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Interferon-α: a gene family in therapeutic use*

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Abstract: Several variants of interferon-α (IFN-α) were isolated and purified to homogeneity. They differed to various degrees in biological properties. However, three IFN-α2 variants showed only minor differences from a variant called IFN-α88 with regard to their ability to inhibit growth and to bind to specific receptors, tested on Daudi cells. Two monoclonal antibodies were studied, which showed overlapping specificity for at least one peptide obtained after HPLC separation of tryptic digests. The monoclonal antibodies could discriminate between sequence differences to a much higher degree than the receptor on Daudi cells. It is concluded that the receptor is degenerate and binds well to different structural variants of IFN and that for therapeutic use, several of the variants will probably have the same biological potency.

Keywords: *Interferon-α; epitope; receptor binding; monoclonal antibodies.*

Introduction

Interferons (IFN) constitute a number of proteins, which share the property of inhibiting replication of a large variety of viruses. Several other functions are known, of which cell growth inhibition has attracted interest for its therapeutic potential in tumour disease. Based on serological criteria, comparisons of sequences and studies on receptor binding they have been categorised as IFN-α, IFN-β and IFN-γ. IFN-α and IFN-β have a 30% homology at the amino acid level and share a cell surface receptor, while IFN-γ has a separate receptor.

IFN-α has been extensively studied as a therapeutic agent in human disease and several IFN-α proteins have been registered in different countries. However, a large number of variants exist. By direct physical mapping 18 loci have been defined [1] and studies of restriction fragment length polymorphisms has given an estimate of 17 loci per haploid chromosome [2]. More than 40 different genes have been isolated from cDNA or genomic libraries differing with between 1 and more than 20 amino acids of the 165/166 in the mature protein [3].

A considerable genetic variation has therefore been inferred. The therapeutic use of IFN-α will therefore involve some obvious problems. Are the allelic variants functionally equal? Do individuals respond in the same way to different IFN proteins? Will the use of

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a specific variant have immunological consequences in individuals with a different genotype?

To start to address these and related questions, the authors have studied several variants of IFN-α either from natural sources, or by recombinant DNA technology. Only minor consequences were observed concerning the ability of IFN-α variants to inhibit cell growth, whereas the abilities of monoclonal antibodies to recognise variations in amino acid sequences were found to be dramatically different.

Materials and Methods

Preparation of leukocyte IFN followed the Cantell procedure [4] with minor modifications. The subtypes were separated by an immune affinity step, followed by separation on FPLC®, Mono S (Pharmacia AB, Uppsala, Sweden [4]).

Cloning, sequencing and expression in *Escherichia coli* have been described [5, 6] as well as purification [2]. To ascertain purity and quantity of the IFN-α subtypes produced in *E. coli*, after purification they were examined by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), and quantitated by densitometric scanning [2]. Only IFN preparations showing one homogenous band on silver-stained SDS-polycrylamide gels were used in the experiments. Purified IFN-α88 was for some experiments cleaved with trypsin at 37°C for 60 min. The resulting fragments were separated on reversed-phase HPLC.

Cell growth inhibition and receptor binding to Daudi cells were estimated as previously described [7].

Results

After human buffy coat leukocytes had been induced by Sendai virus, several molecular species of IFN-α could be identified. Figure 1 shows several bands by SDS-PAGE after separation by an immune affinity step and cation exchange chromatogaphy on Mono S. They differed in apparent molecular size ranging between 19 and 24 kDa. Some of them could be analysed separately and were compared with IFN-α variants produced by recombinant DNA-technology.

In this study four variants were produced in *E. coli* using a vector with a synthetic ribosome binding site [5]. The IFN-α2c gene was isolated from a cDNA library from Namalwa cells [6], while the other α2 variants (IFN-α2a and b) were derived from this

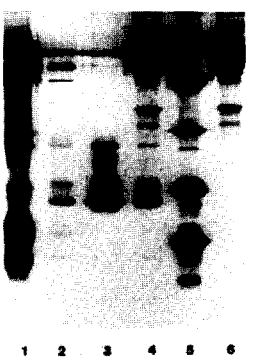


Figure 1
Purification of leukocyte IFN. Leukocyte IFN was prepared according to standard procedures and concentrated according to the procedure of Cantell [4]. (1) Crude IFN; (2) Eluate from antibody column; (3) pooled material after gel filtration; (4) purified material after addition of 1.5 mg ml^{-1} albumin; (5) molecular weight markers; and (6) albumin added to the final product.

variant by *in vitro* mutagenesis [2]. They were all produced in the same *E. coli* strain and the quantity was estimated by densitometric scanning.

Table 1 shows the results from assays on biological functions on Burkitt's lymphoma cell line Daudi. The IFN-α variants tested were all highly potent in growth inhibition. The 19 kDa IFN has at least 20-fold lower anti-viral activity than the others (not shown) and a corresponding lower ability to inhibit cell growth. The potency of the others fell within the same range, although with minor differences, e.g. between IFN-α2a and b versus α2c as previously described [2]. The differences in receptor binding are obvious. However, the IFN-α2 variants were of the same order of magnitude, and differed from IFN-α88 by a factor of five. Calculations of receptor occupancy [8] showed that only a minor fraction of the cellular binding sites were occupied to achieve 50% inhibition of cell growth. Thus, although some differences in biological effects and especially on receptor binding dynamics were observed, the biological dose–response relationship for the different IFN-α2 subtypes and IFN-α88 was in the same range.

To further characterise the different variants, three monoclonal antibodies were tested and compared with a goat antiserum. Table 2 summarises the results. Clearly the monoclonal antibodies were able to discriminate between the different types. The finding that antibody 3-A3-2 inhibited IFN-α88 but not IFN-α2, while the reverse was true for antibody 9-1-1, was independently confirmed by the finding of a similar ability of

Table 1
Biology properties of IFN-α variants

IFN-type	Growth inhibition*	kDa (pM)	Receptor occupancy (%)
19K (α1)	3.1	7600	0.04
24K	0.9	60	1.48
IFN-α2a	0.16	890	0.02
IFN-α2b	0.16	1060	0.02
IFN-α2c	0.45	1090	0.04
IFN-α88	0.25	190	0.13

*Assayed on Daudi cells; pM concentration, when thymidin uptake was reduced to 50% of control.

Table 2
Summary of neutralisation tests of different antibodies tested on IFN-α variants

Antibody	Type of interferon		
	leukoc.	α2	α88
mab 9-1-1	++	++	−
mab 2-2-1	(+)	++	(+)
mab 3-A3-2	(+)	−	++
Goat serum	++	++	++

Daudi cells were given graded doses of the IFN proteins used in the presence of graded doses of the antibodies. The range of doses of IFN:s were chosen to cover the range from total growth inhibition to no response. (++) indicates that complete neutralisation occurred in some of the IFN-doses used; (+) that only minor or partial neutralisation was achieved; and (−) that in none of the doses tested could neutralisation be observed.

the antibodies to compete with iodinated IFN-α2 and IFN-α88 to specifically bind to the cell surface binding sites.

This indicates that the antibodies could interfere with the receptor binding surface of these two IFN proteins. To explore this question, the molecules were linearised and digested with trypsin, as shown in Fig. 2. The 13 peptides (approximately) obtained after HPLC separation were identified by sequencing. It turned out that antibody 9-1-1 could upon immunoblotting bind to peptide T3 (positions 14–23), and to some extent peptides T4 (positions 24–31), T10 (positions 127–134) and T12 (positions 136–145). The sequences of these fragments are shown in Fig. 3.

When the IFN-α2 variants and IFN-α88 are compared these sequences differ in four or five positions in peptides T3 and T4, one in T10 while T12, linked to T4 by a disulphide bond, is identical.

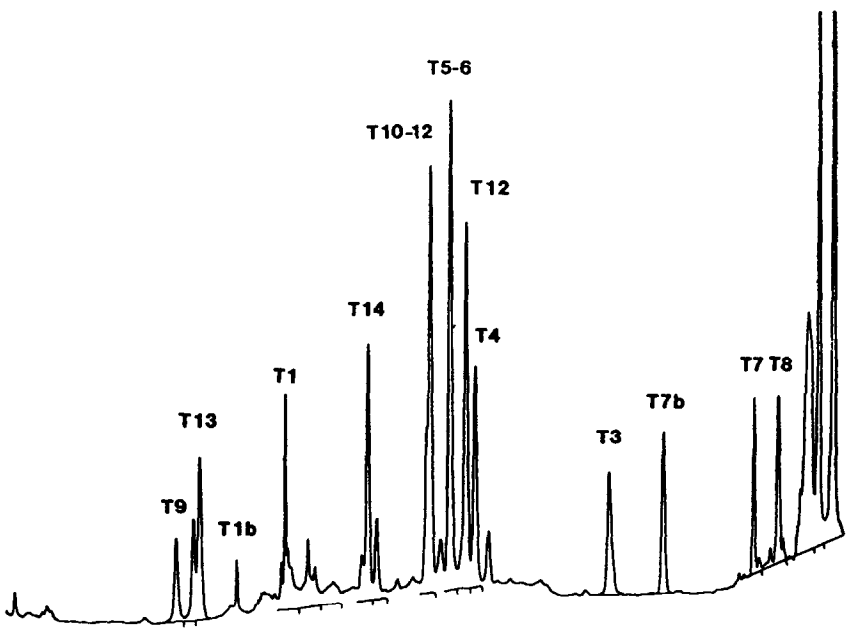
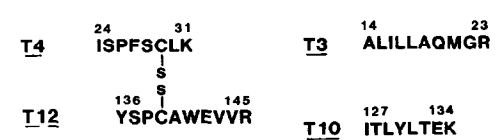


Figure 2
HPLC separation of peptide fragments obtained after tryptic digestion. IFN-α88 was purified to homogeneity and digested with trypsin at 37°C for 60 min. The fragments obtained were separated on a reversed-phase HPLC system, the different peaks collected and identified by partial sequencing. The identities of relevant fragments are given in the text.

Figure 3
Peptides identified by monoclonal antibody 9-1-1. The peptides were resolved by HPLC after trypsin digestion of IFN-α88 and identified by sequencing. The single-letter amino acid code is used. The IFN-α2 variants differ from IFN-α88 with a L in position 27 in peptide T4 and a K in position 132 in peptide T10. The peptide T3 in IFN-α2a and b has the sequence TLMLLAQMRK, while in IFN-α2c position 23 has R as IFN-α88.



Discussion

This paper reports that some IFN-α subtypes have different abilities to inhibit cell growth of Daudi cells. Thus, the 19 kDa protein, very similar to IFN-α1 in its anti-viral properties, was less potent than the others. One amino acid difference in position 34 between the IFN-α2 variants tested gave a five-fold difference in biological effect as previously pointed out [2]. IFN-α88, showing 30 to 31 amino acid differences from IFN-α2 had higher affinity to the binding sites, although the biological response was in the same range.

Two monoclonal antibodies studied might have the ability to interfere with what seems to be the receptor binding region of the IFN proteins. This is based on the finding that these antibodies competed with receptor binding of iodinated ligands.

One of the antibodies (9-1-1) could bind to small tryptic fragments of IFN-α88. Thus, four peptides comprising positions 14–23 (T3), 24–31 (T4), 127–134 (T10) and 136–145 (T12), could be identified with the antibody. The peptide comprising positions 14–23 gave the strongest signal and reacted also with the other antibody (3-A2-3). IFN-α2 and IFN-α88 differed from each other only in positions 14, 16, 22, 26 and 132 in these peptides. These four tryptic fragments are conserved in all the IFN-α subspecies, the latter two fragments having the highest degree of homology [1].

It is not yet known whether the epitope recognised by the monoclonal antibody 3-A3-2 overlaps with that of monoclonal antibody 9-1-1, rather we assume that also other parts of the molecule are involved in the epitopes. Thus, the antibodies seem to inhibit receptor binding of either IFN-α2 and IFN-α88, but both recognise peptide T3 from IFN-α88. It is the subject for ongoing studies to define whether amino acid differences in this fragment contribute to the epitope difference defined by the functional assay.

The differences between the sequenced IFN-α variants in the biological assays used were subtle, and mostly on the level of receptor binding. Obviously the differences between the ligands were more easily resolved by the monoclonal antibodies used than by the biological effects measured. It therefore seems likely that the binding site for IFN-α is degenerate allowing for wide structural differences between the ligands. Hence it is not unexpected that IFN-β with only about 30% homology on the amino acid level [9] binds to the same receptor. A similar degeneracy seems to be the case for IL-1α and β, which binds to the same receptor, although the homology is only 20% [10]. Thus, the structural differences might have small consequences for the biological effects, but may not be trivial, e.g. for galenical preparations, pharmacodynamic properties etc. As antibody reactivity seems more efficient in recognising structural differences than receptor interaction/biological response, it may turn out to be important to monitor immune reactivity during therapeutic use of IFN-α2 variants.

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