimated there are only 1500 new cases of juvenile laryngeal papillomatosis (JLP) annually in the USA, but as this was the first papillomavirus infection in which IFN treatment was tried, it is considered first.

3.1 Juvenile Laryngeal Papillomatosis

Initial uncontrolled studies suggested that leucocyte IFN treatment was beneficial in children with JLP, but no firm conclusions could be drawn because of the small numbers involved (Gobel et al. 1981; Goepfert et al. 1982; Haglund et al. 1981; McCabe & Clark 1983). Recently, there have been 2 large multicentre controlled trials. In one (Kashima et al. 1988; Leventhal et al. 1988), 66 children with severe JLP were enrolled in a 1-year crossover study and were randomised to one of two treatment arms. Patients in one arm received lymphoblastoid IFN for a 6-month period (5 MU/m^2 daily for 28 days and then 3 times a week for the next 5 months) and were then observed for the next 6 months; those in the other arm, were given IFN similarly for 6 months but after an initial 6-month observation period. All the children were examined by endoscopy every 2 months during the study and any papillomas were removed surgically.

The results showed that patients in both groups improved significantly while receiving IFN. Antibodies neutralising the IFN were detected, often transiently, in the serum of 20% of these children (Weck et al. 1989), an incidence much higher than seen in any other category of patient treated with this type of IFN; these antibodies were of low titre, and did not appear to influence the clinical outcome (Thurmond et al. 1991). Many of the patients continued treatment with this IFN at the end of the 1-year study period, or were put back on treatment. Four years later, 59 of these patients were

traced; the initial clinical benefits had been sustained with 24 patients (41%) having had a complete remission, and a further 27 (46%) a clinically significant partial response (Leventhal et al., unpublished data).

In another study (Healy et al. 1988), 123 JLP patients were randomly assigned either to receive human leucocyte IFN (2 MU/m^2 daily for 1 week, and then 3 times a week for 1 year), or to be observed with surgery as required. It was reported that the papillomas grew significantly more slowly during the first 6 months in the IFN-treated group than in those under observation, but the difference diminished during the second 6 months in spite of continued IFN administration. It is not clear whether the different duration of response in these 2 large studies reflected the IFN dose or type (lymphoblastoid versus leucocyte), or the study design.

3.2 Condyloma Acuminata (Genital Warts)

The reported annual incidence of genital warts in 2 recent surveys was 70 to 100 cases per 100 000 of population but, as with other sexually transmitted diseases, the incidence seems to be increasing (Chuang et al. 1984; Department of Health and Social Security 1985). The many treatments used include topical applications of caustic agents like podophyllin (podophyllum-resin) or trichloroacetic acid, and physical removal of the visible lesions by cautery, cryotherapy, laser or conventional surgery (Eskelinen & Mashkilleyson 1987). Such treatments may cause pain, scarring and systemic toxicities, and recurrence rates as high as 50 to 60% have been reported. In a minority of patients, condylomas are resistant to treatment, or rapidly recur, and these have represented a difficult management problem. It appears that a significant proportion can be successfully treated with an IFNα preparation, although the treatment is relatively expensive and there are often some systemic side effects.

Early reports from open studies suggested that condyloma acuminata responded to treatment with various types of IFN (Ikić et al. 1975; Scott & Csonka 1979). These observations have since been confirmed in numerous randomised, double-blind and placebo-controlled trials with IFNα preparations of human cell or recombinant origin. Comparisons between the results from the different clinical trials are difficult because there are so many variables, such as the site, type and size of the lesions; the basis for patient selection; the clinical end-point chosen; and the type of IFN used, the dose and duration of treatment. Also, 2 routes of administration have been explored, with IFN injected either directly with the warts or intramuscularly or subcutaneously at a remote site.

In studies with intralesional administration of preparations of recombinant IFNα-2 (Eron et al. 1986; Hatch 1986; Vance et al. 1986) or natural IFNα (Friedman-Kien et al. 1988; Geffen et al. 1984), the lesions completely resolved in between 10 and 62% of patients. The highest rate was achieved in a study with leucocyte IFN (Friedman-Kien et al. 1988) in which the median age of the patients was 30 years, and the median number of warts was 4 (range 2 to 14); these factors suggest that the lesions were relatively recent and thus unlikely to be refractory to treatment.

Intralesional administration initially gives a relatively high local concentration of IFN in the injected wart, but the occurrence of the usual systemic side effects shows that there is also absorption from this site. Whether this is sufficient to eliminate subclinical infections elsewhere is not known, but there are reports of relapse rates of up to 25% (Friedman-Kien et al. 1988), and of lack of response in uninjected warts (Geffen et al. 1984). Thus, theoretically, IFN given systemically may have advantages, and its efficacy has been demonstrated in a number of studies (Gall et al. 1985, 1986; Kirby et al. 1986; Reichman et al. 1988; Weck et al. 1986). In several studies (Gall et al. 1985, 1986; Weck et al. 1986), lymphoblastoid IFN was administered to a combined total of 62 males and 115 females who had longstanding refractory condylomas. The doses ranged from 1 MU/m^2 to 3 MU/m^2 given daily for 14 days and then 3 times a week for 4 weeks. Overall, the lesions decreased in size by 50% or more in 55 to 94% of patients.

In many patients, these results were accompanied by the usual side effects of IFN. These were dose related and were considered acceptable by most of the patients, although in a few the dose was reduced or treatment stopped. Neutralising antibodies have not been a problem.

In a recent multicentre study (Condylomata International Collaborative Study Group 1991), no benefit was seen from a 4-week course of IFNα-2a in patients with recurrent genital and/or perianal condylomas. This result emphasises the need for more work to define the role of IFNα in the treatment of these lesions. Perhaps the best approach will include their use in combination with other therapies, for example laser treatment as suggested by the results of another recent trial (Peterson et al. 1991).

3.3 Skin Warts

Skin warts in other parts of the body resulting from a human papillomavirus infection are relatively common. A small proportion of the many patients with such nongenital warts fail to respond even to repeated local treatments with a variety of conventional agents, with resultant great frustration to patient and physician alike. It has been shown in a number of studies that preparations of human leucocyte, lymphoblastoid and recombinant IFNα and of IFNβ can be beneficial in such patients (e.g. Eron et al. 1986; Gibson 1986; Gibson et al. 1984; Niimura 1983). The best regimen in terms of dose, route and frequency of administration, and duration of treatment remains to be defined with each of these, but experience with human lymphoblastoid IFN suggests that intralesional administration is the most advantageous route in the treatment of severe and persistent skin warts (Gibson 1988). A single injection of between 3 and 5MU into the base of the largest or most troublesome wart each week for 12 weeks gave an impressive rate of clearance (21 patients out of 27; 78%), and there was also improvement in both nearby and distant warts. Such intralesional injections are briefly painful, the extent depending on the site involved, and there are the usually mild influenza-like side effects associated with treatment with such a dose of IFN. Nevertheless, because there is no clinically detectable local tissue damage and the success rate is relatively high, this regimen has proved acceptable to most of the patients involved. Berman et al. (1986) reported mean responses of 86.1% in the treated warts of 4 patients given intralesional injections of 0.1MU of IFNα-2b, and 38.0% in 4 given placebo.

4. *Interferons in HIV Infection*

IFN inhibits the growth *in vitro* of a number of retroviruses including HIV. IFNα and -β produce dose-related suppression of HIV replication in peripheral blood mononuclear cells *in vitro* at physiologically achievable concentrations, whereas IFNγ does not (Hartshorn et al. 1987a; Ho et al. 1985; Yamamoto et al. 1986). However, in T and monocyte-macrophage cell lines all the interferons show anti-HIV activity (Crespi 1989; Hartshorn et al. 1987a; Kornbluth et al. 1989; Yamada et al. 1988).

IFNα and -β appear to affect predominantly the later stages of HIV replication, notably virus assembly and release. IFNα treatment of HIV-infected cell cultures results in a reduction in virus released into the culture supernatant but an increase in cell-associated virions (Poli et al. 1989). Removal of the IFN leads to the release of preassembled virions, resulting in higher concentrations than in cultures never treated with IFNα, so that prolonged administration would seem to be required to control HIV replication effectively.

Given the encouraging *in vitro* results it appears paradoxical that IFNα can often be detected in the sera of patients with advanced HIV disease (Buimovici Klein et al. 1986; De Stefano et al. 1982; Eyster et al. 1983). This IFN is unusual in that it is predominantly acid-labile and its production correlates with declining immune status. Once it is present, down-regulation of IFNα receptors appears to occur, leading to decreased responsiveness to exogenously administered IFN, a phenomenon seen in patients with Kaposi's sarcoma (Oettgen et al. 1986; Vadhan-Raj et al. 1986). The presence of

this endogenous IFN may explain the lack of efficacy and toxicity in the first of the studies described below.

A study in 67 AIDS patients comparing 2 different IFNα doses (36MU or 3MU 3 times weekly) with placebo failed to detect any differences in efficacy or toxicity variables over a 12-week treatment period (Interferon Alpha Study Group 1988). In a placebo-controlled study with high-dose (35MU daily) IFNα for 12 weeks in patients with much earlier disease, antiviral effects were noted in terms of numbers of patients becoming HIV culture-negative (Lane et al. 1990). However, the high dose was not well tolerated. One study with IFNβ at doses of 90 and 180MU daily in asymptomatic patients showed reductions in p24 antigen (a marker of HIV infection) but tolerance was poor (Borucki et al. 1990). Pilot studies conducted with IFNγ in ARC and AIDS patients have not produced any encouraging results (Baron et al. 1988; Pennington et al. 1986). Overall these results suggest that IFNs as single agents are not appropriate for the treatment of HIV-infected patients who because of the nature of the infection require prolonged, if not lifelong, therapy.

IFNα and IFNβ have, however, been shown to have a role in treating a subset of patients with the AIDS-associated malignancy, Kaposi's sarcoma (de Wit et al. 1988; Miles et al. 1990; Oettgen et al. 1986; Vadhan-Raj et al. 1986; Volberding et al. 1987). The known antiproliferative effects of IFN combined with its anti-HIV activity made it an ideal agent to try in this tumour. High doses (20 MU/m^2 or more) have induced response rates of 20 to 40%. Patients most likely to benefit are those with higher CD4 counts and patients without significant HIV-associated symptoms.

There is strong synergistic inhibition of HIV *in vitro* with combinations of IFNα or -β and the nucleoside analogues zidovudine or dideoxycytidine (Hartshorn et al. 1987b; Vogt et al. 1988; Williams & Colby 1989). Zidovudine and dideoxycytidine are both reverse transcriptase inhibitors, acting at an earlier stage in the HIV lifecycle than IFN, a factor which may account for the synergistic activity.

Clinical studies of IFNα and zidovudine in various dose combinations have been conducted in patients with Kaposi's sarcoma (Bratzke et al. 1988; Fischl et al. 1991; Gustavo et al. 1990; Kovacs et al. 1989; Krown et al. 1990a). Mixed results have been obtained from these trials, with tumour response rates varying from 20% to greater than 80%. Higher response rates than would have been expected with IFN alone were reported in patients with low CD4 counts (Fischl et al. 1991; Krown et al. 1990a). In general, the higher dose combinations showed better responses but were less well tolerated. Additive myelotoxicity and hepatotoxicity were frequently observed and were commonly dose limiting. Coadministration of the growth factor GM-CSF is one possible method of counteracting the neutropenia. This triple combination is under investigation; preliminary results indicate that prevention of neutropenia is possible but that it may potentiate the subjective side effects of IFN (Krown et al. 1990b).

Perhaps the greatest potential of the combination of IFN and zidovudine will prove to be in early HIV disease using much lower doses than those being used in Kaposi's sarcoma. Preliminary results with low doses of both IFNα and zidovudine (1.5MU and 400mg daily, respectively) in patients with early HIV disease have shown significant reductions in p24 antigen, and these lower doses have been well tolerated (Orholm et al. 1989). Further studies with low-dose combinations compared with zidovudine alone are now under way, and it is hoped these will establish the role of this combination on disease progression.

In summary, although IFN has proved to be useful for the treatment of Kaposi's sarcoma, it does not appear to have a role as a single agent for the treatment of HIV disease. Perhaps the greatest value of IFN will be when it is used in combination with other antiviral agents. The combined use of zidovudine and IFNα appears to have a role in the management of patients with Kaposi's sarcoma, although the optimal doses for maximum antitumour effect with minimum toxicity remain to be fully defined. The value of such a combination for

the treatment of earlier manifestations of HIV disease remains to be determined.

5. Interferon in Hepatitis B

5.1 The Natural History of Chronic Hepatitis B Infections

Although most individuals recover completely from an infection with the hepadnavirus hepatitis B (HBV), about 5% become chronic carriers, and some of these develop chronic active hepatitis. It is estimated that there are about 300 million carriers in total worldwide, with 50 million new infections annually, and more than 1.5 million deaths from the long term sequelae, cirrhosis and primary liver cancer. Of all the treatments thus far tried in chronic hepatitis B, the use of IFNα seems the most promising.

The questions about how and when to use IFNα preparations in chronic hepatitis B are best considered in relation to its pathogenesis and clinical course. A chronic infection typically runs a prolonged course over many years, which can be followed in terms of the presence and amounts of various HBV proteins in the patient's serum: these are the surface antigen (HBsAg), the virus DNA polymerase, the core protein (HBcAg) and the so-called e antigen (HBeAg) derived from the core protein (Hull & McGeoch 1989). The corresponding serum antibodies are also useful markers for following the course of the infection.

In chronic hepatitis B, there is an initial phase of immune tolerance, characterised by abundant virus replication in the liver cells. The serum contains high levels of HBeAg, HBV DNA and DNA polymerase; there is little or no histological evidence of liver inflammation; and biochemical abnormalities are minimal. Most paediatric patients are in this phase. Since continued HBV replication usually has serious late consequences, as discussed below, it is logical to treat with an antiviral agent such as IFNα at this stage. IFNα may also help to break immune tolerance: if this occurs, whether spontaneously or as the result of treatment, cytotoxic T cells recognise the virus antigens and increased levels of HLA Class 1 antigens on the surface of infected liver cells and lyse them. If all are eliminated there is full recovery from the infection, but if HBV continues to replicate in some hepatocytes, these in turn are then lysed by the T cells, and progressive liver damage results. Thus, the prime objective of any treatment is to stop HBV replication, usually monitored in terms of the loss of HBeAg and other markers of virus replication from the serum, and sometimes followed by the development of anti-HBe antibodies; such changes are associated with loss of biochemical markers of liver inflammation and improvement in liver histology (Hoofnagle et al. 1981; Liaw et al. 1983; Perillo et al. 1990; Realdi et al. 1980). Suppression of virus replication will also reduce the infectivity of a carrier, a result particularly important for health care personnel and for women of childbearing age. About 90% of the offspring of carrier mothers who are HBeAg positive become carriers of hepatitis B virus, in contrast to only 8% of those born to HBeAg-negative carrier mothers (Oon 1988).

In some chronic HBV hepatitis patients, especially children, there is active HBV replication, shown by high serum titres of HBeAg and HBV DNA, but no liver inflammation. Whether these patients have a defect in their immune response and are thus protected against liver damage, or will in due course develop liver disease, is as yet unknown. In another important group of patients, neither HBeAg nor anti-HBeAg antibody is found in the serum yet the disease is particularly aggressive, as shown by the liver histopathology. Increasingly, therefore, clinical progress is being monitored in terms of the amounts of circulating virus, measured as HBV DNA by hybridisation techniques. Loss of serum DNA correlates well with the disappearance of HBcAg from the liver cells (Hsu et al. 1987; Eddleston, personal communication).

If virus replication continues, as shown by the continued presence of HBs and HBe antigens in the serum, the liver shows persisting histological evidence of damage, and progression to cirrhosis is likely within a 3- to 4-year period in up to half of such patients (Aldershvile et al. 1982; Andres et al. 1981; Chu et al. 1985; Hadziyannis et al. 1983;

Lindh et al. 1986; Sanchez-Tapias et al. 1984).

Subjects with persistent HBs antigenaemia, particularly those infected in early life, run a relative risk of ultimately developing primary hepatocellular carcinoma (PHC) estimated as between 90 and 200 times greater than that of matched controls with no markers of the disease (Beasley & Hwang 1984). The tumour usually develops 25 to 35 years after the primary HBV infection, although occasionally much sooner, and in subjects who have extensive cirrhosis due to chronic hepatitis B.

5.2 Early Trials

The first reports of clinical trials with various preparations of human IFNα and IFNβ in chronic hepatitis B date back many years (Desmyter et al. 1976; Greenberg et al. 1976). Results from 15 early studies were reviewed by Scott and Tyrrell (1985). Daily or 3-times-weekly injections of IFN usually led to a rapid drop in the levels of circulating HBV DNA polymerase and virus DNA, although these returned in most patients to the previous or even somewhat higher levels if treatment was stopped after only a few weeks. In all these studies, about 30% of treated patients appeared to derive benefit. However, these studies involved only 107 patients in total, and as most did not include a control group to show the level of spontaneous improvement in the absence of IFN treatment, there was no definitive evidence that IFNα treatment gave benefit.

5.3 Recent Clinical Data

More recently, results have been published from a large number of controlled trials in which different IFNα preparations were used, and some general conclusions can be drawn from the remarkably consistent responses obtained.

The great majority of patients show an initial response to IFNα treatment that almost certainly reflects the direct antiviral effects of the preparation: within the first week, the level of circulating HBV DNA falls (see fig. 2). In many patients, virus DNA is completely cleared within a few weeks (Anderson et al. 1987). In 30 to 40% of patients, no DNA can be found even by sensitive hybridisation assays, and these patients also lose circulating HBeAg and seroconvert to anti-HBeAg antibody positive (Alexander et al. 1987; Dusheiko et al. 1986; Hoofnagle et al. 1988; Perillo et al. 1990). This seroconversion is typically associated with a mild hepatitis-like illness with transient rise in liver enzymes. Such an episode occurred consistently in many studies after between 4 and 10 weeks of treatment, which suggests that it was a consequence, and not a spontaneous event. Seroconversion is followed by improvement in the liver histology (Perillo et al. 1990), and some patients go on to clear HBsAg from the blood and to produce anti-HBs antibodies.

In order to achieve seroconversion, treatment with IFNα must be continued for 3 to 4 months; longer treatment does not increase the seroconversion rate (Scully et al. 1987). Trials of low doses of IFNα given over the long term as a suppressive therapy are under way, but no reliable results are available as yet.

The doses used in the various trials have varied widely, from 1 up to 100MU daily (Yokosuka et al. 1985). Perillo et al. (1990) found that a dose of 5Mu of IFNα given 3 times weekly led to seroconversion in 38% of those treated, whereas a dose of 1Mu was no more effective than placebo, and a higher dose did not appear to give better results. A 5MU dose is on the whole well tolerated: all patients develop fever and malaise, but usually only after the first 3 or 4 injections. With long term treatment, chronic lethargy and depression become a problem in a minority of patients (Renault et al. 1987).

In terms of criteria for choosing patients for treatment, there are 2 considerations. Theoretically, the earlier the patient is successfully treated in the course of the disease, the lower the number of hepatocytes that will contain integrated HBV sequences. In a recent trial, about 10% of patients cleared both HBeAg and HBsAg, and were also found to be free of circulating HBV DNA as tested by the polymerase chain reaction (Perillo et al. 1990). However, the early immune tolerance phase

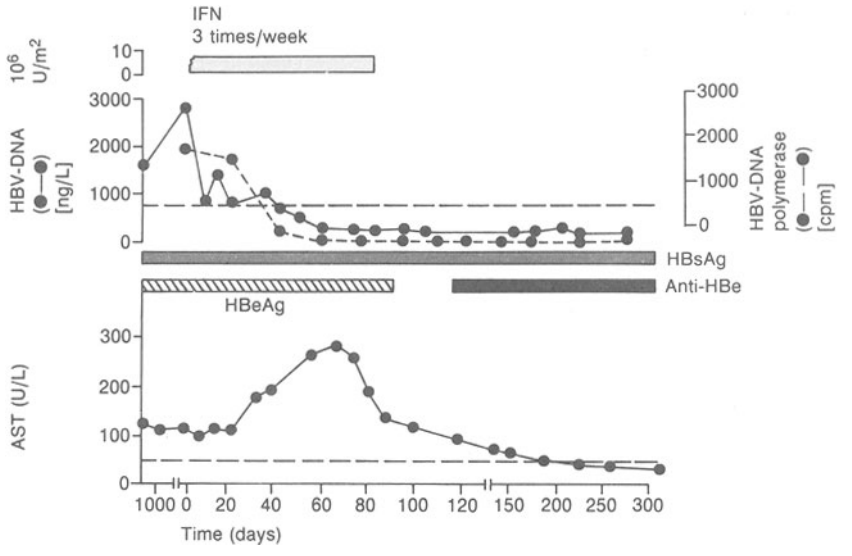

Fig. 2. Typical changes in serological and virus markers in a patient with chronic active hepatitis B successfully treated with an IFNα preparation (reproduced with permission from Prof. H.C. Thomas, St Mary's Hospital, London). The horizontal bars indicate the period of interferon treatment, and the times during which HBsAg, HBeAg and anti-HBe could be detected in the serum of this patient. *Abbreviations:* AST = aspartate amino transferase; HBV-DNA = hepatitis B virus DNA; HBeAg = hepatitis B e antigen; HBsAg = hepatitis B surface antigen; Anti-HBe = antibody to HBe antigen.

of chronic hepatitis B virus infection is characterised by low serum levels of liver enzymes, and the levels of these are the best guide to the likely response to IFNα therapy: high levels of serum alanine amino transferase (ALT) and aspartate amino transferase (AST) are associated with an increased response rate (Hoofnagle et al. 1988; Perillo et al. 1990). It has therefore been suggested that only patients with serum ALT levels greater than twice the normal should be treated with IFNα (Hoofnagle 1990). Thus, the later in the course of the disease that a patient is treated with IFNα, the more likely he or she is to respond, but the greater the likelihood that there will by then be liver damage and that most hepatocytes will contain integrated HBV DNA, with a consequent correspondingly high risk of later development of hepatocellular carcinoma. The choice of when to treat with IFNα must therefore be made by balancing the likelihood of response against the not insubstantial cost of the treatment. It has been suggested that the response to IFNα is less in Asiatic patients, especially children (Lai et al. 1987), than in Caucasians, but in more recent studies adult male Chinese have been shown to benefit (Liaw et al. 1988). Patients with HIV infection respond relatively poorly (McDonald et al. 1987).

Successful IFNα treatment is clearly not a simple reflection of its antiviral effect. The virus is eliminated only following some change in the balance of the host immune response, the nature of which is not yet understood, and there remains the problem that the majority of patients do not clear HBV when treated with IFNα, perhaps because the antiviral effects of IFNα are blocked by the terminal portion of the polymerase protein, as shown *in vitro* by Foster et al. 1990). At present, there is no way of increasing the response above the 30 to 40% level seen in most of the clinical trials, even though a number of approaches have been tried. The most promising of these appeared to be the use of IFNα after pretreatment with steroids, but early reports of success with this protocol (Perillo

et al. 1988) seemed not to be confirmed in a later study (Perillo et al. 1990). However, results from a recent study (Y.F. Liaw, to be published) suggest that this regimen can indeed improve the results in certain categories of patients. Chinese children treated with IFNα-2 who developed relatively high titre neutralising antibodies were found less likely to respond than those with low titre antibodies or none (Lok & Lai 1991; Lok et al. 1990).

It has been noted in Italy that up to 30% of all chronic carriers have antibodies to HBe in their serum and no detectable HBeAg. Nearly 90% of these have active disease with a characteristically severe liver pathology, even though few hepatocytes express HBcAg. Within an 8-year period, the histological picture changes from one of chronic persistent hepatitis to chronic active hepatitis and finally to cirrhosis. These patients represent another category of patient urgently needing treatment. Here, the benefits of IFN treatment can best be assessed in terms of the total loss of HBV DNA from the serum. Alberti and his colleagues (1988) have obtained very encouraging results in preliminary trials with lymphoblastoid IFN in such patients, and further confirmatory studies are urgently needed.

To summarise, there is a good rationale for using IFNα preparations to treat patients with chronic hepatitis B: between 25 and 40% of patients can be expected to derive substantial benefit. Although still better results may be obtained in the future from their use in combination with other antiviral or immunomodulatory agents, IFNα preparations used on their own are at present the best treatment available for this important disease.

6. Interferon in Hepatitis C

Chronic non-A, non-B (NANB) hepatitis may also progress to cirrhosis, and IFN preparations have been tested in such patients. Many of these patients have antibodies to the recently identified hepatitis C virus, and in these at least, there seems to be an association between infection and development of primary hepatocellular carcinoma.

In early studies in which only short IFNα courses were given, liver transaminase levels in the serum normalised during treatment but soon afterwards returned to previous elevated levels (Thompson et al. 1987). In a more recent study with IFNα given 3 times a week for 24 weeks, serum alanine aminotransferase levels fell to normal or near-normal levels in 28% of those who received a dose of 1MU and in 46% of those who received 3MU; in the latter group there were improvements in liver histology, with regression of lobular and periportal inflammation. After the treatment course, about half of the responders relapsed (Davis et al. 1989). In a similar trial in which the dose was 2Mu given 3 times each week for 6 months, 48% of the patients showed a complete response in terms of a normal geometric mean level of serum AST during the course of treatment; 33% had normal enzyme levels at the end of treatment, but at follow-up after 6 to 12 months only 10% had sustained this response (DiBischelie et al. 1989).

Several studies are in progress in which higher doses of IFNα are being given for longer periods. When lymphoblastoid IFN was given 3 times weekly at a dose of 3MU, the preliminary results showed a response in 5 of 7 patients (71%) at the sixteenth week of treatment (Jacyna et al. 1989).

Since the low doses employed are well tolerated, there is considerable optimism that IFNα will become the treatment of choice for this important disease, and indeed its use in hepatitis C infections has already been approved in a number of countries.

7. Conclusion

When the interferons were discovered in 1957, it seemed to some that they would prove as successful for the treatment of viral infections as the antibiotics had been in the realm of bacterial diseases. Such hopes have been disappointed as far as acute viral infections are concerned, probably because treatment cannot be started in time. In contrast, as discussed in the preceding sections, IFNα preparations have proved of considerable value for the treatment of certain chronic viral infections affecting millions of people. Although much still remains to be learned about how and when they are

best administered in each condition, there seems no doubt that in the years to come, IFNα will be used with benefit in an increasing number of patients.

References

Alberti I, Fattovich G, Pontisso P, et al. Interferon treatment of antiHBe positive and HBV DNA positive chronic hepatitis B. Chemoterapia 7 (Suppl. 3): 15-19, 1988

Aldershvile J, Dietrichson A, Skinhoj P, et al. and the Copenhagen Hepatitis Acute Programme. Chronic persistent hepatitis: serological classification and meaning of the hepatitis B e system. Hepatology 2: 243-246, 1982

Alexander GJM, Brahm J, Fagan EA, et al. Loss of HBsAg with interferon therapy in chronic HBV infection. Lancet 2: 66-69, 1987

Anderson MG, Harrison TJ, Alexander G, et al. Randomised controlled trial of lymphoblastoid interferon for chronic active hepatitis. Gut 28: 619-622, 1987

Andres LL, Sawhney VK, Scullard GH, et al. Dane particle DNA polymerase and HBeAg: impact on clinical, laboratory and histological findings in hepatitis B-associated chronic liver disease. Hepatology 1: 583-585, 1981

Antonelli G, Currenti M, Turriziani O, et al. Neutralizing antibodies to interferon-α: relative frequency in patients treated with different interferon preparations. Journal of Infectious Diseases 163: 882-885, 1991

Aoki FY, Crawley JCW. Distribution and removal of human serum albumin-technetium 99M instilled intranasally by drops and spray. British Journal of Clinical Pharmacology 3: 869-878, 1976

Baron G, Ashmen M, Fischl M, et al. Immunomodulation of patients with AIDS related complexes (ARC) during therapy with recombinant interferon gamma (IFNG). Fourth International Conference on AIDS, Stockholm. Abstract no. 3510, 1988

Baron S, Isaacs A. Absence of interferon in lungs from fatal cases of influenza. British Medical Journal 1: 18-20, 1962

Beasley RP, Hwang LY. Epidemiology of hepatocellular carcinoma. In Vyas GN et al. (Eds) Viral hepatitis and liver disease, pp. 209-224, Grune and Stratton, Orlando, 1984

Berman B, Davis-Reed L, Silverstein L, et al. Treatment of Verrucae Vulgaris with α_2 interferon. Journal of Infectious Diseases 154: 328-330, 1986

Billiau A. The main concepts and achievements in interferon research: a historical account. In Billiau A (Ed.) Interferon 1, general and applied aspects, pp. 22-58, Elsevier, Amsterdam, 1984

Borucki MJ, Von Roenn JH, Williams RN, Pollard RB. A multicentre open-label study of subcutaneously administered recombinant interferon beta (rIFN beta) in patients at risk for progression to AIDS. Sixth International Conference on AIDS, San Francisco. Abstract no. SB463, 1990

Bratzke B, Stadler R, Tiel H, et al. Combination of interferon (r alpha 2) and zidovudine for therapy of HIV-associated Kaposi's sarcoma (KS). Fourth International Conference on AIDS, Stockholm, Abstract no. 3631, 1988

Buimovici-Klein E, Lange M, Klein RJ, et al. Long term follow up of serum interferon and its acid stability in a group of homosexual men. AIDS Research 2: 99-108, 1986

Casato M, Laganà B, Bonomo L. Clinical effects of interferons in essential mixed cryoglobulinemia. In Indiveri F et al. (Eds) Proceedings of the First International Symposium on Biological Response Modifiers, San Remo, November 1989, Esculapio, Bologna, pp. 49-54, 1990

Chu CM, Karayiannis P, Fowler MJ, et al. Natural history of chronic hepatitis B virus infection in Taiwan; studies of hepatitis B virus DNA in serum. Hepatology 5: 431-434, 1985

Chuang T-Y, Perry HO, Kurland LT, Ilstrup KM. Condyloma acuminatum in Rochester, Minn., 1950-1978. Archives of Dermatology 120: 469-475, 1984

Condylomata International Collaborative Study Group. Recurrent condylomata acuminata treated with recombinant interferon alfa-2a. Journal of the American Medical Association 265: 2684-2687, 1991

Crespi M. The effect of interferon on cells persistently infected with HIV AIDS 3: 33-36, 1989

Davis GL, Balart LA, Schiff ER, et al. Treatment of chronic hepatitis C with recombinant interferon alpha. New England Journal of Medicine 321: 1501-1505, 1989

De Stefano E, Friedman RM, Friedman-Kien AE, et al. Acid-labile human leucocyte interferon in homosexual men with Kaposi's sarcoma and lymphadenopathy. Journal of Infectious Diseases 146: 451-455, 1982

Department of Health and Social Security. Sexually transmitted diseases. Genitourinary Medicine 61: 204-207, 1985

Desmyter J, De Groote J, Desmet VJ, et al. Administration of human fibroblast interferon in chronic hepatitis B infection. Lancet 2: 645-647, 1976

DiBischelie AM, Martin P, Kassianides C, et al. Recombinant interferon alpha therapy for chronic hepatitis C: a randomised double-blind placebo-controlled trial. New England Journal of Medicine 321: 1506-1510, 1989

Douglas RB, Moore BW, Miles HB, et al. Prophylactic efficacy of intranasal alpha$_2$ interferon against rhinovirus infections in the family setting. New England Journal of Medicine 314: 65-70, 1986

Dusheiko GM, Paterson AC, Pitcher L, et al. Recombinant leucocyte interferon treatment of chronic hepatitis B: an analysis of two therapeutic trials. Journal of Hepatology 3 (Suppl. 2): S199-S207, 1986

de Wit R, Schattenkerk JCM, Boucher CAB, et al. Clinical and virological effects of high dose recombinant interferon-alpha in disseminated AIDS related Kaposi's sarcoma. Lancet 2: 1214-1217, 1988

de Wit R, Schattenkerk KME, Boucher CAB, et al. Clinical and virological effects of high dose recombinant interferon alpha in disseminated AIDS-related Kaposi's sarcoma. Lancet 2: 1214-1217, 1988

Eron LJ, Judson F, Tucker S, et al. Interferon therapy for condylomata acuminata. New England Journal of Medicine 315: 1059-1064, 1986

Eskelinen A, Mashkilleyson N. Optimum treatment of genital warts. Drugs 34: 599-603, 1987

Eyster ME, Goedert JJ, Poon M-C, Preble OT. Acid labile alpha interferon. New England Journal of Medicine 309: 583-586, 1983

Finter NB, Fantes KH, Lockyer MJ, et al. The DNA content of crude and purified human lymphoblastoid (Namalwa cell) interferon prepared by Wellcome Biotechnology Limited. In Hopps HE & Petricciani JC (Eds) Abnormal cells, new products and risks, pp. 125-128, Tissue Culture Association, Gaithersburg MD, 1985

Finter NB, Fantes KH. The purity and safety of interferons prepared for clinical use: the case for lymphoblastoid interferon. In Gresser I (Ed.) Interferon 2, pp. 65-79, Academic Press, London, 1980

Finter NB. Why are there so many subtypes of alpha-interferons? Journal of Interferon Research, Special Issue, pp. 185-194, 1991

Fischl MA, Uttamchandani RB, Resnick L, et al. A phase 1 study of recombinant human interferon-alpha 2a or human lymphoblastoid interferon-alpha n1 and concomitant zidovudine in patients with AIDS-related Kaposi's sarcoma. Journal of Acquired Immunodeficiency Syndrome 4: 1-10, 1991

Foster GR, Goldin RD, Ackrill AM, Kerr IM, Thomas HC, et al. Hepatitis B Pol gene product inhibits the cellular response to interferon. Journal of Interferon Research 10 (Suppl. 1): S15, 1990

Freund M, von Wussow P, Diedrich H, et al. Recombinant human interferon (IFN) alpha-2b in chronic myelogenous leukaemia: dose dependency of response and frequency of neutralising anti-interferon antibodies. British Journal of Haematology 172: 350-356, 1989

Friedman-Kein AE, Eron LJ, Conant M, et al. Natural interferon alpha for treatment of condylomata acuminata. Journal of the American Medical Association 259: 533-538, 1988

Gall SA, Hughes CE, Mounts P, et al. Efficacy of human lymphoblastoid interferon in the therapy of resistant condyloma acuminata. Obstetrics and Gynaecology 67: 643-651, 1986

Gall SA, Hughes CE, Trofatter K. Interferon for the therapy of condyloma acuminatum. American Journal of Obstetrics and Gynaecology 153: 157-163, 1985

Geffen JR, Klein RJ, Friedman-Klein AE. Intralesional administration of large doses of human leukocyte interferon for the treatment of condylomata acuminata. Journal of Infectious Diseases 150: 612-615, 1984

Gibson JR, Harvey SG, Kemmett D, et al. Treatment of common and plantar viral warts with human lymphoblastoid interferon-α: pilot studies with intralesional, intramuscular and dermojet injections. British Journal of Dermatology 115 (Suppl. 31): 76-79, 1984

Gibson JR. Intralesional lymphoblastoid interferon alpha for the treatment of cutaneous, non-genital viral warts. Archives of Dermatology 122: 1098-1099, 1986

Gibson JR. The treatment of viral warts with interferons. Journal of Antimicrobial Chemotherapy 21: 391-393, 1988

Gobel U, Arnold W, Wahn V, et al. Comparison of human fibroblast and leukocyte interferon in the treatment of severe laryngeal papillomatosis in children. European Journal of Paediatrics 137: 175-176, 1981

Goeddel DV, Yelverton E, Ullrich A, et al. Human leukocyte interferon produced by E. coli is biologically active. Nature (London) 287: 411-416, 1980

Goepfert H, Sessions R, Gutterman J, et al. Leukocyte interferon in patients with juvenile laryngeal papillomatosis. Annals of Otology, Rhinology and Laryngology 91: 431-436, 1982

Greenberg HB, Pollard R, Lutwick L, et al. Effect of human leukocyte interferon on hepatitis B virus infection in patients with chronic active hepatitis. New England Journal of Medicine 295: 517-522, 1976

Gresser I, Tovey MG, Bandu TM, et al. Role of interferon in the pathogenesis of virus diseases as demonstrated by the use of anti-interferon serum. I. Rapid evolution of encephalomyocarditis virus infection. Journal of Experimental Medicine 144: 1305-1315, 1976a

Gresser I, Tovey MG, Maury C, Bandu MT. Role of interferon in the pathogenesis of virus diseases as demonstrated by the use of anti-interferon serum. II. Studies with herpes simplex, Moloney sarcoma, vesicular stomatitis, Newcastle disease and influenza viruses. Journal of Experimental Medicine 144: 1316-1323, 1976b

Gugliotta L, Catani L, Vianelli N, et al. Efficacy of interferon alpha-2a treatment in essential thrombocythaemia. Journal of Interferon Research 9 (Suppl. 2): S112, 1989

Gustavo G-C, Lovet C, Navarro-Carrola E, et al. Survival of 30 patients with AIDS related Kaposi's sarcoma treated with AZT and alpha 2a interferon. Sixth International Conference on AIDS, San Francisco. Abstract no. SB515, 1990

Hadziyannis SJ, Lieberman HM, Karvountzis GG, et al. Analysis of liver disease, nuclear HBcAg, viral replication and hepatitis B virus DNA in liver and serum of HBeAg vs anti-HBe positive carriers of hepatitis B virus. Hepatology 5: 656-662, 1983

Haglund S, Lundquist P, Cantell K, et al. Interferon therapy in juvenile laryngeal papillomatosis. Archives of Otolaryngology, Head and Neck Surgery 197: 327-332, 1981

Haller O, Arnheiter H, Lindenmann J, Gresser I. Host gene influences sensitivity to interferon action selectively for influenza virus. Nature (London) 283: 660-662, 1980

Hartshorn KL, Neumeyer D, Vogt MW, et al. Activity of Interferons alpha, beta, and gamma against human immunodeficiency virus replication in vitro. AIDS Research and Human Retroviruses 3: 125-133, 1987a

Hartshorn KL, Vogt MW, Chou TC, et al. Synergistic inhibition of human immunodeficiency virus in vitro by azidothymidine and recombinant alpha-A interferon. Antimicrobial Agents and Chemotherapy 31: 168-172, 1987b

Hatch KD. Evaluation of interferon alpha-2 in the treatment of condyloma acuminatum. Journal of Reproduction Physiology 31: 979, 1986

Hayden FG, Albrecht JK, Kaiser DL, Gwaltney JM. Prevention of natural colds by contact: prophylaxis with intranasal alpha$_2$-interferon. New England Journal of Medicine 314: 71-75, 1986

Hayden FG, Gwaltney Jr JM. Intranasal interferon-alpha$_2$ for prevention of rhinovirus infection and illness. Journal of Infectious Diseases 148: 543-550, 1983

Hayden FG, Gwaltney Jr JM. Intranasal interferon-alpha$_2$ treatment of experimental rhinoviral colds. Journal of Infectious Diseases 150: 174-180, 1984

Hayden FG, Mills SE, Johns ME. Human tolerance and histopathologic effects of long term administration of intranasal interferon-alpha$_2$. Journal of Infectious Diseases 148: 914-921, 1983

Hayden FG, Winther B, Donowitz G, et al. Human nasal mucosal responses to topically applied recombinant leukocyte A interferon. Journal of Infectious Diseases 156: 64-72, 1987

Healy GB, Gelber RD, Trowbridge AL, et al. Treatment of recurrent respiratory papillomatosis with human leukocyte interferon. New England Journal of Medicine 319: 401-407, 1988

Henle W. Interference phenomena between animal viruses: a review. Journal of Immunology 91: 203-236, 1950

Higgins PG, Al-Nakib W, Barrow GI, Tyrrell DAJ. Recombinant human interferon-gamma as prophylaxis against rhinovirus colds in volunteers. Journal of Interferon Research 8: 591-596, 1988

Higgins PG, Al-Nakib W, Willman J, Tyrrell DAJ. Interferon-beta ser as prophylaxis against experimental rhinovirus infection in volunteers. Journal of Interferon Research 6: 153-159, 1986

Higgins PG, Phillpotts RJ, Scott GM, et al. Intranasal interferon as protection against experimental respiratory coronavirus infection in volunteers. Antimicrobial Agents and Chemotherapy 24: 713-715, 1983

Ho DD, Hartshorn KL, Rota TR, et al. Recombinant human interferon alpha-A suppresses HTLV-III replication in vitro. Lancet 1: 602-604, 1985

Hoofnagle JH, Dusheiko GM, Seef LB, et al. Seroconversion from hepatitis B e antigen to antibody in chronic type B hepatitis. Annals of Internal Medicine 94: 744-748, 1981

Hoofnagle JH, Peters M, Mullen KD, et al. Randomized controlled trial of recombinant interferon in patients with chronic hepatitis B. Gastroenterology 95: 1318-1325, 1988

Hoofnagle, JH. Chronic hepatitis. Editorial. New England Journal of Medicine 323: 337-339, 1990

Hovanessian AG. The double stranded RNA-activated protein kinase induced by interferon: ds RNA-PK. Journal of Interferon Research 9: 641-647, 1989

Hsu HC, Su IJ, Lai MY, et al. Biologic and prognostic signifi-

cance of hepatocyte hepatitis B core antigen expression in the natural course of chronic hepatitis B virus. Journal of Hepatology 5: 45-50, 1987

Hull R, McGeoch DJ. Some highlights of virus research in 1988. Journal of General Virology 70: 2825-2842, 1989

Idéo G, Bellati G, Pedraglio E, Leandro G. One year of therapy of non A, non B/C chronic hepatitis with recombinant α-2a interferon (r-IFN) or lymphoblastoid α interferon (1-IFN). Abstracts of the 25th Meeting of the European Association for the Study of Liver Disease. Journal of Hepatology 11 (Suppl. 2): S31, 1990

Ikić D, Bosnić N, Smerdel S, et al. Double-blind clinical study with human leukocyte interferon in therapy of condylomata acuminata. Proceedings of the Symposium on Clinical Use of Interferon, Yugoslav Academy of Sciences and Arts, Zagreb, pp. 229-233, 1975

Interferon Alpha Study Group. A randomised placebo-controlled trial of recombinant human interferon alpha 2a in patients with AIDS. Journal of Acquired Immune Deficiency Syndrome 1: 111-118, 1988

Isaacs A, Lindenmann J. Virus interference 1. The Interferon. Proceedings of the Royal Society Series B 147, pp. 258-267, 1957

Jacyna MR, Brooks MG, Loke RHT, et al. Randomised controlled trial of interferon alpha (lymphoblastoid) in chronic non-A, non-B hepatitis. British Medical Journal 289: 80-82, 1989

Johnston MD. Sources of interferon for clinical use: alpha-interferons from human lymphoblastoid cells. In Finter NB & Oldham RK (Eds) Interferon 4: in vivo and clinical studies, pp. 81-87, Elsevier, Amsterdam, 1985

Kashima H, Leventhal B, Clark K, et al. Interferon alpha-N1 (Wellferon) in juvenile onset recurrent respiratory papillomatosis: results of randomised study in twelve collaborative institutions. Laryngoscope 98: 334-340, 1988

Kirby P, Wells D, Kiviat N, Corey L. Phase I trial of intramuscular recombinant human gamma interferon for refractory genital warts. Journal of Investigative Dermatology 86: 485, 1986

Kojima S, Futatski T, Hatano Y, Koide T. Purification and characterisation of human interferon-alpha subtypes from Namalwa lymphoblastoid cells. Journal of Interferon Research 9 (Suppl. 2): S178, 1989

Kornbluth RS, Oh PS, Munis JR, et al. Interferons and bacterial lipopolysaccharide protect macrophages from productive infection by human immunodeficiency virus in vitro. Journal of Experimental Medicine 169: 1137-1151, 1989

Kovacs JA, Deyton L, Davey V, et al. Combined zidovudine and interferon-alpha therapy in patients with Kaposi sarcoma and the acquired immunodeficiency syndrome (AIDS). Annals of Internal Medicine 111: 280-287, 1989

Krown SE, Gold JWM, Niedzwiecki D, et al. Interferon-alpha with zidovudine: safety, tolerance, and clinical and virologic effects in patients with Kaposi sarcoma associated with the acquired immunodeficiency syndrome (AIDS). Annals of Internal Medicine 112: 812-821, 1990a

Krown SE, Paredes J, Bundow D, Flomenberg N. Combination therapy with interferon-alpha, zidovudine and recombinant granulocyte-macrophage colony stimulating factor (GM-CSF): a phase 1 trial in patients with AIDS associated Kaposi's sarcoma. Sixth International Conference on AIDS, San Francisco. Abstract no. SB513, 1990b

Lai CL, Lok AS, Lin HJ, et al. Placebo controlled trial of recombinant alpha interferon in Chinese HBsAg carrier children. Lancet 2: 877-880, 1987

Lane HC, Davey V, Kovacs JA, et al. Interferon alpha in patients with asymptomatic human immunodeficiency virus (HIV) infection. Annals of Internal Medicine 112: 805-811, 1990

Leventhal BG, Kashima HK, Weck PW, et al. Randomised surgical adjuvant trial of interferon alpha-N1 in recurrent papillomatosis. Archives of Otolaryngology, Head and Neck Surgery 114: 1163-1169, 1988

Liaw LF, Lin SM, Sheen IS. Treatment of chronic type B hepatitis in South East Asia. American Journal of Medicine 85 (Suppl. 2A): 147-149, 1988

Liaw YF, Chu CM, Chen T, et al. Clinical and histological events preceding hepatitis B e antigen seroconversion in chronic type B hepatitis. Gastroenterology 84: 216-219, 1983

Lindh G, Weiland O, Svedmyr A, et al. Long term follow-up of 60 patients with chronic hepatitis B. I. Seroconversion in the hepatitis B e-system, frequency of delta infection and histological outcome. Liver 6: 7-12, 1986

Lok ASF, Lai CL. Incidence, neutralizing activity and clinical significance of interferon antibodies in chronic hepatitis B patients receiving recombinant α-interferons. In Hollinger FB, et al. (Eds) Viral hepatitis and liver disease, in press, 1991

Lok ASF, Lai C-L, Leung EK-Y. Interferon antibodies may negate the antiviral effects of recombinant α-interferon treatment in patients with chronic hepatitis B infection. Hepatology 12: 1266-1270, 1990

McCabe B, Clark K. Interferon and laryngeal papillomatosis: the Iowa experience. Annals of Otology, Rhinology and Laryngology 92: 2-7, 1983

McDonald JA, Caruso L, Karayiannis P, Scully L, Harris JR, et al. Diminished responsiveness of male homosexual chronic hepatitis B carriers with HTLV-III antibodies to recombinant alpha-interferon. Hepatology 7: 719-723, 1987

Miles SA, Wang H, Cortes E, et al. Beta-interferon therapy in patients with poor prognosis Kaposi sarcoma related to the acquired immunodeficiency syndrome (AIDS). Annals of Internal Medicine 112: 582-589, 1990

Nagata S, Taira H, Hall A, et al. Synthesis in E. coli of a polypeptide with human leucocyte interferon activity. Nature (London) 284: 316-320, 1980

Niimura M. Intralesional human fibroblast interferon in common warts. Journal of Dermatology (Tokyo) 10: 217-220, 1983

Oettgen HF, Real FX, Krown SE. Treatment of AIDS associated Kaposi's sarcoma with recombinant alpha interferon. Immunobiology 172: 269-274, 1986

Oon CJ. Hepatitis B virus (HBV): the challenges ahead. Annals of the Academy of Medicine (Singapore) 17: 257-260, 1988

Orholm M, Pedersen C, Mathiesen L, et al. Suppression of p24 antigen in sera from HIV-infected individuals with low dose alpha-interferon and zidovudine: a pilot study. AIDS 3: 97-100, 1989

Pennington JE, Groopman JE, Small GJ, et al. Effect of intravenous recombinant gamma interferon on the respiratory burst of blood monocytes from patients with AIDS. Journal of Infectious Diseases 153: 609-612, 1986

Perillo RB, Schiff ER, Davis GL, et al. A randomized controlled trial of interferon alpha-2b alone and after prednisolone withdrawal for the treatment of chronic hepatitis B. New England Journal of Medicine 323: 295-301, 1990

Perrillo RP, Regenstein FG, Peters MG, De Schryver-Kecskemeti K, Bodicky CJ, et al. Prednisolone withdrawal followed by recombinant alpha-interferon in the treatment of chronic type B hepatitis. Annals of Internal Medicine 109: 95-100, 1988

Pestka S, Langer JA, Zoon KC, Samuel CE. Interferons and their actions. Annual Review of Biochemistry 56: 727-777, 1987

Petersen CS, Bjerring P, Larsen J, Blaakaer J, Hagdrup H, et al. Systemic interferon alpha-2b increases the cure rate in laser treated patients with multiple persistent genital warts: a placebo-controlled study. Genitourinary Medicine 67: 99-102, 1991

Phillpotts RJ, Higgins PG, Willman JS, et al. Intranasal lymphoblastoid interferon (Wellferon) prophylaxis against rhinovirus and influenza virus in volunteers. Journal of Interferon Research 4: 535-541, 1984

Phillpotts RJ, Scott GM, Higgins PG, et al. An effective dose regimen for prophylaxis against rhinovirus infection by intra-

nasal administration of interferon-alpha$_2$. Antiviral Research 3: 121-136, 1983

Poli G, Orenstein JM, Kinter A, et al. Interferon-alpha but not AZT suppresses HIV expression in chronically infected cell lines. Science 244: 575-577, 1989

Realdi G, Alberti A, Rugge M, et al. Seroconversion from hepatitis B e antigen to anti-HBe in chronic hepatitis B virus infection. Gastroenterology 79: 195-199, 1980

Reichman RC, Farchione A, Whitley R, et al. Placebo-controlled trials of three different interferon preparations administered parenterally for condyloma acuminata. Abstract S1430. 28th Intersciences Conference on Antimicrobial Agents and Chemotherapy, Los Angeles, California, October 23, p. 26, 1988

Renault PF, Hoofnagle JH, Park Y, et al. Psychiatric complications of long term interferon alpha therapy. Archives of Internal Medicine 147: 1577-1580, 1987

Samo TC, Greenberg SB, Couch RB, et al. Efficacy and tolerance of intranasally applied recombinant leukocyte A interferon in normal volunteers. Journal of Infectious Diseases 148: 535-542, 1983

Samo TC, Greenberg SB, Palmer JM, et al. Intranasally applied recombinant leukocyte A interferon in normal volunteers. II. Determination of the minimal effective and tolerable dose. Journal of Infectious Diseases 150: 181-188, 1984

Sanchez-Tapias JM, Vilar JH, Costa J, et al. Natural history of chronic persistent hepatits B: relationship between hepatitis B virus replication and the course of the disease. Journal of Hepatology 1: 15-27, 1984

Scott GM, Onwubalili JK, Robinson JA, et al. Tolerance of one month intranasal interferon. Journal of Medical Virology 17: 99-106, 1985

Scott GM, Phillpotts RJ, Wallace J, et al. Purified interferon as protection against rhinovirus infection. British Medical Journal 284: 1822-1825, 1982

Scott GM, Tyrrell DAJ. Antiviral effects of interferon in man. In Finter NB & Oldham RK (Eds) Interferon 4 in vivo and clinical studies pp. 181-215, Elsevier, Amsterdam, 1985

Scott G, Csonka G. Effect of injections of small doses of human fibroblast interferon into genital warts: a pilot study. British Journal of Venereal Diseases 55: 442-445, 1979

Scully LJ, Shein R, Karayiannis P, et al. Lymphoblastoid interferon therapy of chronic HBV infection: a comparison of 12 *vs* 24 weeks of thrice weekly treatment. Journal of Hepatology 5: 51-58, 1987

Steis RG, Smith JW, Erber WJ, et al. Resistance to recombinant interferon alpha 2a in hairy cell leukaemia associated with neutralising anti-interferon antibodies. New England Journal of Medicine 318: 1409-1413, 1988

Strander H, Cantell K. Production of interferon by human leucocytes in vitro. Annales Medicinae Experimentalis et Biologiae Fennae 44: 265-273, 1966

Thompson BJ, Doran M, Lever AML, et al. Alpha interferon therapy for non-A, non-B hepatitis transmitted by gamma-globulin replacement therapy. Lancet 1: 539-541, 1987

Thurmond LM, Brand CM, Leventhal BG, Finter NB, Johnston JM. Antibodies in recurrent respiratory papillomatosis patients treated with lymphoblastoid interferon. Journal of Laboratory and Clinical Medicine, in press, 1991

Vadhan-Raj S, Wong G, Gnecco C, et al. Immunological variables as predictors of prognosis in patients with Kaposi's sarcoma and the acquired immunodeficiency syndrome. Cancer Research 46: 417-425, 1986

Vance JC, Bart BJ, Hansen RC, et al. Intralesional recombinant alpha-2 interferon for the treatment of patients with condyloma acuminatum or verruca plantaris. Archives of Dermatology 122: 272-277, 1986

Vogt MW, Durno AG, Chou T-C, et al. Synergistic interaction of 2', 3'-dideoxycytidine and recombinant interferon-alpha-A on replication of human immunodeficiency virus type 1. Journal of Infectious Diseases 158: 378-385, 1988

Volberding PA, Mitsuyasu RT, Golando JP, et al. Treatment of Kaposi's sarcoma with interferon alpha-2b (Intron A). Cancer 59: 620-627, 1987

von Wussow P, Hartman F, Freund M, et al. Leukocyte-derived interferon-alpha in patients with antibodies to recombinant IFM-alpha 2b. Lancet 1: 882-883, 1988

von Wussow P, Jakschies D, Freund M, et al. Treatment of anti rIFN-alpha 2 antibody positive CML patients with natural interferon-alpha. Journal of Interferon Research 9 (Suppl. 2): S113, 1989

Weck PK, Leventhal BG, Brand C, et al. Detection and incidence of neutralising antibodies to Interferon-alpha-nl. Journal of Interferon Research (Suppl. 1): S37-S43, 1989

Weck P, Trofatter KF, Reichman RC, et al. Interferon alpha-N1 (Wellferon) in severe recurrent genital warts: report of a multistudy program. In Schellekens H & Cantell K (Eds) Biology of the interferon system, pp. 485-491, Elsevier, Amsterdam, 1986

Weitz G, Bekisz J, Zoon K, Arnheiter H. Purification and characterization of a human Mx protein. Journal of Interferon Research 9: 679-689, 1989

Williams GT, Colby CB. Recombinant human interferon-beta suppresses the replication of HIV and acts synergistically with AZT. Journal of Interferon Research 9: 709-718, 1989

Yamada O, Hattori N, Kurimura T, et al. Inhibition of growth of HIV by human natural interferon in vitro. AIDS Research on Human Retroviruses 4: 287-294, 1988

Yamamoto JK, Barre-Sinoussi F, Bolton V, et al. Human alpha- and beta-interferon but not gamma- supresses the in vitro replication of LAV, HTLV and ARV-2. Journal of Interferon Research 6: 143-152, 1986

Yokosuka O, Omata M, Imazeki F, et al. Changes of hepatitis B virus DNA in liver and serum caused by recombinant leukocyte interferon treatment: analysis of intrahepatic replicative hepatitis B virus DNA. Hepatology 5: 728-734, 1985

Zoon KC, Miller D, Bekisz J, et al. Chemical characterisation of human lymphoblastoid interferon-alpha species. Journal of Interferon Research 9 (Suppl. 2): S184, 1989

Correspondence and reprints: Dr *N.B. Finter,* The Wellcome Research Laboratories, The Wellcome Foundation Ltd, Langley Court, South Eden Park Road, Beckenham, Kent BR3 3BS, England.