

# INTERFERONS AND THEIR THERAPEUTIC APPLICATIONS

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## Special points of interest:

- Interferon (IFN) was originally described more than 40 years ago
- The optimism regarding the potential of IFN as an antiviral therapeutic agent has not been fulfilled
- Achievements made during recent years indicate that IFN-alpha should be regarded as an important part of the therapeutic arsenal in malignant diseases.

## Interferons and their therapeutic applications

Interferon (IFN) was originally described more than 40 years ago by Isaacs and Lindeman as a substance that is produced upon stimulation of cells by viruses, and that has the ability to protect cells from infection with viruses of any kind, that is to *interfere* with viral infections. The antiviral activity of IFN was thus found to be non-specific, a fact that led to the idea that IFN might be used therapeutically against all kinds of viral infections. The revolution that antibiotics had meant for bacterial infections, it was reasoned, might be paralleled by therapeutic use of IFN in viral infections

The optimism regarding the potential of IFN as an antiviral therapeutic agent has not been fulfilled and for various reasons it is not until during the last decade that IFN has been established as a potent antiviral agent in chronic viral infections. In parallel with being the object of antiviral research, however, IFN has also been studied with regard to its anti-tumour properties, and it is today becoming a standard treatment in certain malignant diseases.

### Different types of interferons

Although IFN was initially thought to be a single entity, later research has shown that there are multiple molecular species of IFN. Thus, there are three main classes of human IFN:s called alpha, beta and gamma interferons (IFN-alpha, IFN-beta and IFN $\gamma$ ) and a minor class called omega-IFN (IFN- $\omega$ ). There are 13 genes, two of

which are identical, for IFN-alpha, of which there are thus 12 subtypes, but only one gene, and no subtypes for each of IFN-beta and IFN $\gamma$ . IFN-alpha subtypes consist of 165 or 166, IFN-beta of 165 and IFN-gamma of 142 amino acid residues. IFN-alpha and IFN-beta were formerly called type I interferons and IFN-gamma type II or immune IFN.

The reason why there are so many subtypes of IFN-alpha remains enigmatic. However, the various subtypes of IFN-alpha vary markedly regarding their biological activities. Thus, for instance, the most pronounced antiviral activity on a molar basis is found in IFN-alpha-8, and IFN-alpha-1 has certain immunological activities that are absent among other subtypes. It therefore seems plausible that the different IFN-alpha subtypes are indeed separate cytokines which share some activities, notably the antiviral capacity, but otherwise have different functional profiles.

### Cellular origin and production of interferons

Although most cells in the body are capable of producing IFN:s, the different classes of IFN:s are preferentially produced by certain cell types. Thus, IFN-alpha subtypes are preferentially produced by monocyte/macrophages and special "natural interferon-producing" cells that have characteristics in common with natural killer (NK) cells. IFN-beta is preferentially produced by

fibroblasts and IFN-gamma by T cells and NK cells.

IFN:s are part of the innate immunological defence and are produced after introduction in to the body of foreign substances. In particular, viruses have the ability to evoke production of IFN:s but also bacteria, fungi and other “non-self” agents may induce IFN production. IFN-alpha and IFN-beta are very rapidly produced upon stimulation whereas IFN-gamma, which is a major constituent of the antigen-specific T cell response, may be produced at a somewhat later stage of immune responses. However, since NK cells, which are not induced in an antigen-specific manner, also produce IFN-gamma, the formation of this cytokine may also be an early event following stimulation with infectious agents.

#### **Mechanisms of action**

After induction of IFN:s they react with cells that possess specific receptors for the various IFN:s. IFN -alpha and IFN-beta react with the same receptor, which, however, is completely different from the IFN -gamma receptor. Following this interaction a complex series of signal transduction events takes place, resulting, in the end, in the production of a multitude of proteins with different actions. Some of these have the ability to induce antiviral states, others have antiproliferative effects and still others have a variety of immunological effects. Together, all these proteins help to defend the host against various intruders and, also, to suppress the growth of cells with an exaggerated growth potential, such as cancer cells, that arise in the body.

The immunological effects of IFN:s are particularly pronounced regarding IFN-gamma. This cytokine is an important product of the so called T helper type 1 (Th1) cells which are the major effector cells of the cell-mediated immune system. IFN-gamma, that is produced by Th1 cells or NK cells, can induce the expression of both class I

and class II histocompatibility (HLA) antigens on various cells and thereby stimulate immune responses. IFN -alpha and -beta can induce class I but not class II antigens. All interferons have the ability to stimulate cellular cytotoxicity, evoked by cytotoxic T cells, monocytes or NK cells. Together, these actions make interferons powerful immunological modulators. The capacity to stimulate immune responses may explain the fact that autoimmune disorders may be worsened and in certain cases possibly induced during IFN therapy.

#### **Association of interferons with diseases**

In acute viral infections there is usually a rapid IFN response with appearance of sometimes large amounts of IFN-alpha in the blood. This response, however, is short-lived and the IFN disappears from the circulation within a few hours or days. In chronic viral infections there may be a persistent IFN response. Thus, in e.g. HIV infection, IFN-alpha is readily demonstrable in serum in late stages of the disease at levels that increase with progressing disease.

Autoimmune diseases, like systemic lupus erythematosus, are frequently associated with a persistent occurrence of IFN-alpha in the circulation. Since IFN-alpha has immunostimulatory properties and autoantibodies, and sometimes even autoimmune disease, may appear during IFN therapy, it seems possible that IFN-alpha could have a causative role in autoimmune diseases. However, the cause-and-effect relationship is in this case difficult to assess and the pathogenetic mechanisms in autoimmune disease are varying and not fully clarified.

#### **Large scale production of interferon**

In order to use IFN:s for therapeutic purposes, methods to produce large amounts of the substances are required. Early trials with IFN utilised

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semi-purified preparations of IFN-alpha obtained from human cultures of leukocytes that had been stimulated with a paramyxovirus (Sendai virus). After the cloning of IFN genes it became possible to use DNA recombinant techniques to produce large amounts of purified IFN:s in an inexpensive way. Using another line of investigation it was demonstrated that certain lines of lymphoblastoid cells constitutively produced large amounts of IFN-alpha, and these could thus be used for the production of large amounts of IFN.

Presently, there are the following three main types of IFN -alpha preparations that are commercially available.

- Recombinant IFN-alpha2 preparations, which dominate the market are available in different forms of which IFN-alpha2b (Intron A<sup>o</sup>, Schering-Plough) and IFN-alpha2a (Roferon<sup>o</sup>, Roche) have undergone the most extensive clinical trials and are the most widely used.
- Lymphoblastoid IFN-alpha (e.g. Wellferon<sup>o</sup>, Glaxo-Wellcome) contains a variety of IFN -alpha subtypes and is produced by lymphoblastoid cells that are grown in large tanks.
- Leukocyte or natural IFN-alpha is produced by buffy coat cells derived from blood donors and stimulated by Sendai virus. Although highly purified natural IFN-alpha preparations are available (e.g. IFN-alphaN3<sup>o</sup>, Interferon Sciences, USA, or Interferon Alfa active<sup>o</sup>, Bionative, Sweden) these preparations are generally less well studied and the full potential of their therapeutic use has not been established as yet.

IFN-beta has been available in a form produced by cultural human fibroblasts but today mostly recombinant forms, with slightly varying amino acid

sequences are used for therapeutic purposes. IFN-gamma is available only as a recombinant substance.

It is sometimes claimed that natural or lymphoblastoid IFN-alpha preparations should be expected to be therapeutically superior to recombinant preparations for two reasons; firstly because of their contents of multiple subtypes of IFN-alpha and secondly because of the fact that recombinant IFN:s produced by bacteria are not properly glycosophated in comparison with IFN:s produced by human cells. Since only very few comparative studies have been performed regarding recombinant vs. lymphoblastoid IFN-alpha or recombinant vs. natural IFN-alpha, it is not possible, at present to draw any firm conclusions regarding this issue. However, drug resistance may sometimes develop following therapy with recombinant IFN-alpha, due to the formation of antibodies to the particular subtype contained in the preparation. In these cases lymphoblastoid or, in particular, natural IFN-alpha may offer a therapeutic advantage.

#### **Therapeutic use of interferons**

**Side effects**  
Interferons are substances that are produced in the body and have potent biological actions. Many of the symptoms associated with acute viral infections are apparently caused by interferons that are produced in large quantities during the infection, since the symptoms can be reproduced after parenteral administration of exogenous interferon. The common side effects of IFN treatment i.e. the "influenza-like" symptoms fever, chills, nausea fatigue, myalgia and loss of appetite are thus expected events that occur in most subjects, with a severity that depend on the dosage used. These types of side effects usually show a tendency to be less severe with time and are usually tolerable. Other side effects include mental depression which will prompt discontinuation of treatment, alopecia

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and weight loss. These side effects are, however uncommon, as is also appearance of thyroid dysfunction. The latter, that is associated with appearance of thyroid autoantibodies, usually disappears after cessation of IFN-alpha therapy. The risk for autoimmunity appears to be greater following treatment with IFN-gamma than with IFN-alpha, however, and IFN-gamma treatment has even been described to be associated with development of systemic herpes erythematodes.

### **Use of IFN-alpha in viral diseases**

#### *Acute viral infections*

Several trials have been performed in acute viral infections most notably in upper respiratory infections. Since the peak of viral replication, and also the peak of the body's own interferon response usually occurs simultaneously with or before the appearance of symptoms, therapy with exogenous interferon at this stage is not likely to markedly change the course of the infection. In accordance with this notion topical application of IFN-alpha in e.g. viral upper respiratory infections has not been considered to be worthwhile. In very severe acute infections, such as Lassa fever, IFN-alpha therapy has been advocated and in some cases claimed to be successful.

Although IFN is usually relatively ineffective in acute viral infections it has been successfully used for the prevention of respiratory infections in exposed individuals. The side effects following intranasal application of IFN-alpha, such as nose bleeding, however, have precluded its general use as a preventive measure, and likewise, the side effects appearing after parenteral administration of IFN-alpha are usually considered to outweigh the potential benefits of preventive use of IFN:s.

#### *Persistent viral infections*

In contrast to acute infections, chronic viral infections are often amenable to IFN therapy. Among these hepatitis C

virus (HCV) and hepatitis B virus (HBV) infections are the most important and IFN-alpha is currently a standard treatment in HCV and HBV infections.

Large clinical trials have been performed using various types and dosages of IFN-alpha in HCV infections. The trials have been monitored using two types of markers, i.e. serum transaminases and HCV-RNA, the latter being a measure of the virus itself. Quantitative measurements have shown that IFN-alpha therapy results in a rapid initial decline of HCV serum levels followed by a phase of more slowly decreasing levels, that eventually leads to total disappearance of HCV-RNA after several weeks or months of continued treatment in responding individuals. Even after complete virological (disappearance of HCV-RNA) and biochemical (normalisation of liver transaminases) responses, however, there is frequently a relapse of the infection following cessation of treatment.

Using the initially tried dose of 3 million units of IFN-alpha three times a week for a period of 6 months, there was usually a response rate of 40-50% at end of treatment but at follow-up 6 months later about half of the initially responding patients had relapsed. Later studies have shown that it is possible to increase the rate of sustained response significantly by increasing the time of treatment to 12 months and/or by using an initial, so called induction, treatment with daily administration of higher doses of IFN-alpha.

Recently the standard treatment for HCV infection has been changed to include Ribavirin as an addition to IFN-alpha. As compared to 6 month monotherapy with IFN-alpha the sustained response rates using the combination therapy have been found to generally be at least twice as high i.e. 40-50%. Combination therapy is recommended in particular for patients with a high

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viral load (i.e. more than 2-3 million HCV-RNA copies per ml) and for those who have relapsed following IFN-alpha monotherapy. It is also claimed that patients harbouring certain genotypes of HCV that are considered difficult to treat (notably genotype 1b) should undergo primary treatment with the combination of IFN-alpha + Ribavirin.

Although the presently used therapeutic regimens in HCV infection frequently fail to eradicate the virus, they may still be worthwhile. Several studies, most notably one from Japan, have now suggested that the risk of developing hepatocellular cancer may be considerably reduced by IFN treatment, even in cases of persistent infection.

*HBV infections* may be treated with IFN-alpha. A multitude of studies have showed that treatment with IFN-alpha results in an average response of about 40% measured as a change from e-antigen positivity to anti-e positivity (seroconversion) or disappearance of detectable HBV-DNA from the blood. The patients that respond best to treatment are those that have an ongoing immunological response manifested as raised aminotransferase levels and moderate amounts of HBV-DNA in their blood. Generally, it is considered that patients in other stages of the disease, such as in the immunotolerant phase or in late cirrhotic stage respond less well or are resistant to IFN-alpha treatment.

The doses used in HBV infection are usually higher than those generally used in HCV infection and the duration of treatment is usually shorter. Similar response rates, i.e. 40% or higher, may be obtained in children.

During recent years new drugs, in particular nucleoside analogues like Lamivudine<sup>o</sup> or acyclovir-like drugs such as Famciclovir<sup>o</sup> have been found to have profound effects on the replication of HBV. However, the effects of such drugs are usually not long-lasting.

Thus, although HBV-DNA has been found to rapidly decrease following institution of antiviral therapy it has promptly reappeared in the circulation following cessation of treatment. The difference between IFN-alpha and the antiviral drugs in this respect has been interpreted to indicate that the effect of IFN-alpha in HBV-infection is primarily immunomodulatory rather than antiviral. In order to obtain a persistent effect on the hepatitis an immunological action, resulting in a seroconversion (from e- to an-e- positivity) would be necessary. Therefore, trials have been made with a combination of IFN-alpha and antiviral drugs. Future therapy in hepatitis B may possibly, similarly as in hepatitis C, be based on combination therapy.

Hepatitis B and C are the only viral infections where, presently, IFN-alpha is used as a standard treatment. Clear effects on the replication in vivo of HIV-1 have been obtained but since the effect of IFN-alpha in *HIV infection* generally are inferior to those of currently used nucleoside analogues and protease inhibitors, IFN:s have not become a part of the standard treatment in this disease. In HIV-infection complicated by Kaposi's sarcoma, however, IFN-alpha may be used therapeutically. Advantage is in this case taken of both the antitumoral and antiviral and perhaps also of the immunological effects of IFN-alpha.

For similar reasons as in Kaposi's sarcoma IFN-alpha can be used for treatment of papilloma virus infections, both in cases of larynx papilloma and in cases of skin or genital warts. In larynx papilloma, however, a permanent cure is difficult to achieve with this treatment and intralesional injection of IFN-alpha into warts, which is the drug administration of choice is cumbersome and frequently only partially effective.

#### **Interferon-b in multiple sclerosis**

Although multiple sclerosis (MS) is

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considered to be an immunological disease, it has several traits that suggest that the causal agent may be a virus. Early trials with interferons revealed that IFN-gamma may aggravate, and IFN-beta ameliorate disease symptoms. This was taken to indicate that the beneficial effect of IFN-beta are due to the immunomodulatory effects of this substance. It is certainly possible, however, that the antiviral property of IFN-beta may contribute to the therapeutic effect if viruses are indeed involved in the pathogenesis of MS.

Recent large clinical studies have convincingly demonstrated the beneficial effect of two types of recombinant IFN-beta, the glycosylated IFN-beta1a and the non-glycosylated IFN-beta1b carrying one amino acid deletion. Both of these have been found to decrease relapse rates and cause reduction of lesions, detectable by magnetic resonance. Also the progression of neurological disability has been shown in some studies.

The obvious beneficial effects of IFN-beta in MS might be paralleled by similar effects of IFN-alpha. This, however, has not been sufficiently well explored and presently, IFN-alpha is not recommended for use in MS.

#### **Therapeutic use of IFN-gamma**

IFN-gamma is a potent stimulator of cell-mediated (Th1) immunity. As a consequence of this it may have detrimental effects on Th1-cell mediated autoimmune diseases, such as MS or type 1 diabetes mellitus. On the other hand, any disease that is associated with defective cell-mediated immunity may be a potential target for IFN-gamma therapy. The most prominent indication for IFN-gamma, and the one that has been considered to justify approval by medical products agencies in many countries, is *chronic granulomatous disease* that is associated with a severe lack of IFN production. Good results with IFN-gamma therapy have been obtained e.g. in severe mycobacte-

rial infections and mycoses. IFN-gamma has also been tried in atopic dermatitis and in rheumatoid arthritis, but the results have not been uniformly convincing.

#### **Interferon therapy in malignant diseases**

Studies of IFN therapy in tumors have in the majority of cases been conducted using various preparations of IFN-alpha. Early studies in *osteosarcoma* gave promising results and showed that rather crude preparations of leukocyte (natural) IFN-alpha at doses of 3 million units 3 times a week could be given for months-years without causing intolerable side effects. Similarly, the early trials showed that *larynx papilloma* could be successfully treated with these preparations although relapses after cessation of treatment were common.

With the advent of recombinant DNA technology it became possible to produce large quantities of IFN and trials in various malignant diseases were greatly extended. Excellent results using IFN-alpha therapy have then been obtained particularly in *hairy cell leukemia* and in *chronic myelogenous leukemia*. To the list of diseases that constitute indications for IFN-alpha treatment have in many countries been added *multiple myeloma, carcinoma tumors, follicular lymphoma, polycythemia vera* and *malignant melanoma*. There are other drugs for treatment of most of these diseases and combinations of IFN-alpha with such drugs have in many cases proved advantageous.

Generally the doses of IFN-alpha given in malignant diseases are much higher than used in viral diseases, but the duration of treatment is shorter. High doses are chosen because it is believed that the action of IFN-alpha is that of an antiproliferative, cytostatic, drug. Although this may be true in most tumors it is also possible that immuno-

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“IFN-gamma is a potent stimulator of cell-mediated (Th1) immunity”

logic effects, such as stimulation of cytotoxic cells, may play an important role in certain tumors, and lower doses of IFN-alpha may then possibly be required to obtain good effects.

An example of the use of lower doses of IFN-alpha is the currently performed clinical studies of triple therapy with low-dose IFN-alpha, interleukin-2 and histamine in *malignant melanoma*. This disease is poorly sensitive to treatment with IFN-alpha alone but using the mentioned combination of drugs, which is designed to evoke a maximal stimulation of natural killer (NK) cells, a very significant effect on the survival can be achieved.

IFN-alpha has been used as adjuvant therapy, i.e. after surgical removal of primary tumors to prevent development of metastases. Significant effects on the time to relapse and on overall survival of recombinant IFN-alpha have been achieved in malignant melanoma. Generally, however, the side effects associated with the high dose regimen are severe. Several studies are presently performed in order to explore the possibilities of using lower doses of recombinant or natural IFN-alpha for adjuvant therapy in malignant melanoma.

Achievements made during recent years indicate that IFN-alpha should be regarded as an important part of the therapeutic arsenal in malignant diseases. It seems likely that during the coming years the indications for therapy with IFN-alpha, alone and in combination with a variety of other drugs will be extended to comprise a large variety of malignant diseases.

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