New polypeptide, called IFN-α76, produced by E. coli transformed with a newly isolated and characterized human IFN-α gene. The polypeptide exhibits interferon activities such as antiviral activity, cell growth regulation, and regulation of production of cell-produced substances.
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Description

Technical Field
The invention is in the field of biotechnology. More particularly it relates to a polypeptide having interferon (IFN) activity, DNA that codes for the polypeptide, a recombinant vector that includes the DNA, a host organism transformed with the recombinant vector that produces the polypeptide, pharmaceutical compositions containing the polypeptide, and therapeutic methods employing the polypeptide.

Background Art

A principal object of the present invention is to provide a polypeptide having interferon activity that is produced by an organism transformed with a newly isolated and newly characterized IFN-α gene. This polypeptide is sometimes referred to herein as IFN-α76. Other objects of the invention are directed to providing the compositions and organisms that are used to produce this polypeptide and to therapeutic compositions and methods that use this polypeptide as an active ingredient.

Disclosure of the Invention

One aspect of the invention is a polypeptide having interferon activity and comprising the amino acid sequence:

CysAspLeuProGln ThrHisSerLeuGly AsnArgArgAlaLeu IleLeuLeuAlaGln MetGlyArgIleSer HisPheSerCysLeu LysAspArgHisAsp PheGlyPheProGlu GluGluPheAspGly HisGlnPheGlnLys AlaGlnAlaIleSer ValLeuHisGluMet IleGlnGlnThrPhe AsnLeuPheSerThr GluAspSerSerAla AlaTrpGluGlnSer LeuLeuGluLysPhe SerThrLeuLeuTyr GlnGlnLeuAsnAsp LeuGluAlaCysVal IleGlnGluValGly ValGluGluThrPro LeuMetAsnGluAsp SerIleLeuAlaVal ArgLysTyrPheGln ArgIleThrLeuTyr LeuThrGluLysLys TyrSerProCysAla TrpGluValValArg AlaGluIleMetArg SerLeuSerPheSer ThrAsnLeuGlnLys ArgLeuArgArgLys Asp
A second aspect of the invention is a DNA unit or fragment comprising a nucleotide sequence that encodes the above described polypeptide.

A third aspect of the invention is a cloning vehicle or vector that includes the above described DNA.

A fourth aspect of the invention is a host organism that is transformed with the above described cloning vehicle and that produces the above described polypeptide.

A fifth aspect of the invention is a process for producing the above described polypeptide comprising cultivating said transformed host organism and collecting the polypeptide from the resulting culture.

Another aspect of the invention is a pharmaceutical composition having interferon activity comprising an effective amount of the above described polypeptide admixed with a pharmaceutically acceptable carrier.

Still another aspect of the invention is a method of providing interferon therapy to a human comprising administering a therapeutically effective amount of the above described polypeptide to the human.

Brief Description of the Drawings

Figure 1 is a partial restriction map which shows the two XhoII restriction sites that produce a homologous 260 base pair DNA fragment from the IFN-α1 and IFN-α2 structural genes. Data for this map are from Streuli, M., et al Science, 209:1343-1347 (1980).

Figure 2 depicts the sequencing strategy used to obtain the complete DNA sequence of the IFN-α76 gene coding region. Bacteriophage mp7:α76-1
DNA served as the template for sequences obtained with primers A, H and F and bacteriophage mp7:α76-2 DNA was the template for sequences obtained with primers E and G. The crosshatched area of the gene depicts the region that encodes the 23 amino acid signal polypeptide and the open box depicts the region that encodes the mature polypeptide. The scale, in base pairs, is numbered with 0 representing the ATG start codon of preinterferon. The arrows indicate the direction and extent of sequencing with each primer.

Figure 3 is the nucleotide sequence of the structural gene coding for IFN-α76 including some of the flanking 5' and 3' noncoding regions of the gene. The region coding for preinterferon and the mature polypeptide begins with the ATG codon at position 75 and terminates with the TGA codon at position 642.

Figure 4 is a partial restriction map of the coding region of the IFN-α76 gene. The crosshatching represents the region that encodes the 23 amino acid signal peptide and the open box represents the gene coding sequence for the mature polypeptide. The scale, in base pairs, is numbered with 0 representing the ATG start codon of preinterferon.

Figure 5 shows the amino acid sequence of the 23 amino acid signal polypeptide and the 166 amino acid mature IFN-α76 coded for by the gene depicted in Figure 3. The 189 amino acid sequence is displayed above the corresponding nucleotide sequence. Amino acid 24, cysteine, is the first amino acid of the mature IFN-α76 protein.

Figure 6 is the DNA sequence of the E. coli trp promoter and the gene of Figure 3 which was inserted between the EcoRI and HindIII sites of the
plasmid pBR322. The amino acid sequence of Figure 5 is written above the corresponding DNA sequence and the location of the restriction sites used in the construction of the expression plasmid are indicated. Figure 7 is a diagram of the expression plasmid, pGW19.

**Modes for Carrying Out the Invention**

In general terms IFN-α76 was made by identifying and isolating the IFN-α76 gene by screening a library of human genomic DNA with an appropriate IFN-α DNA probe, constructing a vector containing the IFN-α76 gene, transforming microorganisms with the vector, cultivating transformants that express IFN-α76 and collecting IFN-α76 from the culture. A preferred embodiment of this procedure is described below.

**DNA Probe Preparation**

Total cytoplasmic RNA was extracted from human lymphoblastoid cells, Namalwa, which had been induced for IFN production by pretreatment with 5-bromodeoxyuridine (Tovey, M.G., et al, Nature 267:455-457 (1977)) and Newcastle Disease Virus (NDV). The poly(A) (polyadenylic acid)-containing messenger RNA (mRNA) was isolated from total RNA by chromatography on oligo(dT)-cellulose (type 3 from Collaborative Research; Aviv, H., and Leder, P., Proc Natl Acad Sci (USA), 69:1408-1412, (1972)) and enriched for IFN mRNA by density gradient centrifugation on 5%-20% sucrose gradients. Fractions containing IFN mRNA were identified by translating the mRNA by microinjecting aliquots of each fraction into Xenopus oocytes and determining the IFN activity of the products of the translations according to a method

The Namalwa cell human IFN enriched mRNA was used to construct complementary DNA (cDNA) clones in E. coli by the G/C tailing method using the PstI site of the cloning vector pBR322 (Bolivar, F., et al, Gene, 2:95-113 (1977)). A population of transformants containing approximately 50,000 individual cDNA clones was grown in one liter of medium overnight and the total plasmid DNA was isolated.

The sequences of two IFN-α clones (IFN-α1 and IFN-α2) have been published (Streuli, M., et al, Science, 209:1343-1347 (1980)). Examination of the DNA sequences of these two clones revealed that the restriction enzyme XhoII would excise a 260 bp fragment from either the IFN-α1 or the IFN-α2 gene (see Figure 1). XhoII was prepared in accordance with the process described by Gingeras, T.R., and Roberts, R.J., J Mol Biol, 118:113-122 (1978).

One mg of the purified total plasmid DNA preparation was digested with XhoII and the DNA fragments were separated on a preparative 6% polyacrylamide gel. DNA from the region of the gel corresponding to 260 bp was recovered by electroelution and recloned by ligation into the BamHI site of the single strand bacteriophage ml3:mp7. Thirty-six clones were picked at random, the single stranded DNA isolated therefrom, and the DNA was sequenced. The DNA sequences of four of these clones were homologous to known IFN-α DNA sequences. Clone mp7:α-260, with a DNA sequence identical to IFN-α1 DNA (Streuli, M. et al, Science, 209:1343-1347 (1980)) was chosen as a highly specific hybridization probe for identifying additional IFN-α DNA sequences. This clone is hereinafter referred to as the "260 probe."
Screening of Genomic DNA Library

In order to isolate other IFN-α gene sequences, a $^{32}$P-labelled 260 probe was used to screen a library of human genomic DNA by in situ hybridization. The human gene bank, prepared by Lawn, R.M., et al., Cell, 15:1157-1174 (1978), was generated by partial cleavage of fetal human DNA with HaeIII and AluI and cloned into bacteriophage λ Charon 4A with synthetic EcoRI linkers. Approximately 800,000 clones were screened, of which about 160 hybridized with the 260 probe. Each of the 160 clones was further characterized by restriction enzyme mapping and comparison with the published restriction maps of 10 chromosomal IFN genes (Nagata, S., et al., J Interferon Research, 1:333-336 (1981)). One of the clones, hybrid phage λ4A:a76 containing a 15.5 kb insert, was characterized as follows. A DNA preparation of λ4A:a76 was cleaved with HindIII, BgIII, and EcoRI respectively, the fragments separated on an agarose gel, transferred to a nitrocellulose filter (Southern, E.M., J Mol Biol, 98:503-517 (1977)) and hybridized with $^{32}$P-labelled 260 probe. This procedure localized the IFN-α76 gene to a 2.0 kb EcoRI restriction fragment which was then isolated and recloned, in both orientations, by ligation of the fragment into EcoRI cleaved m13:mp7. The two subclones are designated mp7:a76-1 and mp7:a76-2. The -1 designation indicates that the single-stranded bacteriophage contains insert DNA complementary to the mRNA (the minus strand) and the -2 designation indicates that the insert DNA is the same sequence as the mRNA (the plus strand).
Sequencing of the IFN-α76 Gene

The Sanger dideoxy-technique was used to determine the DNA sequence of the IFN-α76 gene. The strategy employed is diagrammed in Figure 2, the DNA sequence thus obtained is given in Figure 3, and a partial restriction enzyme map of the IFN-α76 gene is illustrated in Figure 4. Unlike many genes from eukaryotic organisms, but analogous to other IFN chromosominal genes which have been characterised, the DNA sequence of this gene demonstrates that it lacks introns. Homology to protein sequence information from these known IFN-α genes made it possible to determine the correct translational reading frame and thus allowed the entire 166 amino acid sequence of IFN-α76 to be predicted from the DNA sequence as well as a precursor segment, or signal polypeptide, of 23 amino acids (Figure 5).

The DNA sequence of the IFN-α76 gene and the amino acid sequence predicted therefrom differ substantially from the other known IFN-α DNA and IFN-α amino acid sequences. Nagata, S., et al, (J Interferon Research, 1:333-336, (1981)) describe isolating two IFN-α genes, IFN-α4a and IFNα4b, that differ by five nucleotides which entails 2 amino acid changes in the proteins expressed thereby. The sequence of IFN-αb is given in European Patent Application No. 81300050.2. The IFN-α76 structural gene differs from the IFN-α4b gene by 5 nucleotides which entails 4 amino acid changes in the corresponding proteins: a single nucleotide change creates an amino acid substitution of alanine for threonine at amino acid number 14 of the mature protein; a double nucleotide change creates an amino acid substitution of alanine for glutamine at amino acid number 19 of
the mature protein; a single nucleotide change creates an amino acid substitution of alanine for threonine at amino acid number 51 of the mature protein; and, a single nucleotide change creates an amino acid change of glutamate for valine at amino acid number 114 of the mature protein.

Plasmid Preparation and Host Transformation

Assembly of the plasmid for direct expression of the IFN-α76 gene involved replacing the DNA fragment encoding the 23 amino acid signal polypeptide of preinterferon with a 120 bp EcoRI/Sau3A promoter fragment (E.coli trp promoter, operator, and trp leader ribosome binding site preceding an ATG initiation codon) and using the naturally occurring HindIII site, 142 bp 3' of the TGA translational stop codon, to insert the gene into a vector derived from the plasmid pBR322. The complete DNA sequence of the promoter and gene fragments inserted between the EcoRI and HindIII sites of pBR322 is shown in Figure 6 which also shows the exact location of relevant cloning sites. Details of the construction are described below.

The coding region for mature IFN-α76 encompasses a Sau3A site between codons for amino acids 2 and 3 and an AvaI site between codons for amino acids 39 and 40. The 111 bp Sau3A to AvaI fragment was isolated on a 6% polyacrylamide gel following a Sau3A/AvaI double-digest of the 2.0 kb EcoRI genomic fragment. Similarly, the 528 bp fragment from the AvaI site between codons for amino acids 39 and 40 and the HindIII site 142 nucleotides 3' of the translational stop codon was isolated on a 5% polyacrylamide gel. These two fragments, together with a 120 bp
EcoRI to Sau3A E.coli promoter fragment were ligated together in a four way directed ligation into the EcoRI to HindIII site of pBR322. The promoter fragment, which contains a synthetic HindIII restriction site, ATG initiation codon, the initial cysteine codon (TGT) common to all known IFN-αs, and Sau3A "sticky end", had been constructed previously. The ligation mixture was used to transform E.coli MM294 (Backman, K., et al, Proc Natl Acad Sci (USA) 73:4174-4178 (1976)). The desired correct transformant, one out of 24 screened, was identified by restriction enzyme mapping of colonies which hybridized to a 32p-labelled IFN-α genomic fragment. Figure 7 is a diagram of the final expression plasmid obtained, which is designated pGW19. Other prokaryotic hosts such as bacteria other than E.coli may, of course, be transformed with this or other suitable constructs to replicate the IFN-α76 gene and/or to produce IFN-α76.

IFN-α76 produced in accordance with the invention is believed to be distinct from the corresponding native protein in several respects. Firstly, because the IFN-α76 gene was expressed by bacterial hosts that utilize N-formyl-methionine and/or methionine to initiate translation, some or all of the bacterially produced IFN-α76 molecules are preceded by an N-formyl-methionine or methionine group. Some of the N-formyl-methionine or methionine groups could be removed by natural in vivo bacterial cleavage mechanisms. This would result in a mixture of molecules, some of which would include an initial N-formyl-methionine or methionine and others that would not. All such IFN-α76 molecules, those containing an initial N-formyl-methionine or methionine, those not containing an N-formyl-methionine or methionine and
any mixture thereof, are encompassed by the present invention. Secondly, the amino acid residues of the bacterially produced polypeptide are unsubstituted whereas the residues of the native protein may be substituted with sugar groups, ACTH or other moieties. Also, native IFN-α extracts consist of mixtures of various IFN molecules whereas the bacterially produced IFN-α76 is homogeneous; that is, bacterially produced IFN-α76 does not contain functionally related polypeptides. Accordingly, the invention contemplates producing IFN-α76-containing compositions having biological activity that is attributable solely to IFN-α76 and/or said terminal N-formyl-methionine or methionine derivatives thereof.

15 Cultivation of Transformants

Bacteria transformed with the IFN-α76 gene may be cultivated in an appropriate growth medium, such as a minimum essential medium, that satisfies the nutritional and other requirements needed to permit the bacteria to grow and produce IFN-α76. If the bacteria are such that the protein is contained in their cytoplasm, the IFN-α76 may be extracted from the cells by lysing the cells such as by sonication and/or treatment with a strong anionic solubilizing agent such as sodium dodecyl sulfate. Further purification of the extract may be achieved by affinity chromatography, electrophoresis, or other protein purification techniques.

30 Biological Testing of IFN-α76

IFN-α76-containing cell sonicates were tested in vitro and found to have the following activities: (1) inhibition of viral replication of
vesicular stomatitis virus (VSV) and herpes simplex
virus-1 (HSV-1); (2) inhibition of tumor cell growth;
(3) inhibition of colony formation by tumor cells in
soft agar; (4) activation of natural killer (NK)
cells; (5) enhancement of the level of 2',5'-oligo-
adenylate synthetase (2',5'-A); and (6) enhancement of
the double-stranded RNA-dependent protein kinase. The
sonicates were active in inhibiting viral infection in
both human and other mammalian cells such as hamster,
monkey, mouse, and rabbit cells.

The tests show that IFN-α76 exhibits anti-
viral activity against DNA and RNA viruses, cell
growth regulating activity, and an ability to regulate
the production of intracellular enzymes and other
cell-produced substances. Accordingly, it is expected
IFN-α76 may be used to treat viral infections with a
potential for interferon therapy such as chronic
hepatitis B infection, ocular, local, or systemic
herpes virus infections, influenza and other respira-
tory tract virus infections, rabies and other viral
zoonoses, arbovirus infections, and slow virus
diseases such as Kuru and sclerosing panencephalitis.
It may also be useful for treating viral infections in
immunocompromised patients such as herpes zoster and
varicella, cytomegalovirus, Epstein-Barr virus infec-
tion, herpes simplex infections, rubella, and progress-
ive multifocal leukoencephalopathy. Its cell growth
regulating activity makes it potentially useful for
treating tumors and cancers such as osteogenic sar-
coma, multiple myeloma, Hodgkin's disease, nodular,
poorly differentiated lymphoma, acute lymphocytic
leukemia, breast carcinoma, melanoma, and nasopharyn-
geal carcinoma. The fact that IFN-α76 increases
protein kinase and 2',5'-oligoadenylate synthetase
indicates it may also increase synthesis of other enzymes or cell-produced substances commonly affected by IFNs such as histamine, hyaluronic acid, prostaglandin E, tRNA methylase, and aryl hydrocarbon hydrolase. Similarly, it may be useful to inhibit enzymes commonly inhibited by IFNs such as tyrosine amino transferase, glycerol-3-phosphate dehydrogenase, glutamine synthetase, ornithine decarboxylase, S-adenosyl-1-methionine decarboxylase, and UDP-N-acetylglucosamine-dolichol monophosphate transferase. The ability of the IFN-α76 to stimulate NK cell activity is indicative that it may also possess other activities such as the abilities to induce macrophage activity and antibody production and to effect cell surface alterations such as changes in plasma membrane density or cell surface charge, altered capacity to bind substances such as cholera toxin, concanavalin A and thyroid-stimulating hormone, and change in the exposure of surface gangliosides.

Pharmaceutical compositions that contain IFN-α76 as an active ingredient will normally be formulated with an appropriate solid or liquid carrier depending upon the particular mode of administration being used. For instance, parenteral formulations are usually injectable fluids that use pharmaceutically and physiologically acceptable fluids such as physiological saline, balanced salt solutions, or the like as a vehicle. Oral formulations, on the other hand, may be solid, eg tablet or capsule, or liquid solutions or suspensions. IFN-α76 will usually be formulated as a unit dosage form that contains in the range of 10^4 to 10^7 international units, more usually 10^6 to 10^7 international units, per dose.
IFN-α76 may be administered to humans in various manners such as orally, intravenously, intramuscularly, intraperitoneally, intranasally, intradermally, and subcutaneously. The particular mode of administration and dosage regimen will be selected by the attending physician taking into account the particulars of the patient, the disease and the disease state involved. For instance, viral infections are usually treated by daily or twice daily doses over a few days to a few weeks; whereas tumor or cancer treatment involves daily or multidaily doses over months or years. IFN-α76 therapy may be combined with other treatments and may be combined with or used in association with other chemotherapeutic or chemopreventive agents for providing therapy against viral infections, neoplasms, or other conditions against which it is effective. For instance, in the case of herpes virus keratitis treatment, therapy with IFN has been supplemented by thermocautery, debridement and trifluorothymidine therapy.

Modifications of the above described modes for carrying out the invention, such as, without limitation, use of alternative vectors, alternative expression control systems in the vector, and alternative host microorganisms and other therapeutic or related uses of IFN-α76, that are obvious to those of ordinary skill in the biotechnology, pharmaceutical, medical and/or related fields are intended to be within the scope of the following claims.
Claims

1. A polypeptide having interferon activity and comprising the amino acid sequence:

CysAspLeuProGln ThrHisSerLeuGly AsnArgArgAlaLeu IleLeuLeuAlaGln MetGlyArgIleSer HisPheSerCysLeu LysAspArgHisAsp PheGlyPheProGlu GluGluPheAspGly HisGlnPheGlnLys AlaGlnAlaIleSer ValLeuHisGluMet IleGlnGlnThrPhe AsnLeuPheSerThr GluAspSerSerAla AlaTrpGluGlnSer LeuLeuGluLysPhe SerThrGluLeuTyr GlnGlnLeuAsnAsp LeuGluAlaCysVal IleGlnGluValGly ValGluGluThrPro LeuMetAsnGluAsp SerIleLeuAlaVal ArgLysTyrPheGln ArgIleThrLeuTyr LeuThrGluLysLys TyrSerProCysAla TrpGluValValArg AlaGluLeMetArg SerLeuSerPheSer ThrAsnLeuGlnLys ArgLeuArgArgLys Asp.

2. The polypeptide of claim 1 wherein the polypeptide consists essentially of said amino acid sequence.

3. The polypeptide of claim 1 or 2 wherein the initial cysteine residue of the amino acid sequence is preceded by an N-formyl-methionine group.

4. The polypeptide of claim 1 or 2 wherein the amino acid residues of said sequence are unsubstituted.

5. IFN-α76.

6. A composition having interferon activity and comprising a mixture of:

   (a) a polypeptide having the amino acid sequence:

CysAspLeuProGln ThrHisSerLeuGly AsnArgArgAlaLeu IleLeuLeuAlaGln MetGlyArgIleSer HisPheSerCysLeu LysAspArgHisAsp PheGlyPheProGlu GluGluPheAspGly HisGlnPheGlnLys AlaGlnAlaIleSer ValLeuHisGluMet IleGlnGlnThrPhe AsnLeuPheSerThr GluAspSerSerAla AlaTrpGluGlnSer
and:

(b) a polypeptide having said amino acid sequence wherein the initial cysteine residue of the sequence is preceded by an N-formyl-methionine or methionine group.

7. The composition of claim 6 wherein the amino acid residues of said sequence are unsubstituted.

8. A composition having interferon activity comprising a polypeptide having the amino acid sequence:

CysAspLeuProGln ThrHisSerLeuGly AsnArgArgAlaLeu IleLeuLeuAlaGln MetGlyArgIleSer HisPheSerCysLeu LysAspArgHisAsp PheGlyPheProGlu GluGluPheAspGly HisGlnPheGlnLys AlaGlnAlaIleSer ValLeuHisGluMet IleGlnGlnThrPhe AsnLeuPheSerThr GluAspSerSerAla AlaTrpGluGlnSer LeuLeuGluLysPhe SerThrGluLeuTyr GinGlnLeuAsnAsp LeuGluAlaCysVal IleGlnGluValGly ValGluThrPro LeuMetAsnGluAsp SerIleLeuAlaVal ArgLysTyrPheGlu'ArgIleThrLeuTyr LeuThrGluLysLys TyrSerProCysAla TrpGluValValArg AlaGluIleMetArg SerLeuSerPheSer ThrAsnLeuGlnLys ArgLeuArgArgLys Asp

or a mixture of said polypeptide and a polypeptide having said sequence wherein the initial cysteine residue is preceded by an N-formyl-methionine or methionine group wherein the interferon activity of the composition is attributable to said polypeptide or to said mixture.

9. A DNA unit consisting of a nucleotide sequence that encodes the polypeptide of claim 1 or 5.
10. The DNA unit of claim 9 wherein the nucleotide sequence is:

TGT GAT CTG CCT CAG ACC CAC AGC CTG GGT AAT AGG AGG
GCC TTG ATA CTC CTG GCA CAA ATG GGA AGA ATC TCT CAT
TTC TCC TGC CTG AAG GAC AGA CAT GAT TTC GGA TTC CCC
GAG GAG GAG TTT GAT GGC CAC CAG TTC CAG AAG GCT CAA
GCC ATC TCT GTC CTC CAT GAG ATG ATC CAG CAG ACC TTC
AAT CTC TTC AGC ACA GAG GAC TCA TCT GCT GCT TGG GAA
CAG AGC CTC CTA GAA AAA TTT TCC ACT GAA CTT TAC CAG
CAA CTG AAT GAC CTG GAA GCA TGT GTG ATA CAG GAG GTT
GGG GTG GAA GAG ACT CCC CTG ATG AAT GAG GAC TTC ATC
CTG GCT GTG AGG AAA TAC TTC CAA AGA ATC ACT CT TAT
CTA ACA GAG AAG AAA TAC AGC CCT TGT GCC TGG GAG GTT
GTC AGA GCA GAA ATC ATG AGA TCC CTC TCG TTT TCA ACA
AAC TTG CAA AAA AGA TTA AGG AGG AAG GAT.

11. A cloning vehicle that includes the DNA unit of claim 9 or 10.

12. The cloning vehicle of claim 11 wherein the cloning vehicle is a plasmid.

13. The cloning vehicle of claim 11 wherein the cloning vehicle is the plasmid pGW19.

14. A host that is transformed with the cloning vehicle of claim 11 and produces IFN-α76.

15. The host of claim 13 wherein the host is a prokaryote.
16. The host of claim 14 wherein the host organism is \textit{E.coli}.

17. A host that is transformed with the cloning vehicle of claim 13 and produces IFN-\(\alpha\)76, wherein the host is \textit{E.coli}.

18. A process for producing IFN-\(\alpha\)76 comprising cultivating the host of claim 14 and collecting IFN-\(\alpha\)76 from the resulting culture.

19. A process of producing IFN-\(\alpha\)76 comprising cultivating the host organism of claim 16 and collecting IFN-\(\alpha\)76 from the resulting culture.

20. A process for producing IFN-\(\alpha\)76 comprising cultivating the host organism of claim 17 and collecting IFN-\(\alpha\)76 from the resulting culture.

21. A pharmaceutical composition comprising an effective amount of the polypeptide of claim 1, 2 or 5 admixed with a pharmaceutically acceptable vehicle or carrier.

22. A pharmaceutical composition comprising an effective amount of the composition of claim 6 or 8 admixed with a pharmaceutically acceptable vehicle or carrier.

23. A method of providing interferon therapy to a human comprising administering a therapeutically effective amount of the polypeptide of claim 1, 2 or 5 to said human.
24. A method of providing interferon therapy to a human comprising administering a therapeutically effective amount of the composition of claim 6 or 8 to said human.

25. The method of claim 23 wherein the therapy is for treating a viral infection, providing cell growth regulation, or regulating the production of a cell-produced substance.

26. The method of claim 24 wherein the therapy is for treating a viral infection, providing cell growth regulation, or regulating the production of a cell-produced substance.

27. A method of providing antiviral therapy to a mammal comprising administering a viral infection inhibiting amount of the polypeptide of claim 1, 2 or 5 to the mammal.
Figure 1

Figure 2
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**Figure 3**
Figure 4
1  Met  Ala  Leu  Ser  Phe  Ser  Leu  Leu  Met  Ala  Val  Leu  Val  Leu  Ser  Tyr  Lys  Ser  Ile  Cys
   ATG  GCC  CTG  TCC  TTT  TCT  TTA  CTG  ATG  GCC  GTG  CTG  GTG  CTC  AGC  TAC  AAA  TCC  ATC  TGT

21 Ser  Leu  Gly  Cys  Asp  Leu  Pro  Glu  Thr  His  Ser  Leu  Gly  Asn  Arg  Arg  Ala  Leu  Ile  Leu
   TCT  CTG  GGC  TG T  GAT  CTG  CCT  CAG  ACC  CAC  AGC  CTG  GGT  AAT  AGG  AGG  GCC  TTG  ATA  CTC

41 Leu  Ala  Gln  Met  Gly  Arg  Ile  Ser  His  Phe  Ser  Cys  Leu  Lys  Asp  Arg  His  Asp  Phe  Gly
   CTG  GCA  CAA  ATG  GGA  AGA  ATC  TCT  CAT  TGC  TGC  CTG  AAG  GAC  AGA  CAT  GAT  TTC  GGA

61 Phe  Pro  Glu  Glu  Glu  Phe  Asp  Gly  His  Glu  Phe  Glu  Lys  Ala  Gln  Ala  Ile  Ser  Val  Leu
   TTC  CCC  GAG  GAG  GAG  TTT  GAT  GGC  CAC  CAG  TAC  CAG  AAG  GCT  CAA  GCC  ATC  TCT  GTC  CTC

81 His  Glu  Met  Ile  Gln  Glu  Thr  Phe  Asn  Leu  Phe  Ser  Thr  Glu  Asp  Ser  Ser  Ala  Ala  Trp
   CAT  GAG  ATG  ATC  CAG  CAG  ACC  TAC  GTC  TGC  AGC  ACA  GAG  GAC  TCA  TCT  GCT  GCT  GGG

101 Glu  Gln  Ser  Leu  Leu  Glu  Lys  Phe  Ser  Thr  Glu  Leu  Tyr  Gln  Gln  Leu  Asn  Asp  Leu  Glu
   GAA  CAG  AGC  CTC  CTA  GAA  AAA  TTT  TCC  ACT  GAA  CTT  TAC  CAG  CAA  CTG  AAT  GAC  CTG  GAA

121 Ala  Cys  Val  Ile  Gln  Val  Gly  Val  Glu  Thr  Pro  Leu  Met  Asn  Glu  Asp  Ser  Ile
   GCA  TGT  GTG  ATA  CAG  GAG  GGT  GGG  GAA  GAG  ACT  CCC  CTG  ATG  AAT  GAG  GAC  TCC  ATC

141 Leu  Ala  Val  Arg  Lys  Tyr  Phe  Gln  Arg  Ile  Thr  Leu  Tyr  Leu  Thr  Glu  Lys  Tyr  Ser
   CTG  GCT  GTG  AGG  AAA  TAC  TCC  CAA  AGA  ACT  ATC  CTG  TAT  CTA  ACA  GAG  AAG  AAA  TAC  AGC

161 Pro  Cys  Ala  Trp  Glu  Val  Val  Arg  Ala  Glu  Ile  Met  Arg  Ser  Leu  Ser  Phe  Ser  Thr  Asn
   CCT  TGT  GCC  TGG  GAG  GGT  GTC  AGA  GCA  GAA  ATC  ATG  AGA  TCC  CTC  TCG  TTT  TCA  ACA  AAC

181 Leu  Gln  Lys  Arg  Leu  Arg  Arg  Lys  Asp
   TTG  CAA  AAA  AGA  TTA  AGG  AGG  AAG  GAT

Figure 5
Figure 6
IFN-α76 Expression Plasmid

Figure 7
INTERNATIONAL SEARCH REPORT

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)  
According to International Patent Classification (IPC) or to both National Classification and IPC

IPC³: C 12 N 15/00; C 07 C 103/52; C 12 P 21/02; A 61 K 45/02; 
C 07 H 21/04; C 12 N 1/20 // C 12 R 1/19

II. FIELDS SEARCHED

<table>
<thead>
<tr>
<th>Classification System</th>
<th>Classification Symbols</th>
</tr>
</thead>
<tbody>
<tr>
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<td>C 07 C; C 12 N; A 61 K; C 12 R</td>
</tr>
</tbody>
</table>

Documentation Searched other than Minimum Documentation to the extent that such Documents are Included in the Fields Searched

III. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of Document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to Claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>Nature, volume 290, 5 March 1981. D. Goeddel et al.: &quot;The structure of eight distinct cloned human leukocyte interferon C DNA's&quot; pages 20-26, see the entire document</td>
<td>1,4,8-12</td>
</tr>
<tr>
<td>Y</td>
<td>Nature, volume 287, 2 October 1980, G. Allen et al.: &quot;A family of structural genes for human lymphoblastoid (leukocyte-type) interferon&quot;, pages 408-411, see the entire document</td>
<td>1,2,4</td>
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<tr>
<td>Y</td>
<td>Science, volume 212, 5 June 1981, R. M. Lawn et al.: &quot;DNA Sequence of two closely linked human leukocyte interferon genes&quot;, pages 1159-1162, see the entire document</td>
<td></td>
</tr>
</tbody>
</table>

* Special categories of cited documents:  
* A* document defining the general state of the art which is not considered to be of particular relevance  
* E* earlier document but published on or after the international filing date  
* L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)  
* O* document referring to an oral disclosure, use, exhibition or other means  
* P* document published prior to the international filing date but later than the priority date claimed  
* T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention  
* X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step  
* Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art  
* A* document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search  19th April 1983

Date of Mailing of this International Search Report  11 May 1983

International Searching Authority  
EUROPEAN PATENT OFFICE

Signature of Authorized Officer  
G. L. M. Kruidenberg
FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y EP, A, 0042246 (Cancer Institute of Japanese Foundation for Cancer Research) 23 December 1981, see claims 1-8

1,2,4,8-12

V. OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. Claim numbers because they relate to subject matter not required to be searched by this Authority, namely:

   o) 23-27 (PCT Rule 39.1.iv)

2. Claim numbers because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

VI. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This International Searching Authority found multiple Inventions in this International application as follows:

1. As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application.

2. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the International application for which fees were paid, specifically claims:

3. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/1/10 (supplementary sheet 2) (October 1981)