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INTERFERON AS THERAPEUTIC AGENT

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Summary. — The article summarizes the present status of interferon (IFN) research from therapeurical point of view. This includes its understanding as an antiviral agent and a cell regulator of hormonal- and/or cytokine-type. The IFN seems to represent a part of a balanced multisystem of unsharp contoures. In addition to the analysis of published therapeutical trials, the conclusion is made that without a deeper insight in the functioning of this system, it is difficult to create a rational base for IFN therapy of viral, oncological and/or autoimmune diseases of man.

Key words: *interferon*; *hormones*; *cytokines*; *toxicity*; *paradoxical effects*; *local and systemic application*; *inducer therapy*; *analysis of failures*

At the time when discovered by Isaacs and Lindenmann (1957), interferon (IFN) was considered an autonomous antiviral substance which accompanied the evolution of pathogenic vertebrate viruses. However, intensive studies have soon revealed that IFN has many additional "non-antiviral" effects in the cell which make this popular definition rather incomplete. Also, attempts to position this substance in the framework of the known regulatory systems of the vertebrate organism proved so far impossible (Epstein 1979). The main reason is that, side by side with the primary effects of IFN on the cell, several secondary effects are the consequence of the activation of the body's surveillance-systems, their products such as lymphokines (cytokines), various enzymes, etc. Although the assumption that IFN may have a functional or even structural relationship to known polypeptide hormones received little support (Blalock *et al.* 1980; Oleszak and Inglot 1981; Epstein *et al.* 1982), it seems purposeful as a working hypothesis based on available clinical observations, to consider IFN a cell regulator of hormonal type which mobilizes various mechanisms in order to conserve the homeostasis of the organism. (It is beyond the scope of this review to deal here with IFN as a lymphokine).

However, carrying on this concept further, several questions which are relevant to its physiologic role and therapeutic expectations in the organism should be answered. First, does a physiological level of IFN exist which—by analogy to other hormones and cell-growth factors—changes in answer to adequate stimuli? (Table 1). Second, if such "physiological" IFN does exist,

what is its role in the healthy organism ? Third, has such a substance a therapeutical potential ?

According to present knowledge, the IFN genes are normally silent in the cell and their derepression occurs only after induction (Lengyel, 1982). However, this may reflect only the insufficient sensitivity of our tests. Also lines of lymphoblasts derived from Burkitt tumours are known that continuously release small amounts of IFN into the medium in vitro (Klein, 1973), and IFN can be found in sera of about 15—10 per cent of patients with lupus erythematosus (Panem *et al.* 1982, Lackovič *et al.* 1982 — not published). It cannot be answered yet whether such IFNs are produced as a consequence of mutational depression of IFN genes or induced by the EB virus sequences present in Burkitt tumour cells or a retrovirus (? — in the case of lupus).

In addition to viruses, also non-viral inducers may function as permanent stimulators of the production of "physiological" IFN levels in "strategically" important regions of the animal organism. It has been shown many years ago that various endotoxins that are continually released in the gastrointestinal tract from bacteria may be resorbed into the circulation and stimulate IFN release in various compartments of the body, predominantly in macrophages and lymphocytes (Borecký *et al.*, 1973). A similar role may be played by phages released from bacteria, or lectins from plants ingested by food.

A second system in which a more or less continuous stimulation of IFN production is possible, is the respiratory tract. A similar array of inducers may function as in the gastrointestinal tract.

The immunological organs might represent a third continuous IFN producing system. In addition to agents mentioned above, also hepatal binding protein (a supposed animal lectin) and various proteases seem to be able to function here as inducers (Bocci 1981). The extraordinary reactivity of macrophages and lymphocytes to various inducers in vivo or in culture media may, in some cases, explain the reports on finding "spontaneous" IFN in explanted cultures of macrophages and lymphocytes (Borecký *et al.*, 1973).

Presumably, the production of "physiological" levels of IFN shows a diurnal rhythm and is influenced by external factors such as diet, disease or stress. The main producers of such "physiological" IFNs are apparently cells of the RES. In accordance, there are sporadical indications that IFN production may decline with the advanced age, possibly in parallel with the involution of thymus, accelerating in this way the diseases of senescence (Epstein, 1979).

As to the second question, Reid *et al.* (1981) reported recently that in nude mice treated with anti-IFN serum, tumour cells showed an accelerated growth. There are also indications that in both animals and man IFN regulates the surveillor function of NK-lymphocytes and macrophage systems participating in this way on the first-line defense of the organism (De Clercq, 1982). A convincing answer to the third question will be undoubtedly of extraordinary significance. However, it cannot be given as yet.

Table 1. Comparison of polypeptide hormones with interferons

Polypeptide hormone	Interferons	
	Similarity	Difference
1. The specificity is determined by the tertiary and quaternary structure	Various subtypes have different effect in the same cell type[1]	?
2. The action is mediated by the cAMP-cGMP system	The anticellular effect is mediated through cAMP (?)[2]	?
3. Are present in the organism in "physiological" amounts	? (Insufficient sensitivity of detection methods?)	Appear only after stimulation (?)
4. Are produced by specialized cells or "central" organs	Interferons may differ depending on producing cells	Produced by all vertebrate cells
5. Regulate "normal" cellular functions	? (Regulate surveillor systems?)	Affect infected and transformed cells
6. Their activity is regulated by antagonistic hormones etc.	Detto	
7. Have common precursors	? (Represent a family of substances)	Common precursors not confirmed[3]

[1] Weissman Ch.: I. Internat. Congress on Interferon, Washington D. C. 1980.
[2] Schneck J., Rager-Zisman B., Rosen O. M., Bloom B. R. 1982: Proc. natn. Acad. Sci. U.S.A. 79, 1879–1883.
[3] Blalock J. E., Smith E. M. 1982: Proc. natn. Acad. Sci. U.S.A. 77, 5972.

Interferon as therapeutic agent

The great and feverish interest in the therapeutic exploitation of IFN by the pharmaceutical companies reflects the unsatisfactory situation in the therapy of viral and cancer diseases in general.

However, the exaggerated hopes in finding a general antiviral and anticancer drug in IFN, are hardly justified. IFN cannot replace an acceptable (as yet unavailable) antiviral or anticancer chemotherapeutic substance for

Table 2. The "pendulum" effect of interferon*

	Large doses	Small doses
Virus multiplication	Inhibition	Enhancement (?)
Toxicity of viruses (ds-RNA?)	Inhibition	Enhancement (?)
Multiplication of cells	Inhibition	Enhancement (?)
Antibody production	Inhibition	Enhancement (?)
Graft-survival	Inhibition	Enhancement (?)
Activity of macrophages	Inhibition	Enhancement (?)

* Based on various sources (presented at the VIth Regional Conference on Interferon, 30. 9. to 2. 10. 1980. Lvov, U.S.S.R.)

several reasons. If we accept the hormonal character of IFN, then both unfavourable and selective effects of IFN administration should be taken into account. This follows from the observations on the:

1) paradoxical effects of large doses of IFN on the sensitive cells,
2) dependence of the effect on the presence and number of cell surface receptors for IFN,
3) development of resistance of cells (and viruses ?) to IFN,
4) toxic effects of IFN, and
5) appearance and effect of antiidiotypic antibodies against IFN.

The paradoxical action of "large" amounts of IFN can be demonstrated by its "pendulum" effect, i.e. an opposite effect of large and small doses (Lackovič and Borecký, personal communication) (Table 2). The role of receptors in IFN action can be demonstrated by the blocking effect of anti-ganglioside sera on IFN, by establishment of resistant cell lines and clonal distribution of IFN sensitivity among transformed cells (Fuchsberger *et al.*, 1974, Borecký *et al.*, 1981, etc.). It can be said, however. that in contrast to many antiviral drugs, the development of resistant virus populations has not yet been demonstrated during IFN therapy. On the other hand, it is known that the sensitivity of the same virus strain to IFN in various cell types may differ more than 100 times, reflecting probably the different affinity of various IFN subtypes to cell surface receptors (Moehring *et al.*, 1971, Weissman, 1980).

Table 3. Toxicity of interferon and/or ds-RNA in man

	Interferon	ds-RNA
1. Fever	+ (40.8—100%)	+ (60—100%)
2. Fatigue	+ (95%)	+
3. Weight-loss*	+ (51%)	?
4. Loss of appetite	+ (84%)	+
5. Chilling	+ (51%)	+
6. Psychic disturbances	+	?
7. Leukopenia	+	+
8. Thrombocytopenia	+	+ (50%)
9. Coagulation defects	+	+
10. Transaminase-increase**	+ (62%)	+ (50%)
11. Skin hypersensitivity	+	?
12. Allergization	± (rare)	?
13. Hypotension	?	+ (28%)
14. Arthritis	± (rare)	+ (rare)
15. Alopaecia**	+ (16%)	?
16. Menstruation disorders	± (rare)	?
17. Activation of herpes s.**	+ (7.3%)	?
18. Vomiting, diarrhea etc.	± (rare)	± (rare)

* Impurities?
** Patients with oncological disease
? Insufficient experience
The data are based on publications cited in the text and on own experience.

The fact that IFN may be toxic was accepted only after it had been tested in human patients. However, a) animal experiments already in 1973 demonstrated the hepatotoxicity and, in 1975, the lethality of IFN for newborn mice (Frayssinet *et al.*, 1973, Gresser *et al.*, 1975); b) long-term application of IFN to ZNB/W mice resulted in an accelerated appearance of ascitic tumours (Borecký *et al.*, 1981), and c) anti-IFN serum, surprisingly, proved therapeutic in lymphocytic choriomeningitis (LCM) infected mice (Gresser *et al.*, 1978). These data indicate that IFN is not an indifferent substance for the animal organism.

Similar considerations apply also to the so-called inducer therapy where the patient is stimulated with a suitable inducer to produce his "own" IFN. Inducers have the advantage of inducing a "safe" (non-antigenic and non-toxic ?) IFN and, in addition, at least some of them [double-stranded (ds) RNA] may function as direct antiviral, anti-cell proliferative and immunomodulatory substances as well (Borecký *et al.*, 1978, De Clercq, 1982) From a practical point of view, the inducers of IFN are cheaper then IFN. The therapeutic exploitation of inducers is hampered by fears of their toxicity for man. However, accumulating data suggest that the toxic symptoms ascribed to inducers resemble those observed after IFN administration (Borecký, 1982). For this reason, the possibility that the toxic symptoms after administration of an inducer to an animal might be caused or potentiated at least by the induced IFN itself is very suggestive (Table 3). Admittedly, the inducers may have also an counteracting effect on some of IFN activities (e.g. the immunosuppressive effect) and this may contribute to the unpredictability of therapeutic results with inducers.

The mechanisms participating in the therapeutic effect of IFN are not clear. As mentioned above, according to the present kowledge IFN may exert its antiviral (and anticancerous) effect by several mechanisms. First, IFN may act directly on the infected or cancerous cell. As a result, the cel may be cured (killed ?) by the induced antiviral and/or cell-inhibiting me-l chanisms (Lengyel, 1982). However, IFN proved effective also in treatment of animals which were infected with viruses or inoculated with leukaemic cells that were resistant to IFN in vitro (Gresser, 1972, Kuwata *et al.*, 1976). On the other hand, even in these cases, a direct effect of IFN cannot be excluded since: a) the curative effect in mice infected with IFN-insensitive viruses or resistant cells required a longer treatment and, b) the administration of antilymphocytic serum to mice in an attempt to eliminate the supposed effect of the immune system did not eliminate the curative effect of IFN. Second, the action of IFN may be indirect in the sense that IFN enhances the antigenicity of the infected or cancerous cell, and, in such way facilitates the eliminatory action of immune mechanisms. This view is in line with the growing evidence of the role of NK-lymphocytes and macrophages in the surveillance and early phases of disease. Recently, the activating effect of IFN on NK-cells as well as on the HLA antigens has been confirmed also in man (Lucero *et al.*, 1982). On the other hand, no such enhancing effect on expression of carcinoembryonic antigens by IFN could

be registered in cancer patients. Third, IFN may act on the immune apparatus of the organism and function essentially as an immunomodulator (indirect effect). In comparison with other immunomodulators, it has the advantage of being significantly less toxic and possessing a broad spectrum of activity. Fourth, in most cases the favourable effect of IFN results apparently from a combination of the direct and indirect action on the diseased cells. This view is supported by the results of a study showing sequential activation of antiviral, NK-lymphocytes and antigen expression mechanims (Lucero *et al.*, 1982).

Taken together, the results of therapeutic applications of IFN both in man and animals seem to indicate that the curative mechanism of IFN differs from that of chemotherapeutic agents which, as a rule, act directly on the cell or infectious agent.

The clinical testing of interferon

The fact that the half-life of IFN in the animal organism is very short suggests that its local or regional application might be more effective than systemic administration as it guarantees sufficient concentrations of IFN in the lesion. Nevertheless, the available data of clinical tests with this form of therapy are limited.

In the sixties and seventies, when the available amounts of IFN were rather limited, a few pilot studies in monkeys and volunteers showed encouraging results (Burke, 1979). These data were the starting point for clinical tests. However, it should be kept in mind that these were made in controlled and relatively uniform disease conditions under circumstances rather similar to animal experiments and differing from those in natural diseases of man.

The most favourable experience with IFN application in the early phases of clinical tests was gathered by Yougoslav workers (Ikič *et al.*, 1975—1977) and a few western European clinics which used the Yougoslav IFN preparations. These test included patients with recurrent herpes labialis, recurrent herpes genitalis and papillomatosis laryngis. Only the latter study was "double-blind". The results were, in general, good. In the "double-blind" experiment, only 3 patients in the placebo group but all 10 patients in the IFN treated group were cured. Similar results were obtained in 34 children with primary herpetic gingivostomatitis and 70 patients with keratitis.

Unfortunately, these results contrasted with the results of other authors who used more purified preparations of IFN (Merigan *et al.*, 1975; Kaufman *et al.*, 1976; Jones *et al.*, 1976). In other tests, only high doses of IFN, exceeding those used by Yougoslav authors, were effective and the recurrencies were not affected (Neumann-Haefelin, 1977). Little attention has been paid in these tests to the vehicle of IFN.

During these pioneer studies it became gradually clear that the evaluation of the results of antiviral therapy in man is a rather complicated issue which makes the comparison of results obtained in different places with different preparations and doses of IFN rather difficult. It should be stressed that the

degree of purity of presently used leukocyte IFNs still does not exclude the possibility that non-IFN components (lymphokines ?) present in the preparation are responsible for the therapeutic effect.

Among systemic diseases, most attention was paid to the IFN therapy of chronic hepatitis B. There are several reasons to treat this disease with IFN : 1) There is no evidence that the patients produce detectable serum IFN despite of a chronic viral infection (Tolentino *et al.*, 1975), and 2) the presence of several virus-specific antigens in the serum of patients allows an objective follow up of the therapeutic effect.

In accordance, the first reports dealing with the therapy of 4 patients with chronic hepatitis B in 1976 were encouraging. The doses employed were 300 000 to 3 million units daily. However, when after 10 days the IFN therapy was stopped, the disease reappeared (Greenberg *et al.*, 1976). In a further study 3 types of results were noticed in 11 patients: 1) reversible, partial reduction of Dane particles in 6 of 11 patients without change of the HBsAg level; 2) permanent disappearance of both Dane particles and HBsAg in 3 of 11 patients, and 3) permanent disappearance of Dane particles with a partial decrease of the HBsAg level in 2 of 11 patients (Merigan *et al.*, 1975). The results obtained in the Netherlands and Belgium (with β-interferon) have confirmed the American experience (Billiau and De Somer, 1980). It is worth mentioning that the responsiveness to IFN administration in this disease seems to be sex-linked since favourable effects were registered only in women. The authors of these clinical studies reminded us that the natural course of hepatitis B is still insufficiently understood. In our tests in the Clinics of Infectious and Parasitic Disease in Bratislava, a temporary improvement of disease was registered in 2 out of 6 IFN-treated female patients with chronic hepatitis B. As shown on Table 4, IFN may be useful in treating the acute viral complications, such as varicella, of the underlying chronic (cancer) disease.

Laboratory evidence indicates that the preventive administration of IFN results in clearest antiviral effects. This justifies the attempts to use IFN prophylactically, especially in immunosuppressed patients with organ transplants or tumours. Such patients are at high risk of activation of various viral (especially herpetic) infections. Weimar *et al.* (1978) treated 8 patients with transplanted kidneys with 2×3 million units of IFN weekly. Eight patients in the control group received placebo. The treatment started 1—2 hr before the surgery and continued for 3 months. During a 6-month control period, they found no difference in the number of viral infections or in seroconversions in the two groups. A better result was registered in patients undergoing surgery of the trigeminal nerve. In the IFN treated group, herpes activation was observed in 5 out of 12 patients while in the placebo-treated group 10 out of 11 patients showed activation of herpes labialis. The patients received daily 5 million units of IFN starting 1 day before surgery and ending on the 3rd day after surgery (Pazin *et al.*, 1979).

These results seem to indicate that the contribution of the immunological apparatus to the therapeutic effect of IFN, at least by systemic application,

Table 4. Results of interferon treatment of varicella in children with underlying leukaemia (ALL)*

Patients Number	Age (years)	Severity of disease	Duration of IFN treatment (in days**)	Duration of fever (in days)		Hospitalization (in days)	Exanthems		Antibody production (Trend)	Maximal IFN titre (Range)
				septic	subfebrile		I. wave in days)	II. wave (patients)		
4	4–7	Haemorrhagic	4–5	4	5.7	12.4	7	0	Normal (<8→256)	32–128***
5	4–6	Without	0	5.6	7.2	16.8	8	2	Normal (<8→512)	32–128***

* Dr. E. Urbančoková, University Hospital, Bratislava
** Single dose: 1×10^6 units
*** After 2 or 3 doses

Table 5. Prophylactic use of interferon in leukaemic children exposed to varicella*

Patients		Age (years)	Therapy	Stage	IFN Treatment		Appearance of varicella (Number)
Group	Number				Time	Doses (units)	
1	5	3–11	P-V	Remission	3 days after contact	5×10^6–10^7	1/5**
11	3	3–10	MT	Remission	0 (Gamma-globulin)	—	2/3**
111	3	4–5	P-V, AraC MT, CP	Remission	0	—	1/3**

* Dr. J. Čáp, Children-Clinic, Bratislava
** Disease after 80–120 days
P: Prednison, V: Vincristin, MT: Metothrexate, AraC: Cytocin-arabinoside, CP: Cyclophosphamide

might be essential. It is not known, however, whether enhanced doses of IFN would overcome an immune deficiency.

The results of prophylactic treatment of leukaemic children with IFN after their contact with a varicella case in the Children Clinic in Bratislava are shown on Table 5.

Even greater hopes have been connected with using IFN in the treatment of tumours. The therapeutic results of Scandinavian authors were a potent stimulus for both the support and subsequent development of IFN production in the world. Strander *et al.* (1973) in the years between 1971 and 1973 treated 28 osteosarcoma patients daily with 3 million units of IFN for 1 month and, after surgery, 3 times weekly with 3 million units for 17 months. The control groups consisted of 35 patients hospitalized in the period from 1952—1971 in the same hospital and 23 patients treated with other methods in the years from 1972—1975 in other hospitals ("historical controls"). The comparability of tumours in various groups was controlled by pathologists and clinicians. After 2.5 years 65% of patients in the IFN-treated group were still alive while in the control group the survival was 30%. However, the results of other groups treating advanced or terminal phases of other types of tumours were in general, less satisfactory (Billiau 1981 etc.).

Among tumours responding favourably to IFN, the laryngeal papillomas of children should be mentioned. However, the prolongation of intervals between relapses requires the continuous presence of IFN in the diseased organ (Borecký and Klačanský, 1981 — not published).

Intralesional application of IFN resulting in reduction of the neuroblastoma tumour mass reported recently by Japanese workers (Sawada *et al.*, 1981) seems encouraging but solitary.

These results suport the view that, if the number of IFN molecules at the cell surface is sufficient, a direct (cytostatic) mechanism may be decisive in the curative effect of IFN in cancer.

The succes and failure of interferon therapy

Although inconclusive, the experience so far gained suggests that:

1) In man IFN shows a similar unpredictability of effectiveness as the synthetic antiviral (anticancer) or immunomodulatory substances.
2) Even in cases of a successful therapy in man, the results as yet failed to show such a dramatic effectiveness as observed in some animal and tissue culture models.
3) Both direct and indirect actions of IFN (mediated by the immune system) may participate in the therapeutic activity of IFN in man.
4) The therapeutic effect requires continuous (or sufficiently long) presence of IFN in the lesion.

When one starts to analyse why the results of IFN therapy in man are less encouraging than in laboratory animals, it seems inevitable to pay in the future more attention to the dosage schedule used. This follows also from the data obtained by treatment of cases of leukaemia with different IFN dosage schedules by Hill (1981). The results were "good" when the doses

were substantially higher than used so far (i.e. $0.5-2 \times 10^6$ U/kg for a period of 2 months) and were unsatisfactory when they were 5—20 times lower. However, such high doses represent both an economic and medical problem since (at least theoretically) they may enhance immunosuppression and also the "toxic" effects exerted by IFN. Other requirements for a successful IFN therapy in man can be summarized as follows:

1. The virus or the tumour cell must be sensitive to IFN. (Different types and subtypes of IFN may be of different therapeutic value in treatment of a particular cell type or organ).
2. The amount of IFN introduced into the pathological lesion must exceed the (therapeutically insufficient) amount of locally produced ("own") IFN (dosage requirement).
3. IFN must reach the infectious focus (tumorous lesion) before the agent (cells) disseminates or the tumour burden is too high (timing requirement).

Unfortunately, these requirements are seldom fulfilled because:

1) The natural disease in man differs from the model disease in animals and the virus (or tumour cells) causing the particular model disease may be highly sensitive laboratory artifacts. 2) The effect of IFN may be tampered by antiidiotypic antibodies appearing with different velocity in different patients.
3) The doses of IFN may not be adequate due to the rapid metabolism which is influenced both by the type of IFN (α, β) and the diseased tissue, age, sex and other factors in the recipient.
4) In contrast to model experiments, IFN is usually applied too late in the course of the natural disease. (This applies especially to cancer cases).
5) IFN may not be of adequate quality (purity, subtype composition, etc.).
6) The reactivity of the patient may be insufficient (immunodepressed patients) or exaggerated (allergic patients).

In conclusion, the effort to introduce IFN as an antiviral and/or anticancer remedy yielded as yet little satisfaction. In order to obtain a clear answer it seems, in this respect, inevitable to learn more about the physiological role of IFN in the organism, the diseases that should be influenced and the responsiveness of patients to IFN.

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