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INTERFERON AFTER 30 YEARS

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Summary. — IFN is a product of a growing family of IFN-genes in the vertebrate cell. It is a polypeptide which fits the definition both of lymphokines and/or "local" hormones, and, cannot be anymore considered an "autonomous antiviral factor", as postulated originally. Rather, IFN increasingly seems to play in the organism the role of a "master-lymphokine" that mediates, potentiates or regulates the effects of various lymphokines in differentiation, growth and antigen expression of cells. The antiviral, antiproliferative, cell- and antigen-activating activities of IFN formed the basis for expectations that IFN will be utilized in therapy. These expectations were only partially fulfilled. In some diseases, however, the results of clinical tests are convincing. In such tests, we learned that the successful application of IFN in disease requires other strategies than with cytostatics etc. IFN may be deleterious for the organism when present for a long time in circulation and/or applied in high doses. This follows both from observed side effects of IFN and from the mitigating effect of anti-IFN sera in some diseases.

Key words: *interferons*; *lymphokines*; *production in vivo*; *therapy*; *adverse effects*

Introduction

Since its discovery in 1957 by Isaacs and Lindenmann, interferon (IFN) continuously attracts the interest of scientists. To a great deal, this interest was catalyzed by a relatively fast progress in elucidation of its primary structure, solving the problem of large scale production by recombinant technology and, by a deeper understanding of its biological significance (Rubinstein, 1987; Nagata *et al.*, 1980). In a relatively short time-span of 30 years, the concept of IFN has changed from a broad-spectrum antiviral agent to a cell regulator that incorporates the properties of both lymphokines and hormones, and, as such, presents a new promise in the therapy of viral, neoplastic and, autoimmune diseases (Finter, 1987; Vilček, 1987). In the same time, the new view of IFN has opened new avenues for exploration of the complicated network of cell regulation in the animal organism.

Table 1. PROPERTIES OF IFN GENES OF MAN AND THEIR PRODUCTS (ACCORDING TO WEISSMANN AND WEBER: 1987 AND BOCCI 1987)

	Hu IFN α	Hu IFN β	Hu IFN γ
Number of functional IFN genes	15 + 6 (?)	I	1
Chromosomal location	9	9	12
mRNA 5′ nontranslated region	67—69	73—75	127
mRNA 3′ nontranslated region	240—440	200	584
Number of introns	0	0	3
Length of signal peptide	23*	21	20
Length of mature protein	166	166	146*
Position of cystein in natural protein	1, 29, 99, 139*	17, 31, 141	1, 3
Position of putative N-glycation site	0	80	28, 100
Molec. weight (kD)	18.5—19.4	19	17
Functional form	Monomer	Dimer	Tetramer
Stability at pH2	Stable/but	Stable	Labile
Stability at 56 °C	labile subtypes/	Labile	Labile
Isoelectr. point	5.7—7.0	5.2—6.3	8.7
Receptors	Common	Common	Specific
Location of receptor genes (chromosome)	21	21	6
AV induction	Fast (30—60 min)	Fast	Slow

* Many exceptions

INTERFERONS TODAY: THE INTERFERON GENE-FAMILY

IFNs are polypeptides known in 3 antigenic, physicochemical and functional species, IFN-α, IFN-β and IFN-γ, which are coded by a superfamily of about 24 α, 1 β and 1 γ genes (Weissmann and Weber, 1987). At least 14 of IFN-α genes are potentionally functional nonallelic members. The IFN-α genes can be grouped into 2 subfamilies: the subfamily IFN-αI comprises at least 15 loci (with 1 pseudogene), while the subfamily IFN-αII represents 6 or 7 genes with 5 pseudogenes. Two pseudogenes (IFN-α11 and IFN-α12) may represent two additional subfamilies. The genes of subfamily IFN-αI have been further subdivided into 2 groups on the basis of their relatedness to members of subfamily IFN-αI. Group 1 consists of genes IFN-α1, α13, 2, 5, 6 and group 2 of genes LeIFC, LeIFF, α4b, α7 and α16 (Table 1). However, not all sequences found in subtypes fit this clasification (Weissmann and Weber, 1987). The IFN genes, groups and families are evolutionary developments resulting, most probably, from a common ancestral gene which appeared about 300 million years ago (Pestka and Langer, 1987). The genes in the IFN-αI group differ from each other by values of 8.3 ± 2.5 % for replacement substitutions (which alter the function of polypeptide) and 23 ± 8.3 % for silent substitutions which may remain unrecognized. These differences are larger in IFN-αII group (Weissmann and Weber, 1987).

Some of IFN gene sequences, such as GX-1 and/or IFN-α14 represent remarkable instances of natural hybrid genes, probably, of very recent origin. They may have arisen by crossing-over or gene conversion.

The genes coding for IFN-α and IFN-β are in man located on chromosome 9, while the gene coding for IFN-γ is on chromosome 12.

According to some experts, IFN-α and IFN-β coding regions have a repeat structure, the first half of molecule being homologous to the second half. In contradistinction to IFN-γ, they lack introns.

THE IFN POLYPEPTIDES

Many of IFN genes were cloned and the corresponding recombinant IFN types and subtypes were obtained. They were the first proteins purified to homogeneity (Nagata *et al.*, 1980; Derynck *et al.*, 1980; Taniguchi *et al.*, 1980), (Table 1).

The products of IFN-α genes, preinterferons, are composed of a 23 residue signal sequence (with the possible exception of IFN GX-1 with only 16 residues) and of a mature sequence with 166 aminoacids. The human IFN-α2 is shorter by one while human IFN-αII-1 (IFN "ω") is longer by 6 residues.

The IFN-α molecule (166—165 amino acids) has 4 cysteine residues, while IFN-β (166 amino acids) encodes 3 cysteine residues and IFN-γ (146 amino acids), 2 residues. Interestingly, the replacement of cysteine at position 17 by serine contributed effectively to stability of recombinant IFN-β (Mark *et al.*, 1984) and, as a commercial product, the modified preparation is now used in therapy of human diseases. The IFN-α molecules are rich in leucine and glutamic acid-glutamine (Pestka and Langer, 1987). They are, in general, non glycated. The IFN-α subtypes differ from each other by up to approximately 20 % of their residues (Valenzuela *et al.*, 1985).

On contrary, IFN-γ after separation by HPLC etc appears in 3 species. This heterogeneity is ascribed to varying degrees of glycation (Rubinstein, 1987). IFN-γ (called also "immune" or type II IFN) behaves as a classical broad-range lymphokine: it is produced by activated T-lymphocytes after

Table 2. BIOLOGICAL ACTIVITIES OF IFNS (ACCORDING TO VILČEK et al., 1987)

IFN α/β:	INDUCTION OF AV STATE
	INDUCTION OF 2′-5′-OLIGOADENYLATE SYNTHETASE
	INDUCTION OF eIF-2PROTEIN KINASE
	INHIBITION OF CELL GROWTH
	ACTIVATION OF NK-CELLS
	ACTIVATION OF Tc-LYMPHOCYTES
	INDUCTION OF CLASS I HLA (H2) ANTIGENS
	STIMULATIONS OF B-CELL DIFFERENTIATION
IFN γ:	INDUCTION OF CLASS II (HLA-DR AND H2-Ia) ANTIGENS
	INDUCTION OF Ig Fc RECEPTORS
	ACTIVATION OF MACROPHAGES
	INDUCTION OF MONOCYTIC DIFFERENTIATION
	INHIBITION OF INTRACELLULAR PROTOZOA AND BACTERIA
	STIMULATION OF IL-I AND IL-2 SYNTHESIS
	STIMULATION OF Ig PRODUCTION

immune (specific, antigenic) or mitogenic stimulation, and affects preferentially cellular functions related to immune response. In general, the antiviral (AV) potency of IFN-γ is lower than that of IFN-α/β. It seems possible that IFN-γ exerts its AV activity by induction of IFN-α/β in IFN-γ treated cells (Hughes and Baron, 1987).

The repertoir of biological activities of IFN- γappears to be broader than that of other IFNs: it functions as differentiation factor in monocytes, as modulator of gene expression in fully differentiated cells, as growth regulator in somatic cells etc (Hovanessian *et al.*, 1980; Blalock *et al.*, 1980). Probably, due to its more "classical" lymphokine character, IFN-γ has been, with the exception of malaria, rarely found in man during infectious diseases (Rhodes-Feuilletter *et al.*, 1981), (Table 2).

The molecular weight of IFN-α is between 18.5 and 19.4 kD. In solution, it is predominantly in monomeric form, while the antivirally active IFN-β, with a molecular weight between 31 and 42 kD, is in solution present probably as a dimer since the molecular weight of experimentally determined recombinant IFN-β was 19 kD. The molecular weight of the glycated IFN-γ appears to be around 45 kD (Vilček *et al.*, 1985), while the recombinant monomer had a molecular weight of 17 kD (Pestka *et al.*, 1985). This suggests that the functional form of IFN-γ is a tetramer (or trimer). IFN-γ, again in contradistinction to IFNα/β, is acid- and thermolabile and, (as mentioned) is produced solely by T-lymphocytes. In accordance, patients with profound disturbance of the T-cell system (such as ataxia-teleangiectasia) have a diminished capacity to produce IFN-γ (Paganelli *et al.*, 1984).

IFNs have a very high specific biologic activity: the antiviral (AV) activity of some IFN-α preparations can be detected at levels as low as 10^{-12} to 10^{-13} M and, about 20 molecules per cell seem sufficient to trigger activation (Weissmann and Veber, 1987; Billard *et al.*, 1986). IFNs of IFN-α II-1 group may antigenically differ from other subtypes (Adolf, 1987). This, and the presence of additional 6 amino acides in the primary chain of IFN-αII-1, led once to consider a subtype of IFN-αII-1 as a new, "ω" type of IFN family, as was also the case of "IFN-β2" that is now considered identical with interleukin-6 (IL-6) (Annual Meeting ISIR, Washington, D.C., U.S.A., 1987). While the AV, antiproliferative (AP) and NK-cell stimulating as well as HLA-enhancing activities may be inportant for the therapeutic exploitation of IFNs, the emerging capabilities of IFNs to regulate growth and differentiation of cells support a "physiological" role of IFNs in the organism. The AV and AP effects of IFNs-α/β and γ are synergistic and often mediated through an increase of receptors on cell surface.

Usually, about 10 IFN-α loci are expressed in various normal and leukaemic cells. In consequence, the proportion of various subtypes in natural IFN preparations obtained from blood cells may vary. This may influence the biological activity of natural preparations because the various subtypes often show distinct quantitative patterns of AV, AP, NK-cell stimulating and other activities. For instance, IFN-α1 and IFN-α8 differed in AV potency by a factor of 330 (Pestka *et al.*, 1985).

The recombinant technology made recently possible the construction of man-made hybrid IFNs which were used by Pestka *et al.* (1985) to measure the relative activities of IFN subtypes.

Hybrids prepared from recombinant IFN-αA and IFN-αD were used to define the "specific molecular activity" of 2 subtypes included as the molecules per cell necessary to elicit a specific effect. The comparison showed that, in addition to differences in AV, AP and NK-cell activating potency, new activities of IFN were generated in hybrids. Whereas the AV activity on bovine MDBK cells was similar for all tested IFN species, their activities differed when tested on cells from other species: unlike the parental IFN species, the hybrids were active also on feline and mouse cells. The subtypes of IFN-α may also show different capacity to stimulate NK-cells. The difference between IFN-αB and IFN-αI was, in this respect, more than 1000-fold (Pestka *et al.*, 1985). Also, the hybrid IFN-αA/D was active as NK-cell activator while the parent species were not active (Brunda *et al.*, 1986). Finally, the hybrid IFN-αA/D showed an increased capacity to stimulate the expression of Fc-receptors on macrophages (Yoshie *et al.*, 1985) and had an augmenting effect on resistance of immunosuppressed mice against melanoma (Nishimura *et al.*, 1986). Such experiments showed that small differences in the amino acid composition (lack of 3 amino acids) of identical hybrids may profoundly influence also the depressing effect of IFN on enzyme systems like cytochrome P-450 etc (Renton *et al.*, 1984).

Significant differences were found also between IFN-αD and IFN-αI subtypes in capacity to stimulate surface expression of tumor associated antigens, whereas their AV and AP activities were intact. A hybrid between murine IFN-α1 and IFN-α4 had a 10 to 100 times higher specific antiviral activity than the natural parental subtypes (Zwerthoff *et al.*, 1987). Three regions were found to influence this activity. Such findings led Pestka *et al.* (1985) to conclude that either are the various IFN activities mediated via different cell-receptors (i.e. receptors with different affinities for IFN subtypes), or, different constituents of one common cell-receptor may function as mediators of various activities (Schwarzmeier and Schwabe, 1987).

The construction of hybrid IFNs seems to be prospective also as therapeutic inprovement. Recently, fusion proteins between IFN- and lymphotoxin were constructed which showed enhanced AV and direct cytotoxic activity (Feng *et al.*, 1987).

Since the biological activity of IFN molecule is determined by recognition of its structures by receptors and/or fluid (antibody etc) molecules, the analysis of reactive epitopes on IFN subtypes is important for structure-function analysis.

The features of IFN molecule necessary for recognition by cell-receptors have not been satisfactorily defined (Weissmann and Weber, 1987; Pestka and Langer, 1987). Various binding models were proposed which, however, need experimental verification. At present, it seems that the 10 to 11 amino-acid residues at COOH-terminus are not necessary for the cellular activity of IFN-α/β or IFN-γ and, after binding to cell, the IFN activity remains tem-

porarily accessible to antibody (Arnheiter *et al.*, 1983). In accordance, a monoclonal antibody that recognizes an epitope at this end of molecule did not neutralize the biological activity of IFN-α (Gray and Goeddel, 1983), Garotta *et al.* (1987) found even an increase of biological activity of IFN-γ when a deletion was limited to 10 carboxyterminal aminoacids. Similarly, the biological activity of IFN-α molecule was retained when the NH2-terminus was modified by deleting the first 4 aminoacids or by extending it with the signal peptide in IFN-α2 (Nagata *et al.*, 1980; Trotta and Nagabhushan, 1985). Recently, it has been reported that a relatively conserved region between the amino acids No 128 and No 132 is important for binding of human IFN-γ to specific receptors (Garotta *et al.*, 1985) and, Shearer and Taylor-Papadimitriou (1987) found a similar region between amino acids No 107 and No 113 in human IFN-α2 and, another one, at amino acid position 121. Also, a largely conserved region was found near the carboxylterminus. When a corresponding synthetic fragment, consisting of 56 amino acids found between amino acids No 111 and No 166 of intact IFN-α/β was used for immunization, it stimulated antibodies that bound both to synthetic fragment (used as antigen) and to intact IFN-α1 and IFN-α2 (Arnheiter *et al.*, 1981). Cleavage experiments with thermolysin showed that the AV activity persisted in the "large" (12 kD) fragment of IFN-α2 containing 110 amino acids, while the "small" (5.42 kD) fragment of IFN-α2 (amino acids No 111 to No 153) was only irregularly active, depending mostly on isolation conditions (Ackerman *et al.*, 1984).

THE IFN RECEPTORS

The effect of IFN on the cell is mediated through cellular receptors. Usually, the number of IFN-receptors on established cell lines is higher than on fresh cells taken from organs. An increase of their number was found on hybridomas (Borecký *et al.*, 1984). Lack of binding of IFNs to receptors seems to be the primary basis of species-specificity. As found by competition tests, the receptors are common for IFNs- α and β and different for IFN-γ. (Receptors of Ist and IInd class). Their number on cell surface was estimated to be about 2×10^2 to 6×10^3 per cell for both IFN-α/β and IFN-γ. The dissociation constant for IFN receptors is 1×10^{-9} to 1×10^{-10} M (Pestka and Langer, 1987). The number of receptors may be down-regulated by IFN-γ, while their recovery was inhibited by cycloheximide suggesting proteosynthesis in their formation.

The gene for IFN-α/β receptor is in man located on chromosome No 21. This gene is synthenic with that for SOD-1, the protooncogene ETS, the gene for amyloid β as well as the gene for phosphoribosylglycinamide synthetase (PRGS) (Tan *et al.*, 1973; Moore *et al.*, 1977; Ashley and Cox, 1987; Lovett *et al.*, 1987). In accordance, the cells obtained from patients with 21 trisomy (Down's syndrome) were found to be more sensitive to IFN than are the cells from healthy persons (Funa *et al.*, 1986). In contradistinction, the gene coding for receptor of IFN-γ has been located on chromosome No 6 in the vicinity of HLA-genes, tumor necrosis factor (TNF) gene, and *C-ros (crc)*

and other oncogenes (Ucer *et al.*, 1987). Nevertheless, the presence of chromosome No 6 alone in human cells is not sufficient for protection of cells by IFN-γ suggesting the necessity of additional genetical structures for proper signal transduction. For some activities of IFN-γ such as HLA-induction, a collaboration of chromosomes 6 and 21 seems to be required (Epstein, 1987). The molecular weight of IFN-γ receptor is around 90 kD (Mao *et al.*, 1987). On the importance of receptors in mediating the action of IFN-α/β on cell points the observation that direct injection of IFN-α/β into cells failed to inhibit the replication of vesicular stomatitis virus in treated cells. However, a certain compartmentalization may change the result of microinjection since previous incorporation of IFN-α/β into liposomes and subsequent injection into cell led to activation of AV mechanisms (Pestka and Langer, 1987). At 37 °C, the amount of IFN bound to cell surface that becomes gradually insensitive to trypsin or acid increases. This suggests the consecutive internalization of the IFN-receptor complex. However, others do not find internalization of IFN inevitable for its effect in the cell (Bocci, 1982).

THE INTRACELLULAR TRANSMISSION OF THE IFN SIGNAL

Two IFN-induced, ds-RNA dependent enzymes (2'-5'- oligoadenylate synthetase and the Pi/eIF2 protein kinase) have been identified as regulators of viral and cellular macromolecular synthesis and degradation in the cell. They are activated as a consequence of IFN binding to cell receptor. First a small thermostable compound, 2',5' oligoadenylate (2',5'- An) with 5-terminal phosphate residue is synthesized by the 2',5' oligoadenylate synthetase since after viral infection of the cell its amount rises, while, without ds-RNA, the intracellular level of 2',5' oligoadenylate or Pi/eIF2 protein kinase does not increase.

The 2',5' oligoadenylate synthetase is one of 3 enzymes that are required for the functioning of the 2',5'- An system to which belongs also and endoribonuclease (activated by 2',5'- An structures) and, a phosphodiesterase that catalyzes the hydrolysis of oligonucleotides with 2',5' phosphodiesterase (Pestka and Langer, 1987). The catalysis of the synthesis of oligonucleotides of the general structure pppA/pA/n with $n > 2$ from ADP and ATP is the only known function of 2',5' oligoA-synthetase. However, it is less sure that IFN is its only inducer. The size of the thermostable oligonucleotide extends from dimer to pentadecamer. The induction levels depend on the growth state of the cell and may substantially differ in various cells depending on basal enzyme level, differentiation status, hormonal status etc. The 2',5'- An is known at least in 4 isozyme forms which are present in different proportions in different cells (Lengyel, 1987). The enzyme is encoded by a single gene in man present on chromosome No 11.

The 2',5' An dependent endoribonuclease (2',5' RNAse L) is activated by 2',5'-An. It catalyses the cleavage of both viral and cellular mRNAs on the 3'-side of predominantly UA, UG and UU sequences to yield 3'-phosphate terminated products. The level of this enzyme increases about 2-fold after IFN treatment. Several viruses (EMC etc) may inactivate it in its function.

The 2′,5′-phosphodiesterase (and phosphatase) catalyses the rapid degradation of 2′,5′-An to yield AMP and ATP. Its level in untreated and IFN-treated cells (with exception of mouse L-cells and human Daudi cells) is comparable.

A ribosome associated protein of about 68 kD size, if activated by ds-RNA (or heparin), first phosphorylates itself and then the small (38 kD) subunit of protein synthesis initiation factor (Pi/eIF-2). The phosphorylation results in inhibition of peptide chain inititation. The cellular level of Pi/eIF-2 kinase is extremely sensitive to treatment with IFN. The increase of phosphorylation in IFN-treated cells is dependent on either virus infection or addition of ds-RNA to cells. It is about 30 %, while in non-treated cells it is only 5—10 %. The main target of phosphorylation is the O-phosphoserin-phosphoester linkage (Weissmann and Weber, 1987; Lengyel, 1987). This enzyme is also sensitive to the inhibitory effect of various viruses (Whitaker-Dowling and Youngner, 1986).

While it has been shown that 2′,5′-An and the ds-RNA dependent enzymes efficiently inhibit the protein synthesis in vitro, their antiviral activity in vivo is less clear. This may be the consequence of a rapid degradation since stabilized analogues of 2′,5′- An upon microinjection into cells effectively increased the AV resistance of the cells (Defilippi *et al.*, 1986). Even less clear is the role of Pi/eIF-2. Both a correlation and a lack of correlation has been found between the functional Pi/eIF-2 protein kinase system and various viruses infecting cells. Also, the type of IFN and of cells used played a role in showing correlation or its absence. It is also not clear whether these enzymes are specific gene products induced by IFN.

In addition to above mentioned ds-RNA dependent enzymes, a dozen of proteins appears in IFN treated cells whithin hours which suggests that IFN regulates the expression of several genes. These proteins, resolved by various gel electrophoresis systems, range from 15 to about 120 kD and include HLA antigens thymosin B4, metallothionein and other polypeptides (Pestka and Langer, 1987). Several studies indicate a correlation betwen appearance of these proteins and the development or decline of the AV state (Rubin and Gupta, 1980). Some of them are identical after induction with either IFN-α/β or IFN-γ, but others, such as HLA-DR antigens, are specific for IFN-γ (Epstein, 1987). It should be mentioned that overlapping patterns of polypeptide induction were observed also when either IFNs, or, IL-1 or TNF were used for stimulation of fobroblasts (Epstein, 1987).

Faster appearing changes were observed in unsaturated membrane lipids of IFN treated cells leading to its rigidity. The available evidence suggests that the enhanced activity of prostaglandin synthetase and SOD enzymes are involved in this effect of IFN. In addition, IFN treatment of cells is accompanied by free radical release which are most probably responsible for depression of cytochrome P 450 and activation of macrophages and NK-cells (Das *et al.*, 1986).

WHAT KINDS OF INTERFERONS ARE PRODUCED IN VIVO?

The answer to this question cannot presently be satisfying. In general, production of IFN by a cell is a transient phenomenon and normally IFN cannot be found in the blood. This is often used as argument against the hormonal nature of IFN. However, IFN can be induced by a variety of exogenous and endogenous inducers. In natural conditions they may exert a frequent, if not continuous, low grade stimulation of cells in various parts of body, especially in organs rich in lymphoid tissue which function as a natural filter in the organism. This may result in local production of IFN with short-range effects on neighbour cells such as growth regulation, differentiation, antiinvasive defense etc (Bocci, 1987). Such IFN production can be considered "physiological" in contradistinction to "emergency" production occuring during the acute phases of infectious diseases, or, during massive induction of IFN with therapeutic intentions. In the first case, the production is of paracrine type, in the second-endocrine. The IFN produced by paracrine mechanisms exerts, as mentioned, its effect in the vicinity of the producer cells, does not reach the circulation and cannot be detected in the blood. Such an unorthodox view on production of IFN in healthy conditions is supported by isolation of IFN mRNA from organs of non-stimulated mice (Tovey and Gresser, 1985), by measuring the levels of 2′,5′ oligoA-synthetase in lymphoreticular cells (Hovanessian *et al.*, 1980), by finding mouse macrophages in an induced (antiviral) state (Belardelli *et al.*, 1986), and by finding various organs and cells releasing IFN-α and IFN-γ *in vitro* (Smith and Wagner, 1967; Blach-Olszewska and Cembrzynska-Nowak, 1979).

A regular source of "spontaneous" IFN seems to be also the murine placenta and the human amniotic fluid ("embryonic" IFNs) (Fowler *et al.*, 1980; Lebon *et al.*, 1985).

How much is the "physiological" IFN identical with IFNs produced in man during infection or induction in human cells in laboratories, is, as yet, not known.

A new quality of IFNs may represent the "atypical" IFNs found in pathological conditions such as autoimmune diseases (systemic lupus erythematosus — SLE, psoriasis etc), or AIDS (Hooks *et al.*, 1979; Abb, 1985), or, in congenital rubella (Lebon *et al.*, 1985) and Down's syndrome (Funa *et al.*, 1986). These IFNs are "atypical" in the sense that they seem to be of α-type but are usually acid- (at pH2) and thermo-labile (at 56 °C) (Hooks *et al.*, 1979; Sibbit *et al.*, 1983; Ytterberg and Schnitzer, 1982; Borecký *et al.*, 1986). First they were considered of IFN-γ type which is also acid- and thermo-labile. However, specific anti-IFN-γ serum did not alevays neutralize the IFNs found in SLE, psoriasis or AIDS, while they were neutralized by polyclonal anti-IFNα sera (Preble *et al.*, 1988). Also, tubuloreticular inclusions (TRI) were found by electron microscopy in lymphocytes obtained from patients with autoimmune diseases and/or exposing cultivated Raji-cells (derived from Burkitt's lymphoma) or blood cells of healthy persons to sera of SLE patients containing IFN-α or, to IFNα preparations prepared in laboratory (Bo-

recký *et al.*, 1987; Rich, 1981). IFN-α may induce also polykaryon formation (Rich, 1981; Weinberg *et al.*, 1985). This, together with the known lymphopenic effect of IFN administration suggests the possibility of a direct injurious activity of both "normal" and "atypical" IFNs when present in circulation in elevated concentrations and for longer periods of time. Some authors consider SLE as "hyperinterferonaemia" (Rosenbach *et al.*, 1984 etc.)

However, alpha-IFN of acid- and thermo-labile type may be found also as a regular constituent of normal human leukocyte IFN-α preparations obtained after induction of human blood leukocytes with viruses such as NDV or Sendai (Chadha, 1985; Matsuoka *et al.*, 1985; Borecký *et al.*, 1986). Also the IFN-α/β found in sera of mice after induction with poly I : C is usually thermolabile. However, the thermolabile IFN-α seems to be different from the natural IFN-α found in amniotic fluid (Fowler *et al.*, 1980; Lebon *et al.*, 1985). Hopefully a monoclonal antibody which was recently obtained by us and seems to be capable of differentiating the thermostable human IFNs of alpha type from the thermolabile alpha IFNs of SLE-type, may be useful in its purification and elucidation of its properties (64 — Annual ISIR-Meeting, 1987, Washington, D.C.; Borecký *et al.*, 1989, in press; Kontsek *et al.*, 1989). The monoclonal antibody against the human acid-(thermo)-labile IFN-α has been prepared by using for immunization freshly obtained, high titered (1 : 128 000 per ml) human leukocyte IFN-α after induction with NDV. The proportion of thermostable IFN component in this preparation was about 0.2 per cent. After removal of the majority of NDV from preparation by high speed (30 000 rev/min/90 min) centrifugation, this IFN-α preparation was used for immunization of Balb/c mice. Fourty-eight hours after the third injection, the spleens of immunized mice were used for preparation of splenocytes and fusion experiments with NSO myeloma cells. The reactivity of the obtained monoclonal antibody with various IFNs such as recombinant IFN-α2 (Boehringer) and IFN-α1 (obtained from prof. Bodo, Vienna), IFN-β (Toray Ind.), IFN-γ (Finnish Red Cross) and human acid-(thermo)-labile IFN-α (AL-IFNα) showed that the monoclonal antibody T18 as well as 2 similar monoclonal antibody preparations. T1 and T19, did not inhibit the AP effect of recombinant IFN-α1, α2 LeIFN-α (prepared in the Institute of Virology, Bratislava), or the LeIFN-α prepared by Celltech, U.K., while the monoclonal antibodies against the acid-labile IFN (AL-IFN-α) reacted also with other (available) acid- and thermo-labile IFN-α preparations such as SLE-IFN-α and psoriatic IFN-α. However, it seems important that the neutralization of the acid and thermolabile IFN-α (AL-IFNα) with various polyclonal anti-IFNα sera was not regular suggesting a dependence on the presence of specific antibodies against AL-IFNα in the preparation. Neither the type of producer cell, nor the inducer of the acid- and thermo-labile IFNs are known presently. However, Funa *et al.* (1986) reported to find null lymphocytes producing the acid-labile IFN-α in Down's syndrome. The type of inducer may also play a role in derepression of various IFN genes. It has been reported that (mouse) macrophages produce IFN-γ when stimulated with low molecular weight compounds, and IFN-α and IFN-β when stimulated with viruses or LPS (Kirchner, 1986).

As mentioned above, appearance of IFN-γ in circulation of man in natural conditions was only exceptionally reported. Such was malaria and a diabetic child (Rhodes-Feuilleter *et al.*, 1981; Tovo *et al.*, 1984). Also the reported cases of IFN-production deficiency are rather rare. Ikeda *et al.* (1986), Okabe *et al.* (1986), and, Carvalho *et al.* (1985) reported that patients with active visceral leishmaniasis, chronic hepatitis B and/or myelodysplasia have a reduced capacity to produce IFN-α and IFN-γ. A defective IFN-γ production has accompanied also ataxia-teleangiectasia (Paganelli *et al.*, 1984). In mice, thymic dysfunction may lead to impaired IFN production suggesting a regulating role of thymus in IFN production in vivo (Huygen *et al.*, 1983).

WHAT CAN INTERFERONS DO AND WHAT REGULATES THE REGULATOR?

IFNs exert a wide range of biological and biochemical effects on cells and animal organism. In addition, to AV and AP activity of IFNs, which is associated with activation of several enzymes in treated cells (see above), IFNs influence also the function of the immune system by activating the lymphokine-cascade and subsequently the macrophages, NK- and Tc-cells. The activation of resting macrophages is accompanied by reduction of the mannose-fucosyl receptor and lysozyme level and, accumulation of S-adenoxylmethionine in cells as well as high superoxide release and, Ia (DR) expression (Ezekowitz, 1985; Warfel and Zucker-Franklin, 1986; Bonvini *et al.*, 1986). The enhancing effect of IFN-γ on the cytotoxicity of monocytes (macrophages) and killing by NK-cells seems to be mediated through the "tumor necrosis factor" (TNF) (and possibly free radicals) induced by IFN-γ in cells, while IFN-α may not require such a mediator (Epstein and Philip, 1986). Interleukin-2 (IL-2) plays the role of a cofactor in IFN production and, subsequently, in macrophage and NK-cell activation. On the other side, macrophages may inhibit NK-cell activity through release of IFN-stimulated prostaglandin E (Das *et al.*, 1986). The IFNs are also involved in driving the maturation of resting B-cells to active and enhanced immunoglobulin synthesis through expression of HLA-I and/or HLA-II antigens and alteration of cell membrane on cells (Kirchner, 1986).

Participation on differentiation of cells is an important but less understood function of IFN. Finding of 2',5' oligoA-synthetase and small amounts of IFN in murine myeloid M1 cells induced to differentiate into mature phagocytic cells, however, support the role of IFN in differentiation (Sokawa *et al.*, 1981). Also, in U 937 human cell line stimulated to differentiate with phorbol myristate acetate (PMA), an increase in mRNA hybridizing with an IFN-β probe was found indicating the production of IFN-β during differentiation and its possible participation on this process. In addition, while the differentiation was accompanied by rapid expression of *c-myc* (in mice of *c-fos*) oncogene, IFN depressed the level of oncogenes and, presumably, exerts a feedback regulating effect on this process (Vilček, 1987; Resnitsky *et al.*, 1986; Sariban *et al.*, 1987). The biological effects of IFN may be dose

Table 3. ENDOGENOUS INDUCERS OF IFN

INDUCER	IFN-TYPE	SOURCE OF IFN
IL-1	β	DIPLOID FIBROBLASTS
IL-2	γ	T-LYMPHOCYTES
IL-2	α/β	STEM-CELLS
TNF	β	DIPLOID FIBROBLASTS
H_2O_2	γ	NK-CELLS
CSF-1	β	MACROPHAGES

dependent. Very small ("physiological") doses of IFN in normal cells or, usual doses in leukemic cells had eventuelly a cell growth stimulating effect (Brinchkerhoff and Urye, 1985; Robert *et al.*, 1986). Until recently, however, not sufficient attention was paid to this activity.

For obvious reasons, more studies deal with the cell growth inhibitory (AP) effect of IFNs. The regulatory (inhibiting) role of IFN-β in cell growth found support in studies where the stimulating effect of mitogens was significantly enhanced after addition of anti-IFN-β serum and/or dexamethason (also an IFN inhibitor) to cells (Vilček, 1987; Barrack and Hollenberg, 1981; Arya *et al.*, 1984). It has been also shown that IFN production may participate on the cellular effects of various cytokines. Such are CSF-1 (colony stimulating factor-1), IL-1 (interleukin-1), poly I : C, TNF (tumor necrosis factor), and possibly PDGF (platelet derived growth factor) which function as growth stimulators in normal cells. They were found to induce in cells IFN production which served then as a feedback inhibitor of growth (Vilček *et al.*, 1987). This assumption is supported by the finding that such cell growth stimulators may have also an antiviral effect which was, most probably, mediated through IFN (Table 3).

Other growth factors, such as epidermal growth factor (EGF), fibroblast growth factor (FGF) or platelet derived growth factor (PDGF) may be antagonistic to IFN (Inglot *et al.*, 1980). The dual role of some of them (TNF, PDGF etc) may be determined by character of the cell (normal or transformed) as well as by concentration of the growth factor. Also "classical" hormones may both inhibit and/or augment IFN production and action. Such are the glucocorticoids which besides of IFN, antagonize also the activity of other lymphokines (IL-2) (Kelso and Munck, 1984) and β-endorphins which synergistically increase the activity of NK-cells and production of IFNs (Mandler *et al.*, 1986). In addition to such "natural" regulators, results of some investigations suggest that also exogenous substances such as antibiotics (adriamycin) and, possibly, cytostatics may modulate IFN activity in the organism (Yarvin *et al.*, 1986).

These findings are complicated by identifying β-endorphin and corticotropin activity in IFN-α preparations (Smith and Blalock, 1981), and a release of neopterin (a cofactor in neurotransmitter synthesis) from macrophages treated with IFN-γ (Huber *et al.*, 1984).

The growth regulatory function of IFN follows also from the observation that IFN depressed the stimulatory effect of tumor necrosis factor (TNF) and interleukin-1 (IL-1) on *c-fos* and *c-myc* mRNA levels in human fibroblasts preparing for mitoses (Lin and Vilček, 1987). From these reports, it can be concluded that an interconnection between the IFN and other lymphokine and hormon producing systems becomes increasingly evident. Further, it is a long experience that the AP effect of IFN is reversible and seems to require a functioning cytoskeleton in the cell (Chany, 1987). The reversibility of IFN action may explain the observed transitory therapeutic effects of IFN in patients with papillomatosis, condylomas etc as well as the necessity of prolonged treatment to avoid remissions (Quesada and Gutterman, 1987). Finally, high doses of IFNs may reverse such low dose effects of IFNs as is intertypic synergism (Justesen and Berg, 1986). These findings support the view that lymphokines exert their activities in a dose dependent way.

Interferon may have a dual role also in inflammation. IFNs-α and β were reported as antiinflammatory agents in experiments where carrageenan was used as exogenous inflammatory agent in mice (Mécs *et al.*, 1984; Billiau, 1988). On the other hand, Billiau found evidence that IFN-γ may function as one of proinflammatory cytokines through stimulating interleukin-1 (IL-1) and prostaglandin E production (Karmazyn *et al.*, 1977). Interesting is Billiau's suggestion that IFN-γ may trigger the Shwartzman-Sanarelli reaction in man (Billiau, 1988). This is supported by observation that the antiviral activity of IFN can be inhibited by antiinflammatory agents such as corticosteroids, aspirin or indomethacin. The dual role of IFN in inflammation needs further elucidation. However, several reports on the beneficial effect of higher doses of IFN-α in rheumatic fever received, in this way, a rational basis (Obert, 1987).

Under certain circumstances, IFNs may exert an antimicrobial effect in cells infected with intracellular microbes and/or parazites (Remington and Merigan, 1968). This has found recently an explanation by showing that highly purified or recombinant IFN-γ has a selective and specific tryptophan reducing effect in cultures of cells infected with *Toxoplasma gondii* and/or *Chlamydia psittaci* or *Chlamydia trachomatis*. In addition, it has been shown that IFN exerts *in vivo* also a plasma cholesterol reducing effect most probably mediated through inhibition of a lipoprotein-lipase or heparin-releasable lipase. While such metabolic alterations were observed after administration of IFN-α, they could not be found after IFN-β application (Borden *et al.*, 1987). The participation of IFN in the lipid metabolism is a "new" activity of IFN (see above) which needs further exploration (Das *et al.*, 1986).

INTERFERONS AS THERAPEUTIC AGENTS

As follows from previous chapters, various activities of IFNs allow to consider it as a cell regulator rather than a new type of antiviral or antineoplastic agents. Nevertheless, IFN remains a candidate for therapeutic exploitation in various infectious diseases as well as neoplastic and immune

disorders. However, as with other cell regulators of this type called immunologic (biologic) response modifiers (IRM), the therapeutic expectations by employing IFNs, with some exceptions, were not yet fulfilled. A major difficulty which limits presently the use of IFNs and other IRM in therapy is the complexity of the immune network in animal organism where positive and negative effects of IRM may appear side by side and lead often to a perturbed function of the immune system (Fauci *et al.*, 1987). These difficulties can be overcome only through a better understanding and monitoring of the functioning of the immune system in man.

Controlled clinical studies in cancer patients with IFN were made possible by 1981 when sufficient amounts of recombinant IFN-α became available. These studies established that osteosarcoma, renal cell carcinoma and Kaposi's sarcoma do respond to IFN-α (Quesada *et al.*, 1983; Krown and Real, 1983; Strander *et al.*, 1982). However, the most inpressive therapeutic results were achieved, and later broadly confirmed, with using IFN-α for therapy of hairy cell leukaemia, a rare B-cell disorder for which no effective therapy existed previously (Quesada *et al.*, 1984). More than 100 patients with hairy cell leukaemia were treated with IFN-α up to now with encouraging results. Impressive results were meantime achieved also using IFN for therapy of chronic myelocytic leukaemia (Talpaz *et al.*, 1983). Both of these neoplastic diseases show a high (about 90 %) responsiveness to IFN administration and, after a sufficiently long lasting IFN treatment, a significant improvement. Also other neoplastic diseases proved sensitive to IFN therapy although not in an extent observed in hairy cell and/or myelocytic leukaemia (Table 4). To these belong the B-cell malignancies treated in early phases of disease and without previous therapy (Quesada and Gutterman, 1987). Such malignant B-cells usually respond also to growth factors suggesting that differentiation induced with IFN-α might be the mechanism of curative effect. An other effect of IFN-α in hairy cell leukaemia was the repair of platelet defect which leads to myelofibrosis (Duply *et al.*, 1987). Finally a direct antiproliferative effect of IFN-α on hairy cells cannot be excluded either. In hairy cell leukaemia, the level of IL-2 receptors in the blood of patients shows a correlation with response to IFN-α therapy (Reuben *et al.*, 1986). In the case of solid tumours, however, the therapeutic success of IFN therapy is less impressive.

Of interest are some new therapeutic fields where IFN seems potentially effective. Such is the theumatoid arthritis (see above) where Obert (1987) reported marked improvement in-about 50 % of 111 patients treated with IFN-α for 12 months.

Sone *et al.* (1986) used recently IFN-α for treatment of subacute sclerosing panencephalitis in a young boy with marked improvement, although the disease did not respond to any other kind of therapy.

IFN remains a hopeful remedy for chronic or recurrent viral diseases. The effectivity of IFN therapy in *condyloma acuminata* was, after 13 week 36 % to 7 % in placebo treated group (Eron *et al.*, 1986) and 91 % of patients with genital herpes treated with IFN-α2 were cured in 6 days, vs. 8 days in placebo group (Kuhls *et al.*, 1986). Since such patients are at high risk for

Table 4. PRESENT STAGE OF EFFECTIVITY OF THE IFN THERAPY

I.	HAIRY CELL LEUKAEMIA	UP TO 90 %
	CHRONIC MYELOCYTIC LEUKAEMIA	UP TO 90 %
	HATL	UP TO 42 %
	NON-HODGKIN LYMPHOMA	UP TO 35 %
	CARCINOMA OF PANCREAS	UP TO 30 %
	MYELOMA MULTIPLEX[1]	11–50 %
	MELANOMA[2]	3–20 %
	RENAL CARCINOMA[3]	25 %
	PAPILLOMAS OF VARIOUS LOCATIONS	11–50 %
II.	VIRAL COMPLICATIONS (HERPES, VARICELLA ETC) OF CHRONIC DISEASES (LEUKAEMIA)	
III.	AIDS: (TEMPORARY IMMUNOCORRECTION)	30 %
	KAPOSI'S SARCOMA	40 %

* Based on data of Quesada and Gutterman (1987)
1. Refracterness in about 11 % of cases
2. Depends on the stage of disease
3. Depends on doses

recurrences or visceral dissemination and encephalitis, they may benefit from prophylactic IFN administration also.

Some diseases need further studies for establishing an optimal administration schedule for IFN. Such is AIDS, where 38 % of patients with Kaposi s sarcoma responded to IFN-α with a median duration of 18 months when high doses of IFN-α were applied (30×10^6 units), and only 3 % responded when the dose of recombinant IFN-αA was low. The escalation of low dose IFN to 36×10^6 units resulted in an additional major response rate of 17 % (Real *et al.*, 1986). Four out of 11 patients, who achieved a complete response, remained free of disease when reported, while all partial responders have shown disease progression (Ganser *et al.*, 1986).

An interesting modality of IFN therapy might be its induction directly in patients body using for this IL-2 injections. Merigan (1987) hopes to achieve, in this way, production of IFN-γ and mobilization of T-cells as well as other lymphokines for an effective prophylaxis of recurrent herpes simplex.

Table 5. MECHANISMS OF THE THERAPEUTIC EFFECTS OF IFNs ON CELLS

1. DIRECT:	– ANTIPROLIFERATIVE EFFECT
	– ANTIVIRAL EFFECT
	– INCREASED DIFFERENTIATION
	– REVERSED CELL PHENOTYPE
2. INDIRECT: STIMULATION OF HOST RESISTANCE VIA:	
	– LYMPHOKINE CASCADE
	– NK, LAK-CELL, MACROPHAGE ACTIVATION
	– ENHANCED CELL ANTIGEN EXPRESSION

A prospective way for use of IFN in the future seems to be also its combination with synergistic IRMs such as IL-2, thymic hormones etc.

Although the spectrum of diseases which are candidates for IFN therapy seems broad and still growing (rheumatic fever etc), the problems associated with effective use of IFN in man are open and common to all IRM. They include dosage and timing schedules, mode of application, evaluation of immunological status of patients, monitoring of effectivity and toxicity of IRM in the organism etc. It should be kept in mind that IFN (and IRM) therapy differs from other types of treatment by activating interconnected immunological factors (hormones, lymphokines etc) with direct pleiotypic effects in the organism. With such agents, without a better insight into patients immune status possible complications cannot be safely anticipated. (Table 5).

CAN IFN BE DELETERIOUS?

Gresser et al. (1976) were among the first who turned attention to possible "toxic" effects of IFN in animal organism. Large doses of a mouse IFN-α/β preparation administered by consecutive daily injections into newborn mice caused a progressive growth retardation and finally the death of animal. When the administration of IFN was stopped, the animals recovered. Even more surprisingly, this group of investigators (Riviere *et al.*, 1977) effectively mitigated the LCM virus induced autoimmune disease in mice when a potent anti-IFN-α/β serum was administered to mice concurrently with the LCM virus at birth. The disease was mitigated despite of the fact that the virus titre rose in organs of infected mice. This observation strongly suggested that IFN itself was responsible for many (if not all) symptoms of chronic LCM-disease.

Meantime several authors reported on the presence of an unusual termo- and/or acid-labile type of IFN in various autoimmune diseases of man such as SLE etc. (See above). About at the same time, Skurkovich and Eremkina (1975) reported successful treatment of patients with various autoimmune and allergic disorders using anti-IFN-α serum and, recently, Skurkovich *et al.* (1987) presented data on improvement of haematological findings in 4 patients with AIDS treated with a polyclonal anti-IFN-α serum. Several activities of IFN produced in autoimmune disorders may contribute to development of the disease. Enhanced HLA-antigen expression on treated cells together with release of antigenic constituents from leukocytes damaged by IFN and otherwise, may result in triggering and/or enhancing already stimulated autoantibody production which may then lead to production of immune complexes and damage various organs, as observed in SLE, AIDS, chronic B-hepatitis (Schaff *et al.*, 1986). IFN — when present continuously in the blood — may inhibit macrophages, Tc, LAK and NK-cells activity and lead to release of IL-1 and IL-2 (Borecký *et al.*, 1987). The accelerating effect of IFN in autoimmune disorders was manifested in IFN-treated NZB/W mice both by onset and severity of the autoimmune disease (Borecký *et al.*,

1981 etc). Recently, it has been reported and confirmed by observations of others that IFN-α may cause an exacerbation of psoriasis (Quesada, 1986). Also, IFN-γ was proposed as the "match that lights the fire" in Shwartzmann-Sanarelli phenomenon (Billiau, 1988 see above).

An aggravating effect of IFN is suspected also in the symptomatology of demyelinating diseases such as multiple sclerosis. This follows from the observation that IFN-γ enhanced the HLA-DR antigen expression on astrocytes which subsequently were destroyed by specifically sensitized T-lymphocytes (Fontana *et al.*, 1984).

Based on its structural and antigenic relatedness to β-endorphins and corticotropin (Blalock and Smith, 1985), the suggestion that IFN may participate on symptoms of psychiatric diseases predominantly as a result of its neurological and psychological side effects during long-term administration to patients is of obvious interest. Such side effects may be potentiated by lymphokines which were released from lymphocytes after β-endorphin treatment both in vitro and in vivo (Wolf and Peterons, 1986). These discoveries support the existence of a circuit between the immune and neuroendocrine system and may open new fields for IFN therapy.

Finally, numerous results of local application of IFN for prevention and/or treatment of rhinovirus and other respiratory infections suggested the possibility that many of flu-like symptoms appearing in influenza and other infectious diseases may be caused by concurrently produced IFN-α (Monto *et al.*, 1986).

CONCLUDING REMARKS

30 years ago, IFN started its existence as an autonomous antiviral agent which the vertebrate (?) organism evolved during evolution to protect itself from intracellular infectious genetic entities — viruses. This definition after the 70-ies was abandoned when the structure of IFN has been elucidated and its relationship to other polypeptide cell regulators was established. Nowadays, IFN competes with thymus-factors for the position of the "master lymphokine" in a rather complex network of polypeptide lymphokines that seem to regulate the growth, differentiation and normal functioning of cells in the organism. IFN is not autonomous anymore. Its release is, as a rule, accompanied by the release of various antagonistic and/or synergistic lymphokines and growth factors. It is involved in their activity often as a feedback inhibitor. IFN becomes an established remedy in some diseases such as hairy cell leukaemia, but we must more learn how to apply it properly to man and animals, It shows interconnections with some classical hormones such as β-endorphins, glucocorticoids, insulin and others.

IFN is not completely innocent. It is an emergency factor which appears among the first when the body needs it but its continuous presence in the circulation may be deleterious (as in SLE). IFN excites still a non diminishing number of scientists because it is a fascinating product of the Nature.

References

Abb, J. (1985): *Med. Microbiol. Immunol.* **174**, 205—211.
Ackerman, S. K., Zur Nedden, D., Heintzelman, M., Hunkapiller, M., Zoon, K. (1984): *Proc. natn. Acad. Sci. U.S.A.* **81**, 1045—1047.
Adolf, G. R. (1987): *J. Interferon Res.* **7**, 758.
Arnheiter, H., Ohno, M., Smith, M., Gutte, B., Zoon, K. C. (1983): *Proc. natn. Acad. Sci.* U.S.A. **80**, 2539—2543.
Arnheiter, H., Thomas, R. M., Leist, T., Fountoulakis, M., Gutte, B. (1981): *Nature* **294**, 278—280.
Arya, S. K., Wong-Staal, F., Gallo, R. C. (1984): *J. Immunol.* **133**, 273—276.
Ashley, P. L., Cox, D. R. (1987): *Gene Workshop* **9** (in press)
Barrack, E. R., Hollenberg, M. D. (1981): *J. Cell Physiol.* **108**, 445—454.
Belardelli, F., Gessani, S., Borghi, P., Proietti, E., Boraschi, D., Kawade, Y., Gresser, I. (1986): *J. Interferon Res.* **6**, 10.
Billard, C., Sigaux, F., Castaigne, S., Valensi, F., Flandrin, G., Degos, L., Falcoff, E., Aguet, M. (1986): *Blood* **67**, 821—826.
Billiau, A. (1988): *Immunology Today* **8**, 37—40.
Blach-Olszewska, Z., Cambrzynska-Nowak, M. (1979): *Acta med. biol. Germ.* **38**, 765—773.
Blalock, J. E., Georgiades, J. A., Langford, M. P., Johnson, H. M. (1980): *Cell Immunol.* **49** 390—394.
Blalock, J. E., Smith, E. M. (1985): *Immunology Today*, **6** 115.
Bocci, V. (1982): *Surv. Immunol. Res.* **1**, 137—143.
Bocci, V. (1987): *Pharmac. Ther.* **34**, 1—49.
Borden, E. C., Rosenzwieg, I. B., Byrne, G. I. (1987): *J. Interferon Res.* **7**, 591—596.
Borecký, L., Kontsek, P., Novák, M., Lackovič, V. (1989): *Acta virol.* (in press).
Borecký, L. Lackovič, V., Čiampor, F., Rovenský, J., Ujházy, V., Maťoška, J., Švec, J., Poverennyj, A. M. (1981): *Acta virol.* **25**, 283—295.
Borecký, L., Novák, M., Kontsek, P.: VI. International Congress of Virology, Sendai, Japan, 1.—5. 9. 1984.
Borecký, L., Lackovič, V., Marcinka, K., Rovenský, J. (1986): In: B. Filipič (Ed.): *Yougoslave Colloqium on Interferon.* Slovenian Microbiol. Society, Ljublnaja.
Borecký, L., Lackovič, V., Mistríková, J., Tomovič, J., Koči ková, D., Rovenský, J., Žitňan, D. (1987): *J. Interferon Res.* **7**, 798.
Brinchekerhoff, C. E., Urye, P. M. (1985): *J. Immunol.* **134**, 3142—3146.
Brunda, M. J., Tarnowski, D., Davatelis, V. (1986): *Int. J. Cancer*, **37**, 787—793.
Carvalho, E. M., Badaro, R., Reed, S. C., (1985): *J. clin. Invest.* **70**, 2066—2069.
Chadha, K. C. (1985): Serono Symposia Publications from Raven Press, **24**, 35—41.
Chany, Ch. (1987): *J. Interferon Res.* **7**, 569—574.
Das, U. N., Huang, Y. S., Begin, M. E., Horrobin, D. F. (1986): *Science* **14**, 1069—1074.
Defilippi. P., Huez, G., Verhaegen Lewalle (1986): *Febs. Lett.* **198**, 326—332.
Derynck, R., Content, J., De Clercq, E., Volckaert, G. (1980): *Nature* **285**, 542—547.
Duply, E., Sigaux, F., Bryckaert, M. C. (1987): *J. Haematol.* **65**, 107—110.
Epstein, L. B. (1987): *J. Interferon Res.* **7**, 487—495.
Epstein, L. B., Philip, R. (1986): UCLA *Symp. Mol. Cell. Biol.* **50**, 197—208.
Eron, L. J., Judson, F., Tucker, S., (1986): *New Engl. J. Med.* **315**, 1059—1064.
Ezekowitz, R. A. B. (1985): *Immunol. Lett.* **11**, 135—140.
Fauci, A. S., Rosenberg, S. A., Sherwin, S. A. (1987): *Ann. Intern. Med.* **106**, 421—433.
Feng, G. S., Taylor, M. W., Shepart, M., Gray, P. (1987): *J. Interferon Res.* **7**, 702.
Finter, N. B. (1987): *J. Interferon Res.* **7**, 497—500.
Fontana, A., Fiers, W., Wekerle, H. (1984): **307**, 273—276.
Fowler, A. K., Reed, C. D., Giron, D. J. (1980): *Nature*, **286**, 266—267.
Funa, K., Gazdar, A. F., Mattson, K. (1986): *Clin. Immunol. Immunopathol.* **41**, 159—164.
Ganser, A., Bruchor, W., Brodt, H. R. (1986): *Onkologie* (*Basel*), **9**, 163—166.
Garotta, G., Ozman, L., Dobeli, H., Gentz, R., Le Grice, S., W. Bannwarth, W., Hochuli, E., Talmadge, K. (1987): *J. Interferon Res.* **7**, 685.
Gray, P. W., Goeddel, D. V. (1983): *Proc. natn. Acad. Sci. U.S.A.* **80**, 5842—5846.
Gresser, I., Tovey, M. G., Bandu, M. T., Maury, C., Brouty-Boyé, D. (1976): *J. exp. Med.* **144**, 1305—1315.

Hooks, J. J., Moutsopoulos, H. M., Geis, S. A., Stahl, N. I., Decker, J. L., Notkins, A. L. (1979): *New Engl. J. Med.* **301**, 5—8.
Hovanessian, A. G., Meurs, E., Aujean, O., Vaquero, C., Stefanos, S., Falcoff, E. (1980): *Virology*, **104**, 195—204.
Huber, C., Batchelor, J. R., Fuchs, D. (1984): *J. exp. Med.* **160**, 310—316.
Hughes, T. K., Baron, S. (1987): *J. Interferon Res.* **7**, 603—614.
Huygen, K., Zhe-Xi, Z., De Clercq, E. (1983): *Proc. Soc. exp. Biol. Med.* **172**, 260—264.
Ikeda, T., Lever, A. M. L., Thomas, H. C. (1986): *Hepatology*, **6**, 962—969.
Inglot, A. D., Oleszak, E., Kisielow, B. (1980): *Arch. Virol.* 292—296.
Isaacs, A., Lindenmann, J. (1957): *J. Interferon Res.* **7**, 429—438.
Justesen, J., Berg, K. (1986): *J. Interferon Res.* **6**, 445—454.
Karmazyn, M., Horrobin, D. F., Manku, M. S. (1977): *Lancet*, **2**, 307.
Kelso, A., Munck, A. (1984): *J. Immunol.* **133**, 784—791.
Kirchner, H. (1986): *Antiviral Res.* **6**, 1—7.
Kontsek, P., Borecký, L., Lackovič, V., Kontseková, E., Šujanová, Z., Novák, M. (1989): *Acta virol.* **33**, 254—261.
Krown, S. E., Real, F. X. (1983): *N. Engl. J. Med.* **308**, 1071—1076.
Kuhls, T. L., Sacher, J., Pineda, E. (1986): *J. infect. Dis.* 437—442.
Lebon, P., Daffos, F., Checoury, A. (1985): *J. clin. Microbiol.* **21**, 775—778.
Lengyel, P. (1987): *J. Inferon Res.* **7**, 511—519.
Lin, J. X., Vilček, J. (1987): *J. Biol. Chemistry* **262**, 11908—11911.
Lovett, M., Goldgaber, D., Ashley, P., Cox, D. R., Gajdusek, D. C. Epstein, C. J. (1987): *Biochem. Biophys. Res. Commun.* **144**, 1069—1076.
Mandler, R. N., Biddion, W. E., Mandler, R., Serrate, S. A. (1986): *J. Immunol.* **136**, 934—939.
Mao, C., Ballotti, R., Aguet, M., Falcoff, E., Merlin, G. (1987): *J. Interferon Res.* **7**, 765.
Mark, D. F., Lu, S. D., Creasey, A. A. (1984): *Proc. natn. Acad. Sci. U.S.A.* **81**, 5662—5666.
Matsuoka, H., Kakui, Y., Tanaka, A. (1985): *J. gen. Virol.* **66**, 2491—2494.
Mécs. I., Koltai, M., Tóth, S., Agoston, E. (1984): In L. Borecký, V. Lackovič (Eds): *Physiology and Pathology of Interferon System.* Karger, Basel.
Merigan, T. C. (1987): *J. Interferon Res.* **7**, 635—639.
Monto, A. S., Shope, T. C., Schwartz, S. A., Albrecht, J. K. (1986): *J. infecti, Dis.* **154**, 128—133.
Morre, E. E., Jones, C., Kao, P. T., Dates, D. (1977): *Am. J. hum. Genet.* **29**, 389—396.
Nagata, S., Mantei, N., Weissman, Ch. (1980): *Nature*, **287**, 401—408.
Nishimura, J., Mitsui, K., Tanak, Y. (1986): *Clin. exp. Metastasis* **4**, 35—44.
Obert, H. J. (1987): *J. Interferon Res.* **7**, 696.
Okabe, M., Minagawa, T., Nakane, A. (1986): *Scand, J. Haematol.* **37**, 111—117.
Paganelli, R., Capobianchi, M. R., Matricardi, P. M., Cioe, L., Seminara, R., Dianzani, F., Aiuti, F. (1984): *Clin. Immunol. Immunopathol.* **32**, 387—391.
Pestka, S., Langer, J. A. (1987): *Ann. Rev. Biochem.* **56**, 727—777.
Preble, O. T., Fook, A. H., Quinnan, G. V., Vilček, J., Friedman, R. M., Steis, R., Gelmann, E. P., Sonnabend, J. A. (198): *Annals New York Academy of Sciences*, 65—75.
Quesada, J. R. (1986): *Lancet*, **1**, 1466—1468.
Quesada, J. R., Gutterman, J. U. (1987): *J. Interferon Res.* 575—581.
Quesada, J. R., Reuben, J., Manning, J. T., Hersh, E. M., Gutterman, J. U. (1984): *N. Engl. J. Med.* **310** 15—18.
Quesada, J. R., Swanson, D. A., Trindade, A., Gutterman, J. U. (1983): *Cancer Res.* **43**, 940—947.
Real, P. X., Oettgen, M. F., Krown, S. E. (1986): *J. clin. Oncol.* **4**, 544—551.
Remington, J. S., Merigan, T. C. (1968): *Science*, **161**, 804—806.
Renton, K. W., Singh, G., Stebbing, N. (1984): *Biochem. Pharmacol.* **33**, 3899—3902.
Resnitsky, D., Yarden, A., Zipori, D., Kimchi, A. (1986): *Cell* **46**, 31—40.
Reuben, J., Ip, S., Quesada, J. R. (1986): *Blood* **68** Suppl. 1, 231a.
Rhodes-Feuilletter, A., Druilhe, P., Canivet, M., Gentilini, M., Peries, J. (1981): *CR Acad. Sci. Paris*, **293**, 635—637.
Rich, S. A. (1981): *Science* **213**, 772—775.
Riviere, Y., Gresser, I., Guillon, J. C., Tovey, M. G. (1977): *Proc. natn. Acad. Sci. U.S.A.* **74**, 2135—2193.
Rosenbach, T. O., Moshonov, S., Zor, U., Yaron, M. (1984): *Clin. Rheumatol.* **3**, 361—364.
Rubin, B. Y., Gupta, S. L. (1980): *J. Virol.* **34**, 446—454.

Rubinstein, M. (1987): *J. Interferon Res.* **7**, 545—551.
Sariban, E., Mitchell, T., Griffin, J., Kufe, D. W. (1987): *J. Immunol.* **136**, 1954—1958.
Schaff, Z., Hoofnagle, J. H., Grimley, P. M. (1986): *Hepatology* **6**, 966—970.
Schwarzmeier, J. D., Shwabe, M. (1987): *J. Interferon Res.* **7**, 760.
Shearer, M., Taylor-Papadimitriou, J. (1987): *J. Interferon. Res.* **7**, 760.
Sibbitt, W. L., Mayhews, P. M., Bankhurts, A. D. (1983): *J. clin. Invest.* **71**, 1230—1239.
Skurkovich, S. V., Eremkina, E. I. (1975): *Ann. Allergy* **35**, 356—360.
Skurkovich, S. V., Skurkovich, B., Bellanti, J. A. (1987): Clin. *Immunol. Immunopathol.* **43**, 362—373.
Smith, E. M., Blalock, J. E. (1981): *Proc. natn. Acad. Sci. U.S.A.* **78**, 7530—7534.
Smith, T. J., Wagner, R. R. (1967): *J. exp. Med.* **125**, 559—577.
Sokawa, Y., Nagata, K., Ichikawa, Y. (1981): *Exp. Cell. Res.* **135**, 191—197.
Sone, S., Maeda, K., Haginoya, K. (1986): *Brain Develop.* **8**, 636—640.
Strander, H., Aparisi, T., Blomgren, H., Brostrom, L. A., Cantell, K., Einhorn, S., Ingimarsson, S., Nilsonne, U., Soderberg, G. (1982): *Rec. Results Cancer Res.* **80**, 103—107.
Talpaz, M., McCredie, K. B., Mavligit, G. M. (1983): *Blood* **62**, 689—692.
Tan, Y. H., Tischfield, J., Ruddle, F. H. (1973): *J. exp. Med.* **137**, 317—330.
Taniguchi, T., Mantei, N., Schwarzstein, N., Nagata, S., Muramatsu, I., Weissmann, Ch. (1980): *Nature* **285**, 547—549.
Tovey, M. G., Gresser, I. (1985): International Symposium on the Interferon, Rome, Program and Abstracts, pp. 3.
Tovo, P. A., Ceritti, F., Palomba, E. (1984): *Acta Paediatr. Scand.* **73**, 785—788.
Trotta, P. P., Nagabhushan, T. L. (1985): In F. Dianzani, G. B. Rossi (Eds): *The Interferon System.* Serono Symposia Publications from Raven Press, 24, pp 231—235.
Ucer, U., Wiegmann, K., Scheurich, P., Kronke, M., Aguet, M., Hubner, K., Knowles, B. B., Pfizenmaier, K. (1987): *J. Interferon Res.* **7**, 764.
Valenzuela, D., Weber, H., Weissmann, C. (1985): *Nature*, **313**, 698—700.
Vilček, J. (1987): *J. Interferon Res.* **7**, 537—543.
Vilček, J., Kelker, H. C., Le, J., Yip, Y. K. (1985): *Raven Press*, pp 299—313.
Vilček, J., Kohase, M., Henrikesen-Destefano, D. (1987): *J. Cell Physiol.* **130**, 37—43.
Warfel, A. H., Zucker-Franklin, D. (1986): *J. Immunol.* **137**, 651—655.
Weinberg, J. B., Hobbs, M. M., Misukonis, M. A. (1985): *Blood* **66**, 1241—1246.
Weissmann, Ch., Weber, H. (1987): *Prog. Nucleic Acid Res. Mol. Biol.* **33**, 251—300.
Whitaker-Dowling, P., Youngner, J. S. (1986): *Virology* **152**, 50—57.
Wolf, G. T., Peterson, K. A. (1986): *Otolaringol. Head Neck Surg.* **94**, 224—229.
Yarvin, M., Tkaczore, H. R., Chadha, K. C. (1986): *Antiviral Res.* **6**, 113—122.
H. [9]Yoshie, D., Aso, H., Sakakibara, A., Ishida, N. (1985): *J. Interferon Res.* **5**, 531—540.
Ytterberg, S. R., Schnitzer, T. J. (1982): *Arthritis Rheum.* **25**, 401—406.
Zwarhoff, E. C., Gennissen, A. M. C., Van As, K., Van Heuvel, M., Bosveld, I. J., Trapman, J. (1987): *J. Interferon Res.* **7**, 757.