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CURRENT VIEW ON THE PERSPECTIVES OF INTERFERON THERAPY

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Summary. — In recent years, impressive progress has been made towards the understanding of the molecular biology of interferon. Unfortunately, this progress was not accompanied by a parallel elucidation of the role of interferon in the animal organism. The feeling prevails that what we know about interferon today is only the peak of an iceberg showing interferon types and subtypes as a part of an insufficiently delineated system of cell-regulatory polypeptides (lymphokines, hormones) acting in a concerted way with other regulatory cells (such as NK-cells, macrophages) and their products (such as IL-2 etc.). An imbalance of this system may probably itself lead to disease (autoimmune diseases) and its disturbed functioning may hinder the therapeutic exploitation of its components in substitutive therapy (cancer).

Key words: interferon; types; receptors; mediators; clinical exploitation; helper systems

According to the present view, interferon (IFN) represents a family of related biologically active polypeptides produced by animal cells after stimulation with a variety of inducers. Although the IFNs are known since 1957 (Isaacs and Lindenmann, 1957), a substantial breakthrough in IFN research has been achieved only in the 70-ies when the interferonists (originally virologists) were joined by molecular biologists and the methods of gene--manipulation were introduced into IFN-research. The increased interest of scientists in IFN research was stimulated by accumulating reports which suggested the possibility of its soon therapeutical exploitation. In a time, when the viral and tumour diseases represent the major therapeutic problem in the civilized countries, the broad antiviral spectrum of IFN as well as its ability to inhibit preferentially the multiplication of tumour cells obviously met great expectations.

According to our present knowledge, the IFN family consists of three genotypes (alpha, beta and gamma) and a number of subtypes (Goeddel *et al.*, 1981). So IFN-alpha has at least 20 subtypes as the products of corresponding number of alpha-genes, while IFN-beta is known in 2 (Sehgal, 1982) and gamma in 1 genotype. The IFN-alpha genes can be subdivided into Class I and Class II genes. While Class I genes code for a family of about

15 IFNs with about 77% homology, the IFNs of Class II show only about 50% homology to the Class I products (Goeddel *et al.*, 1985). The amino acid sequence of IFN-alpha shows a 30—50% homology with IFN-beta. IFN--alpha and beta consists of 165—166 amino acids while IFN-gamma has only 146 amino acids (Pestka *et al.*, 1985). The three IFN types correspond in several and differ in other activities. The genes that regulate these activities often function in "linkage groups".

The identification of chromosomes which carry the genes responsible for IFN production is still contradictory. However, studies of hybrid human--mouse cells suggest that the alpha and beta genes are located on chromosome 9 and that of IFN-gamma is in man localized on chromosome 12 (Sagar *et al.*, 1981). The mechanism of regulation of IFN production is also unclear. It still applies that non-induced cell produces no detectable IFN. However, its synthesis can be relatively easily induced by a broad variety of substances of low or high mol. mass. Moreover, even reversible inhibitors of cell macromolecular synthesis such as cycloheximide (CHM) or dichlorribofuranosyl-benzimidazol (DRB) can function as inducers of IFN so as monoclonal antibodies to cell-surface antigens, or, IFN itself may stimulate its own synthesis (Stewart, 1979; Tan, 1981). Such conclusions do not contradict to findings of Italian authors (Bocci *et al.*, 1984) of a constant low level of IFN present at some strategic points in the body fluids, especially in the vicinity of lymphatic glands etc. since our intestines and/or respiratory tract are in a continuous contact with various inducers present in these organs which are mostly bacterial and viral agents or mitogens ingested by food.

The syntnesis and the release of IFN by the cell is most probably a "one shot" phenomenon of short duration. Consequently the production of IFN as well as the activity of IFN genes must be regulated by a hypothetical regulatory protein (repressor "R") which — during induction — is temporarely excluded from its repressive action on IFN-genes. Its existence was deduced from the phenomenon of superinduction of IFN-beta, which follows the treatment of producing cells with inhibitors of transcription and/or translation such as CHM and DRB (Vilček and Ng, 1971; Dianzani *et al.*, 1981). Supposedly, the metabolic inhibitors during superinduction inhibit the preferential production of R m-RNA (or its product) releasing in this way the IFN-genes from repression. A subsequent overproduction of R m-RNA (and its product) would then lead to the important phenomenon of the temporal hyporeactivity (tolerance) i.e. resistance against a new induction of IFN production by the same inducer (Stewart, 1979). This hypothesis of superinduction, however, could not be confirmed in the case of IFN-alpha or gamma suggesting different mechanisms in that case.

Also the phenomenon of hyporeactivity which follows the burst of IFN release is insufficiently understood. Stringfellow (1982) c.f. found that treatment of hyporeactive cells with prostaglandin E renewed their IFN producing activity. The possibility of involvement of prostaglandins in the regulation of IFN production needs, however, further confirmation.

IFN protects the treated cells against viral infection as well as some other cell damaging activities. This follows from the observation that the resistance of cells against virus infection correlated with their IFN producing capacity. For instance, the hamster cells are poor IFN producers while usually very sensitive to various virus infections (Hoffman *et al.*, 1968). A negative correlation between sensitivity to virus infection on one side and IFN producing capacity on the other was also found in comparative studies using 6 human diploid cell lines. However, the mechanism of protection of cells by IFN is far from clear. Already in 1968 it has been recognized that IFN production by the cell and the sensitivity of a cell to IFN are different phenomena (Nabholz, 1969). The gene determining the sensitivity of the cell to alpha, beta and gamma IFNs is localized on chromosome 21, but is influenced also by other chromosomes (No. 16) (Tan *et al.*, 1974). Interestingly, the gene on chromosome 21 is in the vicinity of the superoxiddismutase (SOD) gene, that regulates the killing system of polymorphonuclear leukocytes (Chany *et al.*, 1975).

Not only the sensitivity to the antiviral (AV) effect of IFN correlates with the presence and the number of chromosome 21 in the cells. The presence of additional copies of the chromosome 21 in the cell enhances also the sensitivity of cells against the antiproliferative or priming effect of IFN. In accordance, the blastogenesis of lymphocytes from patients with Down's syndrome (which are trisomic for chromosome 21) could be achieved with concentrations of IFN that were substantially lower than required for blastogenesis of lymphocytes from healthy donors (McPherson and Tan, 1980).

Receptors for IFN

As yet, only the first attempts were made to isolate the IFN-receptors. Their existence seems, however, unevitable (Friedman, 1967, Besançon and Ankel, 1977). The preliminary tests suggest that the receptors for IFN-gamma are of protein nature since they are sensitive to trypsin and resistant to neuraminidase. The increased sensitivity of 21-trisomic cells to IFN could be best explained with an increased number of receptors on cell surface which are coded by the enriched number of genes responsible for the sensitivity to IFN of trisomic cells. Possibly, the IFN receptors — like the receptors of several glycoprotein hormones — have 2 binding sites: one for the binding of the IFN-molecule to the cell surface, the second one for activation of the antiviral mechanism in the cell. Such receptors are most probably common for IFN-alpha and beta (receptors of Ist class) but different from IFN-gamma (receptors of IInd class). This made possible to treat effectively cells which are resistant to one type of IFN with IFN of the other type. It is possible that IFN receptors are not present on all cells in equal amount. Nevertheless, after interaction with IFN, the signal from the receptors carrying cell is probably, through the intercellular bridges, transferred to the receptor-devoid cells (Blalock *et al.*, 1980). Such view is supported by the studies of Burke *et al.* (1978) who found that IFN exerted only an insignificant protective effect on non-differentiated cells which, presumably, carried no or few

IFN receptors, or, had receptors which were in an inefficient configuration. Presumably, the receptors mature during differentiation. An open question is whether various activities of IFN are mediated by specialized receptors.

Intracellular mediators of antiviral, antiproliferative and immunoregulating effects of interferon

In the last years important progress has been made in elucidating the AV effect of IFN. First, it has been experimentally confirmed that IFN alone has no direct AV effect on viruses when injected into the cell. After many years of search, 2'-5'-oligoadenylate synthetase, a phosphokinase, and, a phosphodiesterase were identified as the main mediators of AV effect of IFN (Baglioni, 1979; Revel, 1979; Lin *et al.*, 1980). In accordance, it has been found that 2'-5'-oligoadenylate — (product of 2'-5'-oligoadenylate synthetase) — even in absence of IFN inhibits the reproduction of the infecting virus in the cell. However, even this mechanism does not seem to offer a complete explanation with regard to the AV effect of IFN since c.f. the embryonic HEC-1 cells, or, the erythrocytes of some birds show often high levels of 2'-5'-oligoadenylate synthetase and phosphokinase even in absence of any stimulation (Verhaegen *et al.*, 1980). Nevertheless, such cells are not protected against viral infection. This suggests, that further regulators of the AV IFN effect must exist. The problem can be demonstrated on the aforementioned HEC-1 cells that are resistant to the AV effect of IFN, while their daughter cells are usually sensitive (Burke *et al.*, 1978). In both parental and daughter cells a high level of 2'-5'-oligosynthetase was found. The explanation for the atypical behaviour of the parental cells is sought:

a) In a defective receptor system in HEC-1 cells (possibly together with the Ca^{2+}-calmodulin component of the membrane),
b) In a defective intracellular phosphokinase system that regulates in a specific way the intracellular processes (Smith - Johanssen *et al.*, 1984),
c) In an overfunctioning phosphodiesterase activity that destroys the 2'-5'-oligoadenylate and makes in this way inactive the alpha endonuclease which is supposed to destroy the viral RNA.
d) An additional pathway was suggested by Pottathill *et al.* (1980):
   1) They found that a functioning cyclooxygenase of fatty acids is necessary for the antiviral resistance of the cell. This followed from the finding that an inhibitor of this enzyme (oxyphenylbutazon) inhibited the effect of IFN in mouse cells. In general, however, the effect of prostaglandins on the activity of IFN is rather confusing (Strayer and Carter, 1984).
   2) Also, the infecting virus may regulate the IFN effect since it is known that a) cells are not equally resistant to various viruses, b) the amount of the viral dsRNA regulates also the activation of 2'-5'-oligoadenylate and, concurrently, the preferential degradation of viral RNA in comparison with cellular mRNA (Stitz and Schellekens, 1980).

Even less clear is our understanding of the antiproliferative effect (AP) of IFN which shows large differences when tested in various cells. It is as-

sumed that the AP effect is also mediated through the 2'-5'-oligoadenylate since a) this compound inhibits proteosynthesis, b) has antimitogenic effect, and, c) inhibits the proliferation of cells. The problem is that the amount of 2'-5'-oligoadenylate in the cell may rise or decline also without any IFN tratment. For inst., after application of steroids or during gravidity (Lebleu and Content, 1982).

The immunoregulating effect of IFN seems to require at least 2 types of receptor on lymphocytes, monocytes, macrophages, NK- and K-cells: the specific IFN-binding receptors, and the presence of Fc-receptors (Herberman *et al.*, 1980; Zarling, 1984). IFN induces production of mediators such as IL-2, IL-1 or prostaglandins and may act synergistically or antagonistically with them. However, IFN is not the solely regulator of these cells and the immune cells and the mediators activated by IFN are under the regulatory influence of the immune, hormonal and neural systems. For this reason, we proposed to consider the IFN system a component of a broader multisystem which beside of IFN would include the polypeptide hormones, the immune cell products and the prostaglandines (HIIP-system). It is of interest that recently overlapping antigenicity and sequences of homology between hormones such as ACTH and IFN have been detected (Blalock *et al.*, 1980). The prostaglandins seem to have a modulating (sensitizing) effect on various hormonal and immune reactions (Pottathil, 1980, Schultz, 1980). The concept of HIIP-multisystem based also on experience with the hormonal and immune therapy may help to a better understanding of the multifaced effects of IFN and allows predictions on its therapeutic efficacy. To such predictions belongs the thesis that a therapeutic efficacy higher than 30—40% shoud not be expected in IFN therapy (Borecký, 1984).

Recently it has been found that a 45 amino acids containing N-terminal fragment might be responsible for the known immunosuppressive effect of IFN (Orchansky *et al.*, 1985).

In addition, IFN activates the H-2 and/or HLA/A, B, C as well as the Ia/DR antigen (class I. and II. products) expression on the surface of cells, it may promote or inhibit the differentiation of various cells types, and, seems to play through the Cytochrome P-450 — a role in the detoxication of drugs. The involvement of various factors and systems in the IFN effect may explain also the various paradoxic effects observed during IFN treatment (Stewart, 1979; Pottathill *et al.*, 1980).

Various cells, their developmental stages, presence or absence of various polypeptide hormones, the integrity or deficiency of the immune system, may all modify the effect of IFN in the organism. This makes IFN an interesting and lasting research subject, but at the same time a not easily managable therapeutical tool.

Problem of the clinical exploitation of IFNs

It is generally agreed upon that the antiviral substances are badly needed. IFN offers in this respect a new approach to the therapy of viral and tumour diseases which differs by mechanism and spectrum from the action of chemo-

therapeutic drugs used in virus diseases and which, by its broader significance, can be linked to the discovery of antibiotics in the fortieth years. However, the results of IFN therapy in viral and cancer diseases of man are yet far behind the results seen in the model test with laboratory animals. How to explain this discrepancy?

IFN was up to now not available in sufficient amount and purity, nor the test with IFN in man were sufficiently controlled. However, now it can be stated that the controlled tests showed several virus diseases significantly sensitive to IFN treatment. To such diseases belong:

— First, keratoconjunctivitis, herpes zoster, herpes encephalitis, varicella, hepatitis B, common cold (Borecký, 1984; Kishida *et al.*, 1984; Tyrrel, 1985; Merigan and Cunninghanm, 1985 etc.).
— Second, tumours with more or less confirmed viral aetiology: as papillomatosis laryngis, epidermodisplasia verruciformis, melanoma, and, recently, the hairy cell leukaemia represent also clear indication for IFN treatment (Quesada *et al.*, 1985 etc.).
— Third, an important field of IFN benefit is the oncological patient with exacerbating viral diseases.

Less clear, but important, is the presumed effectiveness of IFN in cervix and/or brain tumours suggested by Yougoslavian and Japanese authors (Ikič *et al.*, 1981; Nagai *et al.*, 1982) when direct topical application of IFN was used.

The answer to the question whether other viral, or tumour diseases, or immune disorders (such as sclerosis multiplex, mammary tumours etc.) will be a promising subject for IFN therapy awaits further controlled trials.

In this connection, we should not forget that there is a great difference between the laboratory and clinical examinations of IFN. While the laboratory infections are performed with known virus strains of known virulence, with known amounts of it etc., in animals of more or less uniform quality, age etc., the clinical trials are performed on genetically ill defined individuals, infected at an unknown time with ill defined virus, treated in various stages of the disease etc. This all needs patiency and sophisticated approaches in evaluating the results of IFN applicability to man.

The differences between the man and animal are reflected also in the problems of toxicity of IFN. Since only newborn animals treated with supereffective doses of IFN showed clear signs of toxicity in laboratory tests (liver etc.), we were inclined to think that IFN is completely avoid of toxicity also for man. This is not true although the side effects of IFN in immunologically integrated persons are usually not dramatic. A visible toxic effect of IFN on the cellular level is the appearance of tubulo-reticular inclusions in some cells during IFN treatment or diseases like SLE. Paradoxically, IFN may prolong the biotransformation of several drugs as a consequence of inhibition of P-450 oxygenase system. Now it is clear that the toxicity shows clear species differences. For instance: rhesus monkeys were resistant while chimpanzees were sensitive to the toxic effects of human IFN (Schelleken and Van der Meide, 1985).

Finally, it should not be forgotten, that the effect of IFN is mediated by various helper cell systems such as NK-cells, macrophages, T_c-lymphocytes and cell products like IL-2, MIF, prostaglandins etc. This means that disturbances of the immune or hormonal system (stress) may deprive the IFN molecule of its helpers. In addition, IFN has its insufficiently characterized antagonists and synergists of various provenience and the former should be as far as possible excluded from the therapy during IFN treatment. The antagonists seem to belong either to cell products like corticosteroids, prostaglandins (some types) or they are vitamin A and drugs like Aspirin, Oxyphenobutazon (a pyrazolidin derivative), Adriamycin, Vincristin, H_2-antagonists (Cimetidin etc.). A certain common property of these drugs is their antiphlogistic activity (Strayer ans Carter, 1984).

Why is IFN a perspective antiviral and antitumour drug?

1. Taken together the present stage of our knowledge indicates that IFN should be considered a bioregulator which has a normalizing effect on cells. Such a definition makes clear that IFM belong to the same category of cell products as the hormones, growth factors and cytokines and their antagonists and its remarkable effects result often from a concerted action of all helper components on diseased cells or organs. (For instance in hairy cell leukaemia IFN restores the NK-cell and macrophage activity.) This makes topical the problem of existence of so called "physiological" IFNs as stressed especially by the Italian school of interferonists (Bocci *et al.*, 1984; Paulesu *et al.*, 1985) and which may be used as an indicator of that integrity.
2. The mode of action of IFN resembles the mode of action of growth hormones such as Gastrin or PDGF which are now increasingly involved into the explanation of the action of oncogene-products that might be pathological analogues of growth factors. IFN may represent their antagonists appearing under pathological conditions.
3. The dependence of the effectiveness of IFN therapy on various immune and hormonal factors suggest that in the therapeutic strategy criteria different from those applied to chemotherapeutic agents should be created.
4. The effectiveness of IFN therapy will be larger when IFN is combined with synergistic drugs. In contradistinction to many cytostatics, however, IFN exerts its effect also as a "last cell killer" and this makes him valuable especially for prevention of metastases.
5. The well documented effectiveness of IFN in hairy cell leukaemia shows clearly that we need reliable tests which would differentiate for therapy suitable and non-suitable cases, and enable the follow up of the therapeutic effect. Similar tests are needed to evaluate the functioning of various branches of the immune system. In this respect we should probably not forget that the present approach to the IFN therapy resembles the first years of insulin therapy when the blood levels of sugar could not be controlled.

In conclusion, it can be said that IFN has opened a new chapter in the therapy of viral and cancer diseases. Data supporting its effectiveness in

well selected diseases are accumulating in parallel with our understanding of both the diseases treated and IFN action.

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