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Purification, Bacterial Expression, and Biological Activities of the Human Interferons

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The structural and functional complexity of the human interferon system has become increasingly evident. More than eight different alpha (leukocyte) interferons are expressed in induced human cells in culture. Many of these have been purified by a combination of methods, including high-performance liquid chromatography. Moreover, at least 12 different human leukocyte interferons have been cloned, and several have been efficiently expressed in *Escherichia coli* and other organisms. The availability of purified species of leukocyte interferon, both natural and recombinant, has allowed structural work to be done, including amino acid sequence determinations, chemical modification studies, and the crystallization of one species. The purified material has also been used for the production of monoclonal antibodies with various specificities that are proving invaluable in rapid assays and purification techniques. Testing of the purified species for their relative potency in antiviral, antiproliferative, and immunomodulatory assays has begun to demonstrate the functional uniqueness and diversity of the purified alpha interferons. Hybrid interferon genes have been synthesized by splicing together parts of various cloned interferon genes. The resulting hybrid proteins have been valuable in establishing structure/function relationships. In several cases, the functional properties of the hybrid protein were novel and unpredicted from the properties of the parental molecules.

Interferon was discovered by Isaacs and Lindenmann during an investigation of the phenomenon of viral interference, wherein infection by one virus produces resistance to subsequent infection by other, apparently unrelated viruses. Their work [1,2] and that of others [3] demonstrated that cells could respond to challenge with a virus or with other substances (subsequently termed *inducers*) by producing a substance that could confer upon other cells resistance to subsequent viral attack. This substance, termed *interferon*, was characterized as a protein that had some species specificity, but that was fairly nonspecific in protecting against a wide range of viruses.

Since that time, the complexity of the system has increased as more interferon species within an organism have been recognized and characterized and biological effects have been documented [4].

The chemical heterogeneity of the interferons has become increasingly evident (Table I). It was found that interferons produced by different cell types were either stable or labile at pH 2. This lead to the designation of types I and II, respectively. Further work demonstrated that there were two main antigenically distinguishable forms within the type I class. The type I and type II designations have now been superceded by a nomenclature based on antigenic properties and protein structure. Corresponding generalizations evolved that the different classes of interferons were produced by different cell types under the action of different inducers. These observations were the basis for the tripartite classification of leukocyte, fibroblast, and immune interferon. Because it is now recognized that some cells can simultaneously produce both leukocyte and fibroblast interferon, the classification based on producer-cell type has been considered inaccurate. Consequently, the human leukocyte, fibroblast, and immune interferons have been designated as α, β, and γ classes, respectively. Subsequent work has demonstrated that there are at least 10 highly related human alpha interferons (IFN-α) each with characteristic chemical and biological properties and each encoded by a different gene. Thus far only a single protein species and gene have been isolated for the human β and γ interferons, although some evidence exists for heterogeneity of the β interferon [5]. There is some evidence that the α, β, and γ classes do not encompass all the human interferons (reviewed in [5]). However, these other species have not yet been clearly defined.

Work on the function of the interferons has demonstrated that they are capable of numerous effects in biological systems (Table II). Besides the antiviral effects, by which the interferons were discovered and functionally defined, the interferons are also potent inhibitors of growth and exhibit a number of immunomodulatory effects. This combination of activities has stimulated tremendous interest in their physiological roles and in their potential therapeutic applications, especially against viruses and tumors.

Considerable progress has been made in dissecting the biochemistry of the antiviral response, particularly through the pathway involving the (2′-5′)oligoadenylate synthetase (reviewed in [6]). The product of this enzyme modulates the action of several enzymes, including the activation of a latent endoribonuclease, ribonuclease L, which can degrade viral RNA. Other antiviral effects, probably distinct from this pathway, have been demonstrated, and it is unlikely that all viruses are inhibited via the same biochemical pathway. Recent work has also shown that at least 12 proteins are induced in cells treated with IFN-α, with an additional 12 proteins induced with IFN-γ [7]. Despite such recent progress, our knowledge of the biochemistry of the antiviral response is at an early stage. Moreover, it is likely that the antiproliferative and immunomodulatory effects of interferon are mediated through currently unknown biochemical pathways.

The ongoing expansion of biological and therapeutic work has been made possible by the availability of pure interferon. The purification and characterization of the leukocyte interferons from natural sources and the cloning and expression of these molecules in bacteria are allowing detailed investigations of the biological effects of the individual species and their interactions. This article summarizes recent advances in the purification and functional understanding of the human α (leukocyte) interferons and progress in defining the functional diversity of the individual molecular species. This article will emphasize the approaches and results obtained in our laboratory.

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Abbreviations:
CML: chronic myelogenous leukemia
DBM paper: diazobenzyloxymethyl paper
HPLC: high-performance liquid chromatography
IFN-α: alpha interferon
IFN-γ: gamma interferon

TABLE I. *Classification and general properties of interferons*

Type	I	I	II
Current nomenclature	α (leukocyte)	β (fibroblast)	γ (immune)
Usual source	Buffy coat leukocytes	Fibroblasts	T-lymphocytes
Stability at pH 2	+	+	−
Number of species	>10	1[a]	1
Glycosylation	−[b]	+	+
Presence of introns in genes	−	−[a]	+

[a] Some evidence for additional β species has been presented. Genes for such species could contain introns [5].
[b] Possible that minor species may be glycosylated.

TABLE II. *Selected biological activities of interferons*

Antiviral
Antigrowth
Antitumor
Immunomodulatory effects:
NK cell enhancement
T-cell functions:
Suppressor T cells
Cytotoxic T cells
Monocyte functions
B-cell functions

PURIFICATION AND PROPERTIES OF NATURAL LEUKOCYTE INTERFERONS

Since the lack of pure interferon was a serious drawback to both basic and clinical studies, purification of the interferons was early recognized as a critical task. While a large number of classical and novel techniques were attempted, the low abundance of the molecule and some of its physicochemical properties prevented its purification and characterization.

Several developments allowed the successful purification of leukocyte interferon in our laboratory. These developments included a more rapid in vitro assay of antiviral activity, which took 16 hours rather than 3 days [8], an efficient inducible system for the production of interferon, and a new purification scheme. The purification scheme was made possible by the use of high-performance liquid chromatography (HPLC), which had been modified for the analysis of amino acids, peptides, and proteins at the picomole level by a sensitive fluorescence assay developed by Sidney Udenfriend, Stanley Stein, and their colleagues in our Institute [9].

The purification started with the induction of interferon in buffy coat leukocytes by Newcastle disease virus. Following several initial steps for the concentration and gross fractionation of the culture medium, the material was purified over a series of HPLC columns with results similar to those shown in Fig 1. Overall purification was about 60,000- to 80,000-fold from the initial incubation medium. These studies demonstrated that human leukocyte interferon is actually a family of closely related proteins [10,11]. Purification of leukocyte interferons by other workers also demonstrated the existence of multiple species [12,13,13*a*]. This purification, the first reported for any leukocyte interferon, produced a sufficient quantity to allow the first chemical analysis and the first amino acid analysis of any interferon. The major problem behind this success was that the starting material came from buffy coats taken from 50 to 100 blood donors per week for several months.

With the demonstration that leukocytes from patients with chronic myelogenous leukemia (CML) were a rich source of interferon when induced, it was possible to use the same HPLC methodology for the purification of several species of leukocyte interferon from CML cells [14]. This purification permitted the amino acid analysis of several individual species [11]. Moreover, it was then possible to look at the functional properties of the various natural species. The ability of the purified natural interferons to protect either bovine MDBK cells or human fibroblasts from killing by viruses was compared (Table III). All purified species protected both bovine and human cells. However, while there is only a four-fold variation in the protection of the bovine cells by the various leukocyte interferons, greater variation was observed in human cells. Specifically, two of the leukocyte species, γ_3 and γ_5, were considerably less active on human cells than were the others. This demonstrated the functional heterogeneity within the leukocyte interferon family. These results also contributed to the growing realization that interferons are not species-specific, but rather have character-

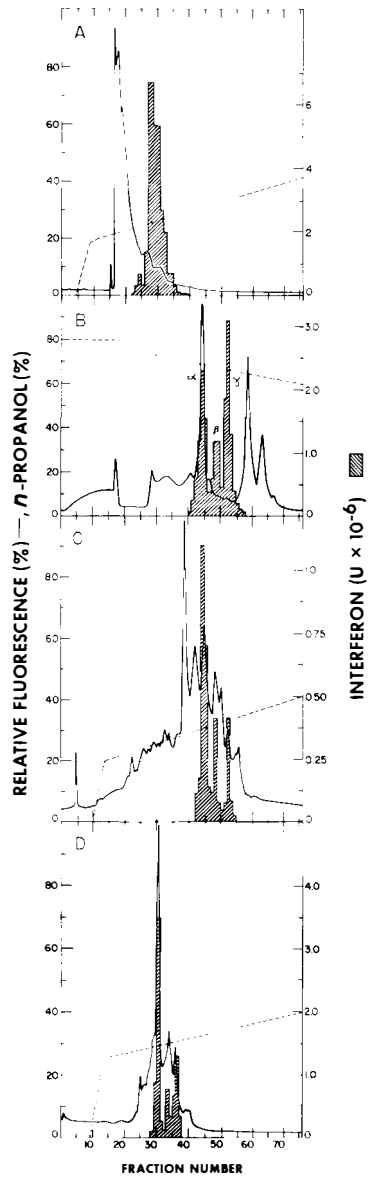

FIG 1. High-performance liquid chromatography of interferon. Following concentration and initial purification, material was purified on LiChrosorb RP-8 at pH 7.5 (*A*). A small part of the column effluent was directed to the fluorescamine monitoring system for detection of protein; fractions were also tested for interferon antiviral activity. The pooled interferon peak of panel *A* was chromatographed on a LiChrosorb Diol column at pH 7.5 (*B*). (The labels α, β, and γ were for laboratory use, and all correspond to HPLC peaks; they do *not* represent leukocyte, fibroblast, and immune interferons, respectively). The abundant peak γ was separated into components by chromatography on LiChrosorb RP-8 at pH 4.0 (*C*). The most abundant peak in panel *C* was rechromatographed on LiChrosorb RP-8 (◗). The major peak in panel ◗ represents a single subtype of leukocyte (α) interferon. (*Reproduced from [10] with permission.*)

istic patterns of activity on cells of different species. All these species exhibited antiproliferative activity [15].

Purification of multiple species was also accomplished with interferon produced by a continuous cell line, KG-1, which had been established from a patient with erythroleukemia [16]. Initial work in our laboratory demonstrated that KG-1 was a good producer of interferon after proper growth and induction [17,18]. Eventually, eight major species of human interferon were purified from these cells [19]. These also showed wide differences in potency on human cells, whereas all were quite active on bovine cells.

The purification of naturally induced human leukocyte interferons conclusively established several concepts: (1) Multiple leukocyte interferon species can be induced simultaneously in cultured human cells. This immediately suggested the existence of multiple genes corresponding to each of these structurally distinct species. (2) These interferons are closely related, having similar, but not identical molecular weights, amino acid content, and tryptic maps. (3) Although all the species were active on both human and bovine cells, the relative specific activities, particularly on human cells, differed considerably. (4) No carbohydrate was detected on five of the purified species examined. This contradicted the previously accepted notion that all interferons were glycoproteins.

In addition to demonstrating that the various human leukocyte interferons differ in their ability to confer antiviral protection on human cells, it was desirable to test their efficacy in other functional assays. Evinger et al [15] compared the ability of the purified species to inhibit cell multiplication of the human lymphoblastoid Daudi cell line (Table IV). It was found that all the species exhibited antiproliferative activity on these cells, although their potencies differed. This supported the notion that the chemical differences of the leukocyte interferon species are reflected in functional differences. In comparing the potency of each leukocyte interferon species in the antiproliferative assay with its potency in the antiviral assay, it was found that the ratios of antiproliferative-to-antiviral activity differed dramatically. A similar conclusion was drawn from experiments on the ability of the leukocyte interferons to stimulate natural killer cells (Table IV). If the various effects of interferon—antiviral, antiproliferative, natural killer cell activation, etc.—were all mediated through the same biochemical pathway, then it might be expected that the potency of a species in one assay would correlate with its potency in another assay. However, this was not observed. Thus the various effects must be mediated through different pathways. Where are the branchpoints in the pathways? How does a single interferon species generate different activities? These questions can be extended to the first molecular event—the binding of interferon to the cellular receptor. In the various assays on different human cell types, is the same cell surface receptor involved, or are there multiple receptors, each corresponding to a distinct biochemical pathway? Do different human cell types have different receptors, or is the same receptor able to trigger activities differentially following its activation? In summary, how is the cell able to respond uniquely to the various leukocyte interferons with resultant individual biochemical responses modulated to various degrees?

TABLE III. *Specific activities of human leukocyte interferons*

Species[a]	Specific activity on bovine MDBK cells (units/mg)[b]	Specific activity on human AG-1732 cells (units/mg)[c]	Relative activity MDBK/AG-1732
α_1	2.6×10^8	2.6×10^8	1.0
α_2	4×10^8	3×10^8	1.3
β_1	3.4×10^8	4.4×10^8	0.8
β_2	4×10^8	2×10^8	2.0
β_3	4×10^8	3×10^8	1.3
γ_1	2.6×10^8	2×10^8	0.7
γ_2	4×10^8	1.5×10^8	2.7
γ_3	3.5×10^8	1.5×10^7	23.3
γ_4	3.5×10^8	4×10^8	0.9
γ_5	0.9×10^8	2×10^6	45.0

[a] The α, β, γ nomenclature is an internal laboratory notation denoting peaks on HPLC columns. This nomenclature is unrelated to that of the three main classes of interferon. Data from [11].
[b] 25 percent.
[c] 50 percent.

EXPRESSION AND CHARACTERIZATION OF HUMAN INTERFERONS PRODUCED IN BACTERIA

Although it was possible to purify sufficient quantities of interferons from induced cells in order to conduct the structural and functional studies described thus far, the purification from human cells was quite laborious. The techniques of recombinant DNA technology presented the possibility of more easily producing large quantities of pure interferons and of getting at other questions concerning the gene structure and regulation of these proteins. Hence the cloning and expression of human α interferons was undertaken.

Plasmids containing cDNA copies of interferon-specific mRNA were constructed as follows [21] (Fig 2): Leukocytes were stimulated by viruses for the production of interferon. The mRNA was extracted from these cells and was transcribed into DNA copies that were then ligated into bacterial plasmids. These were then used to transform *E. coli*. Individual transformed colonies were screened for the presence of DNA complementary to mRNA from interferon-producing cells, but not complementary to mRNA from cells that had not been induced for interferon production (Fig 3). Positive colonies were then examined for the presence of interferon-specific sequences (Fig 4). The denatured plasmid DNA was attached covalently to DBM paper. RNA from interferon-producing cells was then allowed to hybridize to this DNA, and the paper was extensively washed to remove mRNA that was not complementary to the plasmid DNA. The bound mRNA was finally eluted from the

TABLE IV. *Relative activities of purified natural human α interferon species in several assays*

Purified interferon species	Growth inhibitory activity (GIA) (percent of control)	Antiviral activity (units/ml)	ΔGIA/AV unit[a]	Natural killer enhancement[b]
α_1	32	5.1	13.3	Low
α_2	44	7.7	7.3	Low
β_2	63	0.6	62	High
β_3	76	7.3	3.3	High
γ_1	55	6	7.5	High
γ_2	56	1.5	29	Low
γ_3	71	3.0	9.7	Low
γ_4	50	16	3.1	Low
γ_5	54	1.0	46	Low

[a] Abbreviations: GIA, growth-inhibitory activity; AV, antiviral activity; ΔGIA = 100 − GIA. Data from [15].
[b] Data from [20].

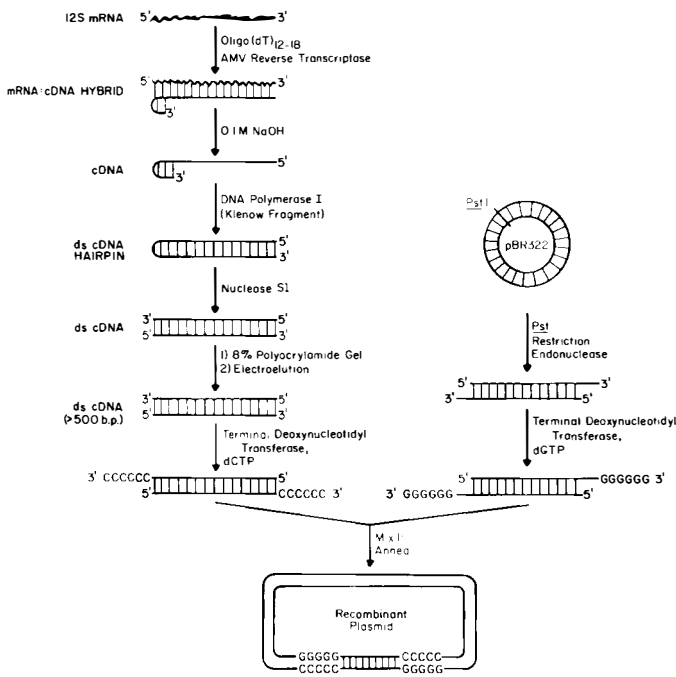

FIG 2. Preparation of interferon recombinant DNA plasmids from mRNA extracted from induced cells. (*Reproduced from [49] with permission.*)

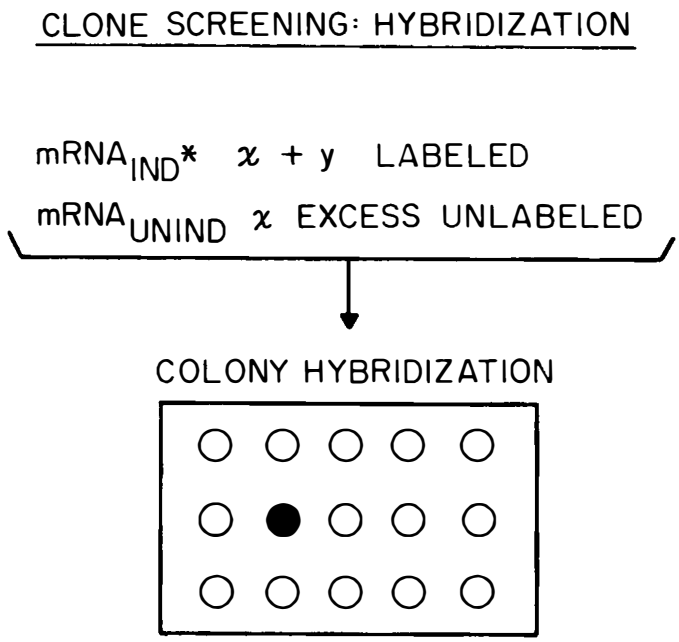

FIG 3. Schematic outline of hybridization procedure. [^{32}P]mRNA from induced cells binds preferentially to sequences specific for the induced cells when excess unlabeled mRNA from uninduced cells is present as a competitor. The induced specific sequences include interferon sequences and other sequences that are induced, including mitochondrial ribosomal RNA. (*Reproduced from [49] with permission.*)

paper and was injected into frog oocytes. Extracts from the frog oocytes were assayed for human interferon activity. The presence of such activity demonstrated that the mRNA that had been injected was specific for interferon and hence that the DNA to which it had hybridized on the filter paper had sequences corresponding to the interferon genes. When the nucleotide sequence of our first bacterial plasmid (p104) containing the coding region for interferon was compared with the available amino acid sequence of purified α interferon, it was discovered that only about 80% of the DNA sequence was in the plasmid [21]. Shortly before, Nagata et al [22] reported the cloning of human leukocyte (α) interferon [23]; even earlier, Taniguchi et al [24] reported the cloning of human fibroblast (β) interferon.

This original plasmid (p104), while not itself containing the full-length copy of interferon cDNA, was instrumental in finding other plasmids that had full-length copies of the α-interferon mRNA [25]. A number of such plasmids containing cDNA from interferon-specific mRNA were identified [26]. From these sequences, the amino acid sequences of the proteins were predicted (Fig 5). The sequences illustrate that the leukocyte interferons are a highly conserved family of proteins. In several cases it has been possible to correlate the natural and recombinant leukocyte interferons (Table V).

The original cDNA clones also provided a probe for identifying DNA from the human genome containing sequences corresponding to the interferon genes. Thus far at least 12 distinct genomic sequences from a human embryonic DNA library in bacteriophage λ have been found in our laboratory and others [25,27–30]. The existence of these distinct genomic sequences confirmed the deduction that the different leukocyte interferons are encoded by a closely related family of genes. Analysis of the genomic DNA also demonstrated that the genes lack introns found in most eukaryotic genes.

The bacterial plasmids containing the interferon cDNA were also used for the engineering of new plasmids that could direct the efficient production of interferon in the bacterial host (Fig 6). First, the cDNA sequence was excised from the bacterial plasmid by restriction endonuclease *Pst*I. Reflecting the general structure of eukaryotic mRNAs, the cDNA not only contained the information for the protein itself, but also was flanked on the 5′ end by a sequence encoding the polypeptide leader sequence required for the export of the interferon from eukaryotic cells. The coding sequence was also flanked on the 3′ end by an untranslated sequence followed by a stretch of polyadenylic acid. The interferon recombinant was reconstructed as shown in Fig 6. The result was that the sequence coding for the leader polypeptide was removed and an ATG initiator codon was added to the 5′ end of the gene.

The gene was ligated into a new bacterial plasmid such that it was now flanked on the 5′ end by the control region of the *E. coli* tryptophan operon (Fig 7). This control region includes the RNA polymerase promoter region and a sequence specifying

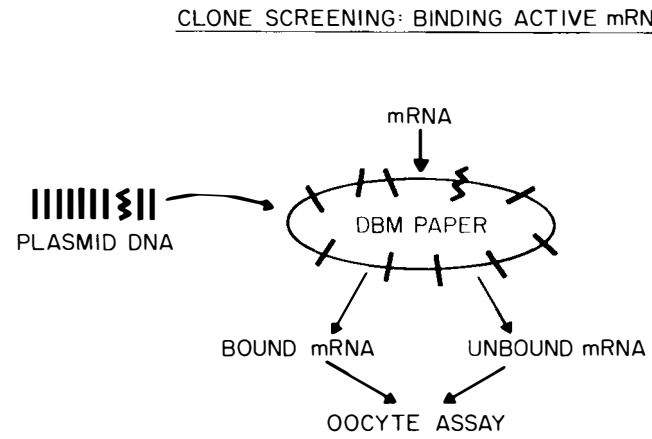

FIG 4. Schematic illustration of the screening of recombinants with DBM paper. Plasmid DNA from one or several colonies is denatured and covalently bound to DBM paper. mRNA from cells synthesizing interferon is hybridized to the filters, which are then extensively rinsed. Strongly bound mRNA is eluted and injected into *Xenopus laevis* oocytes. The production of human interferon by the oocytes implies that the plasmid DNA on the filter contains a sequence homologous to interferon mRNA. (*Reproduced from [49] with permission.*)

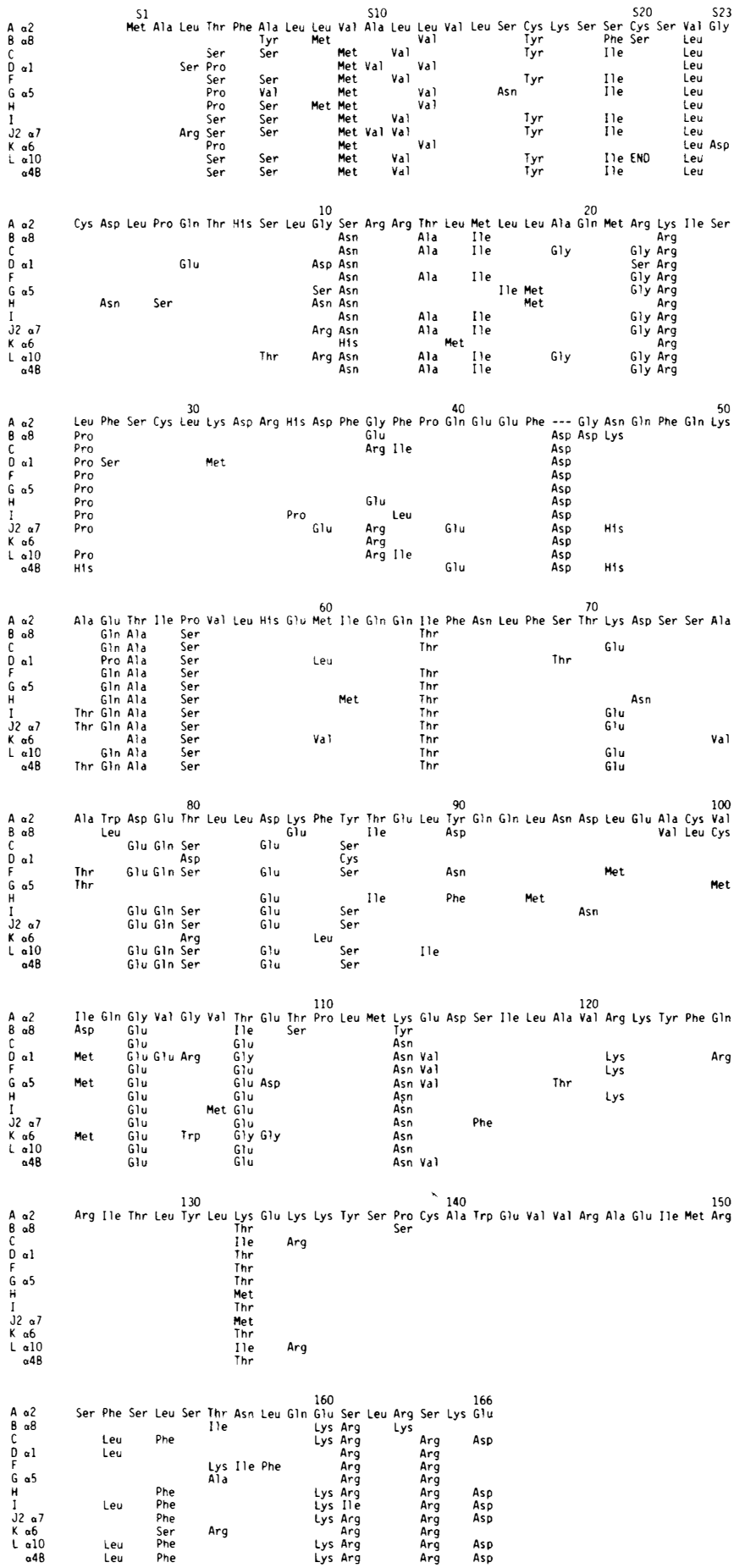

FIG 5. Summary of amino acid sequences of human leukocyte interferons. Sequences were derived from the respective DNA sequences. The entire sequence of IFN-αA is given, including the sequence of the precursor signal peptide (S1-S23). Corresponding residues of the other species are shown only where they differ from those of IFN-αA. Since IFN-αA has only 165 amino acids, whereas the other species have 166 amino acids, a gap in the IFN-αA sequence has been introduced between residues 43 and 44 to provide maximum homology with the other species. Sequences A through L are from the laboratory of the authors and from that of Goeddel and coworkers [26,49–51]. Sequences prefixed by α are from the laboratory of Weissmann et al [29]. Sequences that differ by only a few amino acids are here listed together (e.g., there is a single amino acid difference between A and α2). The α5 sequence is complete, whereas the G sequence begins at residue 34, since only a partial-length clone of this recombinant was isolated; identity of G with α5 is proposed on the basis of the extant sequences. α4B was described by Weissmann et al [29].

TABLE V. *Correlation of natural and recombinant human leukocyte interferons*

Recombinant[a]	Natural: Leukocytes[b]	Natural: Myeloblasts[c]
A	α_1	(a)
B	α_2	b_1
C	β_1	b_2
D	β_2	b_3
F	β_3	c_1
G	γ_1	c_2
H	γ_2	c_3
I	γ_3	d_1
J	γ_4	d_2
K	γ_5	
L		

[a] Corresponding species are connected by lines; from [25,26,31] and unpublished data.
[b] From [11].
[c] From [19]; the a species from myeloblasts have not been purified to homogeneity.

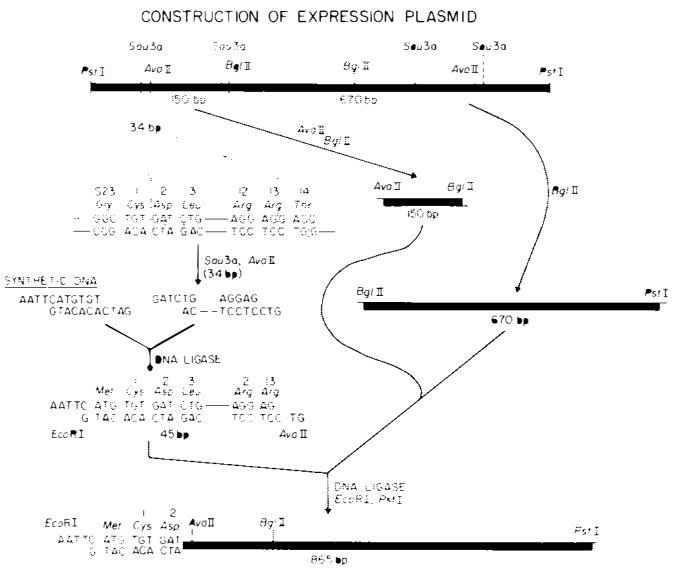

FIG 6. Construction of expression plasmid for human leukocyte interferon. A cDNA recombinant plasmid for leukocyte A interferon was isolated. The *Pst*I insert containing the complete cDNA sequence was excised. The three fragments shown above were isolated. The *Sau*3a-*Ava*II fragments corresponds to amino acids 2 to 13 of the mature interferon. A synthetic DNA was synthesized that restored the TGT codon of *Cys*1, introduced a methionine initiation codon (ATG) to the 5′ side of the *Cys*1 codon, and contained *Eco*R1 and *Sau*3a restriction sites. This was ligated to the *Sau*3a-*Ava*II fragment; the resultant fragment was ligated to the 150-bp *Ava*II-*Bgl*II and 670-bp *Bgl*II-*Pst*I fragments. This final expression fragment contained the coding sequence for a mature leukocyte interferon with an additional methionine at the NH_2-terminal end. This was ligated to the *E. coli* tryptophan promoter-operator for high-efficiency expression in *E. coli*. (*Reproduced by permission from [49]; data from [31].*)

the ribosome binding site. When this "expression vector" was introduced into *E. coli* and the bacteria were cultured on a large scale, the culture produced high yields of a single species of interferon, greater than 2×10^8 units of interferon per liter of culture (about 1 mg). Currently it is possible to produce about 1×10^{10} units (50 mg) per liter with improved expression vectors. For comparison, the purification of interferon from cultured human KG-1 cells yielded a mixture of IFN-α species totaling about 3×10^7 units per liter (approximately 100 μg).

The first crystals of human leukocyte interferon were prepared by Drs. Miller, Kung, and Pestka [32] as a step toward the analysis of its three-dimensional structure by x-ray crystallography. In addition, purified interferon was now available in large quantities for in vitro functional studies, for animal studies, and for human clinical trials.

MONOCLONAL ANTIBODIES TO INTERFERON

In experiments that were progressing simultaneously with the purification of the natural interferons, an array of monoclonal antibodies was produced in collaboration with Theophil Staehelin and his colleagues in Basel [33]. These monoclonal antibodies permitted the development of convenient solid-phase radioimmunoassays for interferon [34], as well as the development of immunoaffinity columns for the rapid purification of interferon from both natural and bacterial sources [35]. Additional possibilities for their use have arisen from the demonstration that many of these antibodies preferentially recognize native interferon rather than heat-denatured or chemically inactivated interferon [36].

INTERFERONS WITH NOVEL PROPERTIES

Plasmids containing interferon-specific DNA sequences have been utilized to create novel synthetic DNA genes by splicing parts of the natural genes together [37–39] (Fig 8). Because of the existence of common restriction endonuclease sites in the

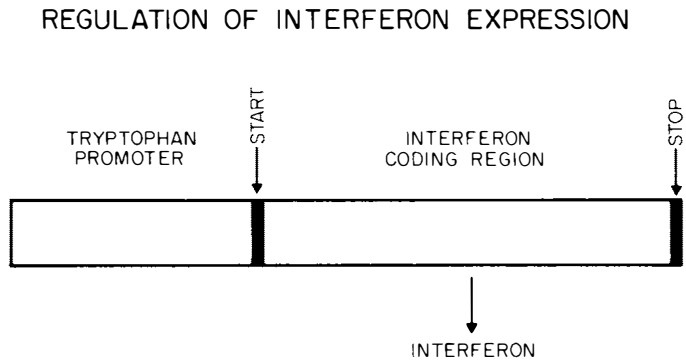

FIG 7. Schematic illustration of the DNA construction for the expression of mature interferon in *E. coli*. Here it is under the control of the tryptophan operator-promoter and has a proper *E. coli* ribosome binding site. (*Reprinted by permission from [49].*)

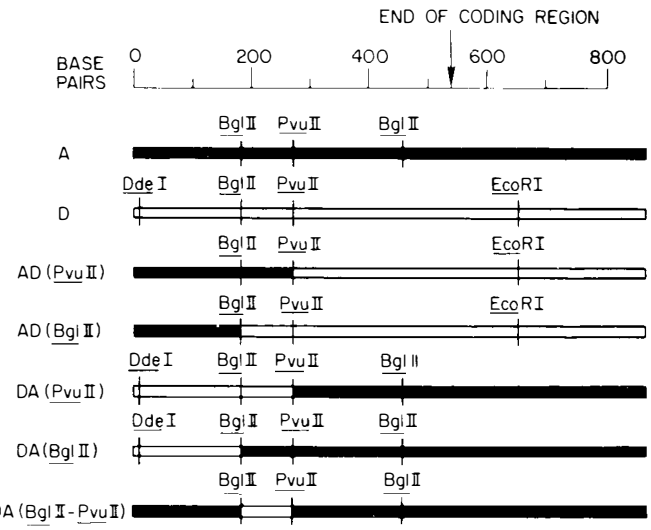

FIG 8. Schematic illustration and restriction maps of the coding regions for the mature proteins of the plasmids pIFN-αA and pIFN-αD and the hybrids constructed from these sequences. (*Reprinted by permission from [49].*)

closely related interferon genes, it was possible to cut the recombinant cDNA molecules at the common sites and to religate the pieces in new combinations to form novel molecules. Several such hybrids were formed from the two interferon clones designated A and D, which were cut by the restriction endonucleases *Bgl*II and *Pvu*II. These new sequences were engineered for efficient expression in *E. coli*, and the resulting proteins were tested functionally.

The potency of the parental and hybrid interferons in the viral cytopathic effect inhibition assay on cells from several species is shown in Table VI. These data represent the number of molecules of interferon per cell for 50% inhibition of viral cytopathic effect. The parental A and D molecules are both quite active on bovine MDBK cells, but they exhibit different activities on human cells and are relatively inactive on mouse L cells. This lack of activity on mouse cells is a general property of human interferons. The hybrid proteins that were spliced at the *Bgl*II or *Pvu*II restriction sites, having the NH_2 terminus of the A molecule and the COOH terminus of the D molecule, had a specificity similar to the parental A molecule on human or bovine cells. Completely unexpectedly, however, these proteins were quite active on mouse L cells, in contrast to all known human interferons. The converse construction, where the NH_2 terminus of D was ligated to the COOH terminus of A, produced proteins that resembled the parental molecules in being relatively ineffective on mouse cells.

These experiments demonstrated that the species specificity of the parental A and D interferons segregated with portions of the molecule. Similarly, when the parental and hybrid proteins were tested for antiproliferative activity on human Daudi cells, their potency ranged over a factor of 5000-fold (Table VII). The ratios of antiproliferative to antiviral activity were seen to vary considerably (Table VII). As discussed earlier, this ability of different leukocyte interferons to activate different cellular responses to various extents suggests that the biochemical pathways of these responses diverge at some point, possibly as early as the interferon-receptor interaction. Finally, the finding that the A/D hybrids were active on mouse cells demonstrated the potential of creating more diversity and possibly novel activities in the leukocyte interferons by manipulating the sequences by recombinant DNA technology.

TABLE VI. *Molecules per cell for 50% inhibition of viral cytopathic effect for various cell lines*[a]

Interferon/ cell type	AG-1732 (human)	MDBK (bovine)	L cells (murine)
A	4,900	1,100	3.1×10^7
D	360,000	6,600	4.3×10^6
A/D (*Bgl*)	5,500	1,200	3,300
A/D (*Pvu*)	4,100	2,400	48,000
D/A (*Bgl*)	1.5×10^6	1,800	$>7.2 \times 10^7$
D/A (*Pvu*)	590,000	5,400	7.1×10^6
A/D/A	36,000	2,000	1.1×10^6

[a] Data from [39].

PURIFICATION AND CLONING OF HUMAN FIBROBLAST (β) INTERFERON

Human β (fibroblast) interferon and its gene have also been investigated. Several laboratories had reported the purification of human β interferon by SDS-polyacrylamide gel electrophoresis as the last step of purification [40,41]. We developed a simple purification procedure involving Blue-Sepharose chromatography followed by HPLC on octyl silica [42]. This procedure produced pure β interferon free of salts, solvents, and SDS. The specific activity of this material was 3×10^8 units per milligram of protein. The amino acid composition and the sequence of the first 19 amino acids were determined. This sequence confirmed and extended those reported earlier for the first 13 [43,44], and 10 residues [45]. Subsequently, this sequence was shown to be identical to that predicted from the nucleotide sequence of the cloned DNA.

The cloning and expression of the β interferon gene was accomplished in collaboration with Goeddel and coworkers [46] by procedures similar to those described for the cloning and expression of the α interferons. A cDNA library was constructed with mRNA from human fibroblasts induced with polyinosinic·polycytidyic acid. A bacterial clone containing the fibroblast cDNA was identified by hybridization to radiolabeled cDNA prepared from 12S mRNA from induced fibroblasts as template for the reverse transcriptase and synthetic oligonucleotides predicted from the NH_2-terminal amino acids as primers. The nucleotide sequence of a plasmid from the cDNA library was determined, allowing deduction of the total amino acid sequence of the fibroblast interferon and of its export signal sequence. An expression plasmid was then constructed that permitted the synthesis in *E. coli* of 8×10^7 units of human β interferon per liter of culture. The interferon thus produced was indistinguishable from natural human β interferon by several criteria, although it lacks the carbohydrate moieties found on the natural β interferon. Since the amino acid sequences of β interferon and the nucleotide sequences of the cDNA cloned in several laboratories are in agreement [23,24,46–48], it seems that there is only a single major species of β interferon encoded by a single gene. Some evidence for molecular heterogeneity has been reported but not fully substantiated [5].

SUMMARY AND CONCLUSION

The human α (leukocyte) interferons are a family of closely related molecules, many of which can be simultaneously induced. At least nine of these natural α interferons have been purified from several human sources. They have been characterized in terms of their species specificity in antiviral assays. In addition, their antiviral, antiproliferative, and natural killer cell stimulatory activities on human cells have been compared. The variations in the absolute activities of the α interferons demonstrate that the chemical diversity is mirrored in functional diversity.

A number of human IFN-α species have been cloned. These

TABLE VII. *Molecules per cell for 50% inhibition of various assays on human cells*

Interferon	Antiviral[a]	Antiproliferative[b]	Natural killer cell enhancement[c]	AV/AP	AV/NK
A	4,900	13,000	118	0.38	41
D	360,000	450,000	2.8×10^5	1.80	1.3
A/D (*Bgl*)	5,500	9,300	58	0.59	95
A/D (*Pvu*)	4,100	30,000	1,300	0.14	3.2
D/A (*Bgl*)	1.5×10^6	910,000	2.5×10^5	1.7	6.0
D/A (*Pvu*)	590,000	4.2×10^6	46,000	0.14	13
A/D/A	36,000	60,000	20,000	0.60	1.8

[a] Measured on AG-1732 cells [39].
[b] Measured on Daudi lymphoblastoid cells [39].
[c] Measured on natural killer cells [52, 53].

have been efficiently expressed in *E. coli* to produce purified interferon proteins. The large-scale production of genetically engineered interferon has made possible further physical and chemical characterization of the proteins, including the crystallization of human leukocyte interferon A. This material has also been used for extensive animal and human clinical trials.

The genes for the natural leukocyte interferon species have been genetically engineered to form hybrid genes coding for corresponding chimeric proteins. The hybrid proteins had some novel and unexpected characteristics, such as activity on mouse cells, which are generally insensitive to protection by human interferons.

The observation that the relative potencies of the leukocyte species, whether natural, recombinant, or hybrid, do not necessarily correlate among the various functional assays suggests that the biological effects are differentially activated and hence must proceed along different biochemical pathways. The branchpoint for this differential activation of pathways is not known, but it could be as early as the recognition step by the cellular receptor, which itself could conceivably be a family of molecules.

The fact that the leukocyte interferon species are functionally distinct, despite their high degree of relatedness, as well as the fact that the genetic recombination of parts of the molecules may lead to new and unexpected functional changes, has several implications for the investigation of the therapeutic properties of interferon. First, for any application, each species will have to be tested for efficacy. Second, the usefulness of genetic engineering technology in this area not only might be in the production of large amounts of interferon, but also might be in the production of novel modified interferons that have such desirable combinations of properties as high specific potency for a particular application and low overall toxicity. The finding of differential activation of biological effects makes this decoupling of desirable and undesirable properties theoretically reasonable. However, any rational approach to the therapeutics of such a varied family of molecules will depend on continuing experiments designed to identify the structural basis of the differential biological responses as well as experiments on the nature of the cellular receptor and the subsequent biochemical mechanisms involved.

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