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Section II. Interferon

Computer-assisted analysis demonstrates that polypeptides induced by natural and recombinant human interferon-α are the same and that some have related primary structures

Jon Weil[1], Charles J. Epstein[1,2], Lois B. Epstein[1,3], Jonathan van Blerkom[4] and Nguyen H. Xuong[5]

[1]*Department of Pediatrics,* [2]*Department of Biochemistry and Biophysics and* [3]*The Cancer Research Institute, University of California San Francisco, San Francisco, CA 94143,* [4]*Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO 80309 and* [5]*Departments of Physics, Chemistry and Biology, University of California, San Diego, La Jolla, CA 92093, U.S.A.*

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The biological effects on diploid and trisomy 21 human fibroblasts of pure human interferon IFLrA, a single IFN-α species produced from cloned DNA, were compared with those of partially purified natural IFN-α. Twelve interferon-induced polypeptides were visualized by two-dimensional gel electrophoresis and autoradiography. Seven of these were shown to have related primary structures and are therefore products of related genes or are related through post-translational modification. Qualitative visual comparisons and computer-aided quantitation of autoradiograms revealed no differences in the patterns of polypeptide induction following treatment with the two types of IFN-α, and the two interferons also induced (2′-5′) oligoisoadenylate synthetase equally. By these criteria, the activities of the two interferons are qualitatively and quantitatively indistinguishable. In addition, the effects of trisomy 21 on IFLrA-induced polypeptide synthesis and on antiviral response were similar to those previously demonstrated with natural IFN-α.

interferon-α; IFLrA; 2-5A synthetase; 2-D gels; computer-assisted; trisomy 21

Introduction

The antiviral products now known as interferon-α (IFN-α)*, which are produced

* The abbreviations used are: IFN-α, interferon-α; IFN-β, interferon-β; IU, International Units; VSV, vesicular stomatitis virus; p90, which indicates the polypeptide of molecular mass 90 000 daltons, for example.

Correspondence and reprint requests to: Dr. Charles Epstein, Department of Pediatrics, University of California, San Francisco, CA, U.S.A.

when human buffy coat leukocytes are treated with Sendai virus, actually comprise a family of at least 13 different molecules distinguished by differences in molecular weight [1], chromatographic affinities [2], and, in some instances, amino acid sequence [3]. The term 'natural' has been used to describe these interferons in order to distinguish their origin from that of the corresponding family of IFN-α's produced in *E. coli* from cloned DNA. Nucleotide sequencing of these DNA clones has demonstrated 13 different IFN-α genes whose protein products are deduced to have approximately 80% homology in their amino acid sequences [4].

The biological significance of the multiple forms of IFN-α has been investigated by comparing the activities of different forms on cells from different mammalian species [5–10], on different human cell lines [7,9], and in inducing different responses in a single cell type [11,13]. In two cases the ratio of antiviral activities against two viruses tested in the same host cell line differed among cloned IFN-α species [12,13]. Different ratios of antiviral activity to antiproliferative activity on human cell lines have also been reported [9]. IFN-α is known to induce several cellular functions [14], and the reports cited above suggest that the relative induction of the different functions may vary depending on the specific IFN-α [9]. Since single IFN-α species produced in *E. coli* are an important source of interferon for experimentation and clinical trials, further comparison of their activities with those of natural IFN-α (a mixture of several species of IFN-α) would be valuable.

An alternative method for comparing the biological activities of different interferon preparations is to investigate the interferon-induced synthesis of polypeptides as visualized by two-dimensional polyacrylamide gel electrophoresis [15,16]. This allows interferon preparations to be compared simultaneously with respect to their activities in inducing the synthesis of several different gene products and thereby permits differences in the relative induction of different genes as well as in overall potency to be detected. In addition, computer-aided analysis of two-dimensional gels can facilitate quantitative comparisons of the patterns of response [17,18].

We report here a comparison of the activity of IFLrA, a single pure IFN-α species produced from cloned DNA, with that of a partially purified natural IFN-α, PIF, on fetal human fibroblasts. The comparison is based on antiviral activity, induction of 2–5A synthetase, and induction of polypeptides visualized by two-dimensional gels. Further, since the gene for the IFN-α receptor (*IFRC*) is located on human chromosome 21 [19], we have also compared the quantitative effects of the two types of IFN-α in trisomy 21 and diploid fibroblasts. Finally, by analyzing polypeptides eluted from the 2-D gels, we present evidence that IFN-α induces the synthesis of a family of seven polypeptides that have similar primary structures.

Materials and methods

Interferon

Natural human IFN-α (PIF) induced in human leukocytes by Sendai virus and partially purified to a specific activity of 4×10^6 IU/mg protein was obtained from Dr. Kari Cantell. Human IFN-α prepared by recombinant DNA methods (IFLrA) was

obtained from Dr. Sidney Pestka. It was purified to homogeneity by Dr. Donna Hobbs and Dr. Hsiang-fu Kung of the Roche Institute of Molecular Biology, Nutley, New Jersey. Its amino acid sequence has been deduced from the base sequence of the cloned DNA [20].

Cell strains

Two pairs of matched trisomy 21 and control diploid human fetal fibroblasts were used [21]. Fetal lung fibroblast strains 153 (trisomic) and 152 (control) were derived from dizygotic twin abortuses. Fetal skin strains 256 (trisomic) and 255 (control) were from unrelated fetuses matched for gestational age and tissue of origin and were used at similar passage number.

Gel electrophoresis and scanning

The induction, radioactive labelling, two-dimensional polyacrylamide gel electrophoresis [22] and autoradiography of IFN-α induced polypeptides has been previously described [16]. The scanning equipment and the computer software and hardware for analysis of autoradiograms of two-dimensional polyacrylamide gels have been described elsewhere [17,18]. Autoradiograms were prepared at three different exposure levels for each of the six gels that were analyzed. The sets of three autoradiograms were analyzed with a computer-coupled scanning system and the data combined to provide estimates of the radioactivity in each computer-identified polypeptide spot [17]. An operator-interactive system was used to match and compare all spots on two gels (153 untreated and 153 treated with natural IFN-α) [18]. No interferon-induced spots which had not previously been identified by visual inspection were found. For quantitation, the interferon-induced spots, plus 20 interferon-invariant control spots selected by visual analysis and situated near the interferon-induced spots, were matched and analyzed on all six gels by means of an operator-interactive procedure which allows rapid analysis of a limited number of spots [18]. The sum of radioactivity in the control spots on each gel was used to normalize comparisons of the radioactivity in interferon-induced spots on the different gels.

Peptide mapping of individual polypeptides eluted from gels was performed by partial proteolysis with *Staphylococcus aureus* V-8 protease (Miles) and one-dimensional SDS-polyacrylamide gel electrophoresis as previously described [23].

Antiviral assays

Antiviral assays were performed and analyzed as previously described [16,24], using bovine vesicular stomatitis virus (VSV) on strain 153 cells.

Enzyme activity

Enzyme activity of (2'-5')-oligoisoadenylate synthetase was measured by the method of Minks and Baglioni [25].

Results and discussion

Induction of polypeptides

We compared the polypeptides induced by 200 IU/ml natural interferon and by purified recombinant interferon IFLrA in two pairs of matched trisomy 21 and control diploid fetal fibroblast strains, and the two interferon preparations were found to have the same polypeptide inducing effects. Figure 1 indicates the location of the twelve IFN-α induced polypeptides that were detected. These consist of one of ~90 000 daltons; a series having molecular masses of 77 000 to 81 000 that are resolved on some gels into seven distinct spots (as shown in Fig. 1B), two of 58 000 and 59 000 that lie close to one another and have an extended distribution in the isoelectric focusing (horizontal) dimension, one of 51 000, and one of 38 000. These molecular masses differ slightly from those previously reported by us and are based on more accurate determinations.

The majority of these polypeptides have previously been identified in our laboratory [16,26], and those of 80 000 and 58 000 daltons have apparently also been identified by others in IFN-α or IFN-β treated human fibroblasts [15,27–29], peripheral blood lymphocytes [29–31] and T-cell and B-cell lymphoblastoid lines [29]. We also previously reported that treatment of fibroblasts with IFN-α led to the induction of a 40 000 dalton polypeptide and to the interconversion of two polypeptides of 39 000 and 37 000 daltons [16]. However, the lot of ampholines we are currently using for isoelectric focusing gives a different pattern on the basic (left-hand) side of the gels from that originally observed, and we can no longer identify these polypeptides in our current gels.

Quantitation of polypeptide induction

Visual inspection of matched gels from cells treated with natural IFN-α and with purified IFLrA did not reveal any differences in either the absolute or relative intensities of the interferon-induced polypeptides listed above. In addition, detailed visual comparisons of gels from such treated cells and from untreated cells did not reveal any differences in the qualitative or quantitative effects of the two types of interferon on the patterns of synthesis of any other polypeptides visualized by the autoradiograms. This contrasts with comparisons between the major classes of interferon, in which we have shown that IFN-γ induces the synthesis of a unique set of polypeptides in addition to those induced in common with IFN-α and IFN-β [32].

To compare further the effects of the two types of interferon we used a semi-automatic, operator-interactive computer system to analyze the two dimensional gels [17,18]. Autoradiograms of gels from cell strains 152 and 153 that had been treated with either natural IFN-α or IFLrA (200 IU/ml) or left untreated as controls were so analyzed. The computer system resolved eight of the IFN-α induced polypeptides (or adjacent polypeptide pairs) described above and calculated their concentrations relative to a set of interferon-invariant control polypeptides. This quantitative analysis confirmed the conclusions drawn from visual comparisons.

The relative extent of induction of each polypeptide or polypeptide pair by the two different types of interferon is presented in Table 1. Relative to polypeptide p80a, each

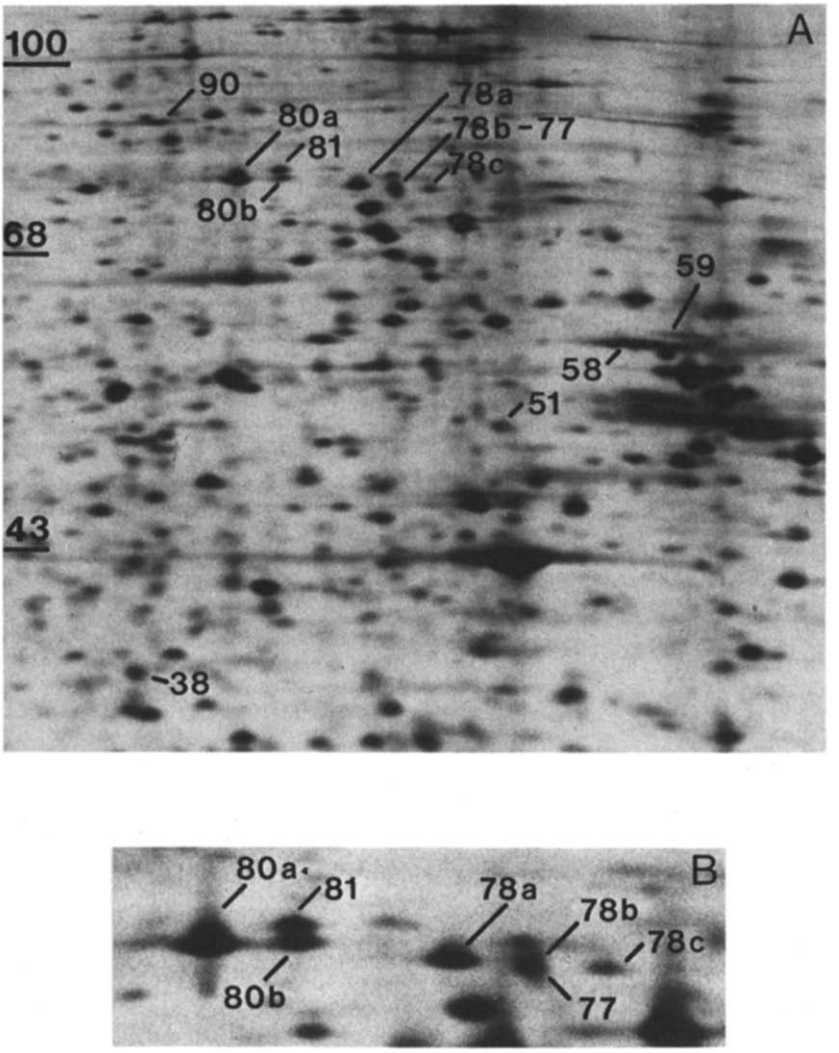

Fig. 1. A. Autoradiogram of diploid strain 255 treated with 200 IU/ml recombinant IFN-α (IFLrA). Polypeptides whose synthesis is induced by IFN-α are identified by their apparent molecular masses in kilodaltons. Only that portion of the gel containing IFN-induced polypeptides is shown. Molecular mass standards were phosphorylase B, bovine serum albumin and ovalbumin, whose locations are indicated on the left, plus carbonic anhydrase (29 kilodaltons) whose location is not shown. B. Enlargement of the region showing seven IFN-α induced polypeptides of approximately 80 kilodaltons. This is from an autoradiogram of trisomic strain 153 treated as in A. and from the same experiment.

polypeptide is induced to approximately the same extent by 200 IU/ml of natural IFN-α and IFLrA in cell strains 152 and 153. The data are analyzed in a different manner in Table 2, which presents a comparison of the extent of induction of each polypeptide by the two interferons. Again, the induction of the different polypeptides is very similar in cells treated with one or the other interferon. This is true both for comparisons among the set of polypeptides of 77 000–81 000 daltons (Table 1, rows 1–4) and for comparisons between the polypeptides in this set and the remaining polypeptides (rows 5–8).

TABLE 1

Relative concentrations of IFN-induced polypeptides following treatment with natural IFN-α and pure recombinant IFN-α (IFLrA)

Cell strain	IFN	Polypeptides							
		p80a	p81-p80b	p78a	p78b-p77	p59	p58	p51	p38
152	IFN-α	1.0	0.31	0.48	0.45	0.32	0.21	0.12	0.36
Diploid	IFLrA	1.0	0.25	0.47	0.48	0.48	0.28	0.16	0.34
153	IFN-α	1.0	0.22	0.45	0.29	0.39	0.17	0.14	0.34
Trisomic	IFLrA	1.0	0.36	0.46	0.49	0.42	0.20	0.09	0.27

Autoradiograms of gels prepared from cell strains 152 and 153 treated with 200 IU/ml natural IFN-α or IFLrA were scanned and analyzed by computer, and the relative concentrations of interferon-induced polypeptides were calculated as described in 'Materials and Methods'. For each gel (i.e., each cell strain and interferon treatment), relative polypeptide concentrations were normalized to that of polypeptide p80a.

TABLE 2

Comparison of polypeptide induction by natural IFN-α or IFLrA (200 IU/ml)

Polypeptide	Induction by natural IFN-α/induction by IFLrA	
	Strain 152	Strain 153
p80a	1.4	1.1
p81-p80b	1.7	0.6
p78a	1.4	1.0
p78b-p77	1.3	0.6
p59	1.1	0.9
p58	0.9	1.0
p51	1.5	1.3
p38	1.1	1.6
Mean ± SD	1.3 ± 0.26	1.0 ± 0.34

Partial peptide mapping

The seven polypeptides with molecular weights of 77 000–81 000 have small, relatively uniform differences in their isoelectric points, as determined from their distribution in the isoelectric focusing dimension (Fig. 1). In addition, the relative concentrations of these polypeptides are approximately the same following induction by natural IFN-α at concentrations ranging from 0.5 to 5 000 IU/ml [16; unpubl. obs.]. These polypeptides could therefore have related primary structures [16]. To investigate this possibility, the polypeptides were eluted from two-dimensional gels, subjected to digestion by *Staphylococcus aureus* V-8 protease, and the resulting polypeptide fragments were analyzed by one-dimensional polyacrylamide gel electrophoresis. As shown in Fig. 2, the polypeptide fragment patterns of p80a and p78 are quite similar, indicating that they have similar primary structures. The polypeptide fragment patterns of p81, p80b, p78c, and the doublet p78b–p77 suggest that they are also related to p80a and p78a. Several interferon-independent polypeptides in the molecular weight range of 43 000–75 000 were also analyzed and gave polypeptide fragment patterns that were clearly different from one another and from those of the IFN-α induced polypeptides. From these results we conclude that all seven of the 77 000- to 81 000-dalton IFN-α-induced polypeptides are related, either as products of related, coordinately induced genes or, more probably, by post-translational modification of a single primary gene product.

The demonstration that these polypeptides are related reinforces the suggestion that the two polypeptides of 58 000 and 59 000 daltons, which have similar molecular masses and distributions in the isoelectric focusing dimension, are also related [16]. The properties of the remaining three polypeptides indicate that they are unrelated to one another or to the polypeptides of 58 000 or 80 000 daltons. Thus the twelve polypeptides appear to represent the products of at least five different unrelated genes. IFLrA induces the synthesis of all twelve polypeptides, confirming that the inducing agent is IFN-α as opposed to possible contaminants in the partially purified natural IFN-α preparations used previously.

Comparison of trisomy 21 and diploid cell strains

Trisomy 21 fibroblasts and diploid controls have the same relative sensitivity to IFLrA as to natural IFN-α. As is shown in Table 3, when polypeptide induction by both types of interferon is measured, the trisomic cells are approximately 1.5 times as sensitive as controls. This is in agreement with the differences in dosage of the IFRC gene [16] and is consistent with the fact that trisomic cells have 50% more receptor molecules, as has now been directly verified by measuring the binding of purified ^{125}I-labelled human IFLrA to diploid and trisomic cells [33]. By contrast, the relative sensitivity of trisomic cells to IFLrA and natural IFN-α is much greater in the antiviral test, with the trisomic strain being about three times more sensitive to IFLrA than is the matched diploid control (Table 4). This indicates an amplification of the gene dosage effect at some step or steps between binding and the antiviral response. The current results demonstrate that this amplification does not result from impurities in the natural IFN-α preparations previously used [16,34,35] and is directly due to the IFN-α itself.

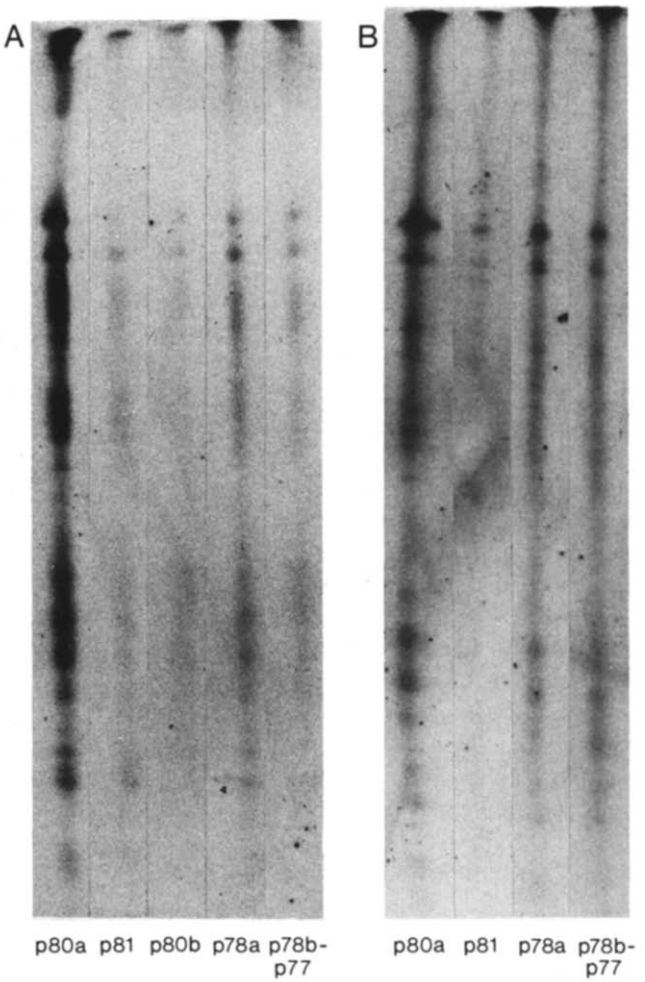

Fig. 2. Autoradiograms of one-dimensional gels of polypeptide fragments obtained by limited proteolysis of specific interferon-induced polypeptides designated in Fig. 1. Individual polypeptides or polypeptide pairs were eluted from two-dimensional gels, subjected to limited proteolysis and analyzed in one-dimensional polyacrylamide gels as indicated in 'Materials and Methods'. A. Polypeptides eluted from a 2-D gel prepared as described in 'Materials and Methods'. B. Polypeptides eluted from a 2-D gel loaded with 1.7×10^7 c.p.m. of ^{14}C-labelled cell extract and fixed with 50% methanol, 12% acetic acid, 0.2% methylene blue. In both cases the autoradiogram of p78c was too faint for reproduction, but visual inspection indicated that it also has the same polypeptide fragment pattern.

Induction of 2-5A synthetase

To investigate further the relative activities of natural IFN-α and IFLrA in human fibroblasts, we measured the induction of the enzyme (2'-5') oligoisoadenylate synthetase (2-5A synthetase) in strains 153 and 152 following treatment with interferon

TABLE 3

Relative polypeptide induction of trisomy 21 and diploid cell strains

Polypeptide	Induction in trisomy 21 strain 153/induction in diploid strain 152	
	Natural IFN-α	IFLrA
p80a	1.5	1.9
p81-p80b	1.0	2.8
p78a	1.4	1.9
p78b-p77	0.9	2.0
p59	1.2	1.4
p58	1.8	1.7
p51	1.4	1.5
p38	1.6	1.1
Mean ± SD	1.4 ± 0.3	1.8 ± 0.5

TABLE 4

Relative antiviral responses of trisomy 21 and diploid cell strains

Experiment	IFN_{50} (diploid strain 152)/IFN_{50} (trisomy 21 strain 153)	
	Natural IFN-α	IFLrA
1	2.1	2.8
2	9.3	3.1
3	5.0	2.4
4	9.6	3.6
Mean ± SD	6.5 ± 3.6	3.0 ± 0.5

Trisomy 21 strain 153 and diploid strain 152 were tested for antiviral response to natural IFN-α and to IFLrA as described in 'Materials and Methods'. IFN_{50} values, representing concentrations of interferon giving 50% protection against the cytopathic effects of the virus, were calculated.

(100 IU/ml). The ratios of enzyme activity after treatment with natural IFN-α to activity after treatment with IFLrA were 0.80 and 0.92 in the two strains, respectively, indicating that the response to the two types of interferon is the same.

The biological activities of IFLrA and natural IFN-α human fibroblasts have thus been compared with respect to seven cellular responses: the synthesis of at least five unrelated polypeptides, the induction of 2-5A synthetase, and antiviral activity. Cooper [31] used two-dimensional gels to compare the activities of IFLrA, natural IFN-α and natural IFN-β in peripheral blood lymphocytes and also found identical responses to the recombinant and the natural interferons. IFLrA has been tested

previously for a variety of different biochemical and biological activities in human cells [6,11,36–40], and in all cases where tested its activity was similar to that of natural IFN-α. The present results extend the analysis of IFLrA-induced polypeptides to human fibroblasts and quantitatively analyze a variety of IFLrA-induced functions in the same cell lines. They provide further evidence that IFLrA induces the same set of intracellular responses and physiological processes, and to the same extent, as does natural IFN-α.

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