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Schneider-Fresenius et al.

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(54) **RECOMBINANT HUMAN BETA INTERFERON WITH ENHANCED SOLUBILITY**

(58) **Field of Search** 530/351; 424/85.4, 424/85.6; 435/69.51, 71.1

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(73) **Assignee:** **Fraunhofer-Gesellschaft zur Forderung der Angewandten Forschung e.V.**, Munich (DE)

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(*) **Notice:** Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

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(21) **Appl. No.:** **09/403,532**

* cited by examiner

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Primary Examiner—Yvonne Eyler

(86) **PCT No.:** **PCT/EP98/02238**

Assistant Examiner—Janet L. Andres

§ 371 (c)(1),
(2), (4) **Date:** **Feb. 22, 2000**

(74) *Attorney, Agent, or Firm*—Barnes & Thornburg

(87) **PCT Pub. No.:** **WO98/48018**

PCT Pub. Date: **Oct. 29, 1998**

(57) **ABSTRACT**

(30) **Foreign Application Priority Data**

Apr. 23, 1997 (DE) 197 17 864

The invention relates to variants of recombinant human beta interferon and to a method for the production thereof, wherein at least one of the following amino acids Leu(5), Phe(8), Phe(15), Leu(47), Phe(50), Leu(106), Phe(111), Leu(116), Leu(120) and Phe(156) is exchanged with hydrophilic amino acid with a hydroxy group, specially serine, tyrosine or threonine, resulting in enhanced hydrophilic property of the protein surface.

(51) **Int. Cl.⁷** **A61K 38/21**; C07K 14/00; C12P 21/04

(52) **U.S. Cl.** **424/85.6**; 424/85.4; 530/351; 435/69.51; 435/71.1

9 Claims, 4 Drawing Sheets

Met 1	Ser	Tyr	Asn	Leu 5	Leu	Gly	Phe	Leu	Gln 10	Arg	Ser	
Ser	Asn	Phe 15	Gln	Cys	Gln	Lys	Leu 20	Leu	Trp	Gln	Leu	Asn 25
Gly	Arg	Leu	Glu	Tyr 30	Cys	Leu	Lys	Asp	Arg 35	Met	Asn	Phe
Asp	Ile 40	Pro	Glu	Glu	Ile	Lys 45	Gln	Leu	Gln	Gln	Phe 50	Gln
Lys	Glu	Asp	Ala 55	Ala	Leu	Thr	Ile	Tyr 60	Glu	Met	Leu	Gln
Asn 65	Ile	Phe	Ala	Ile	Phe 70	Arg	Gln	Asp	Ser	Ser 75	Ser	Thr
Gly	Trp	Asn 80	Glu	Thr	Ile	Val	Glu 85	Asn	Leu	Leu	Ala	Asn 90
Val	Tyr	His	Gln	Ile 95	Asn	His	Leu	Lys	Thr 100	Val	Leu	Glu
Glu	Lys 105	Leu	Glu	Lys	Glu	Asp 110	Phe	Thr	Arg	Gly	Lys 115	Leu
Met	Ser	Ser	Leu 120	His	Leu	Lys	Arg	Tyr 125	Tyr	Gly	Arg	Ile
Leu 130	His	Tyr	Leu	Lys	Ala 135	Lys	Glu	Tyr	Ser	His 140	Cys	Ala
Trp	Thr	Ile 145	Val	Arg	Val	Glu	Ile 150	Leu	Arg	Asn	Phe	Tyr 155
Phe	Ile	Asn	Arg	Leu 160	Thr	Gly	Tyr	Leu	Arg 165	Asn		

(SEQ ID No. 1)

Fig. 1

Met 1	Ser	Tyr	Asn	Xaa 5	Leu	Gly	Xaa	Leu	Gln 10	Arg	Ser	
Ser	Asn	Xaa 15	Gln	Cys	Gln	Lys	Leu 20	Leu	Trp	Gln	Leu	Asn 25
Gly	Arg	Leu	Glu	Tyr 30	Cys	Leu	Lys	Asp	Arg 35	Met	Asn	Phe
Asp	Ile 40	Pro	Glu	Glu	Ile	Lys 45	Gln	Xaa	Gln	Gln	Xaa 50	Gln
Lys	Glu	Asp	Ala 55	Ala	Leu	Thr	Ile	Tyr 60	Glu	Met	Leu	Gln
Asn 65	Ile	Phe	Ala	Ile	Phe 70	Arg	Gln	Asp	Ser	Ser 75	Ser	Thr
Gly	Trp	Asn 80	Glu	Thr	Ile	Val	Glu 85	Asn	Leu	Leu	Ala	Asn 90
Val	Tyr	His	Gln	Ile 95	Asn	His	Leu	Lys	Thr 100	Val	Leu	Glu
Glu	Lys 105	Xaa	Glu	Lys	Glu	Asp 110	Xaa	Thr	Arg	Gly	Lys 115	Xaa
Met	Ser	Ser	Xaa 120	His	Leu	Lys	Arg	Tyr 125	Tyr	Gly	Arg	Ile
Leu 130	His	Tyr	Leu	Lys	Ala 135	Lys	Glu	Tyr	Ser	His 140	Cys	Ala
Trp	Thr	Ile 145	Val	Arg	Val	Glu	Ile 150	Xaa	Arg	Asn	Phe	Tyr 155
Phe	Ile	Asn	Arg	Leu 160	Thr	Gly	Tyr	Leu	Arg 165	Asn		

(SEQ ID No. 2)

Fig. 2

Met 1	Ser	Tyr	Asn	Ser 5	Leu	Gly	Ser	Leu	Gln 10	Arg	Ser	
Ser	Asn	Ser 15	Gln	Cys	Gln	Lys	Leu 20	Leu	Trp	Gln	Leu	Asn 25
Gly	Arg	Leu	Glu	Tyr 30	Cys	Leu	Lys	Asp	Arg 35	Met	Asn	Phe
Asp	Ile 40	Pro	Glu	Glu	Ile	Lys 45	Gln	Ser	Gln	Gln	Ser 50	Gln
Lys	Glu	Asp	Ala 55	Ala	Leu	Thr	Ile	Tyr 60	Glu	Met	Leu	Gln
Asn 65	Ile	Phe	Ala	Ile	Phe 70	Arg	Gln	Asp	Ser	Ser 75	Ser	Thr
Gly	Trp	Asn 80	Glu	Thr	Ile	Val	Glu 85	Asn	Leu	Leu	Ala	Asn 90
Val	Tyr	His	Gln	Ile 95	Asn	His	Leu	Lys	Thr 100	Val	Leu	Glu
Glu	Lys 105	Ser	Glu	Lys	Glu	Asp 110	Ser	Thr	Arg	Gly	Lys 115	Ser
Met	Ser	Ser	Ser 120	His	Leu	Lys	Arg	Tyr 125	Tyr	Gly	Arg	Ile
Leu 130	His	Tyr	Leu	Lys	Ala 135	Lys	Glu	Tyr	Ser	His 140	Cys	Ala
Trp	Thr	Ile 145	Val	Arg	Val	Glu	Ile 150	Leu	Arg	Asn	Phe	Tyr 155
Ser	Ile	Asn	Arg	Leu 160	Thr	Gly	Tyr	Leu	Arg 165	Asn		

(SEQ ID No. 3)

Fig. 3

L5	5'-CT <u>CC</u> CTTGGATTCTACAAAGAAGC-3'	25 (SEQ ID No. 4)
F8	5'-CTTGCTTGGAT <u>CC</u> CTACAAAGAAGC-3'	25 (SEQ ID No. 5)
F15/C17	5'-AGCAGCAATTCTCAGT <u>CC</u> CAGAAGCTCC-3'	28 (SEQ ID No. 6)
C17	5'-AGCAGCAATTTTCAGT <u>CC</u> CAGAAGCTCC-3'	28 (SEQ ID No. 7)
L47	5'-TTAAGCAGT <u>CC</u> CAGCAGTTCCAGAAGG-3'	27 (SEQ ID No. 8)
F50	5'-TTAAGCAGCTGCAGCAGT <u>CC</u> CAGAAGG-3'	27 (SEQ ID No. 9)
L106	5'-GAAGAAAAA <u>TCC</u> GAGAAAGAAGATTTCACC-3'	30 (SEQ ID No.10)
F111	5'-GAAGAAAAACTGGAGAAAGAAGATT <u>CC</u> CACC-3'	30 (SEQ ID No.11)
L116	5'-AAAA <u>TCC</u> ATGAGCAGTCTGCACCTG-3'	25 (SEQ ID No.12)
L120	5'-AAA <u>ACT</u> CATGAGCAGT <u>TCCC</u> ACCTG-3'	25 (SEQ ID No.13)
F156	5'-ACTTTTACT <u>CC</u> ATTAACAGACCTACAGG-3'	28 (SEQ ID No.14)
L5/F3Rev	5'-TTGTAGCTCATATGTAAGTATTTCC-3'	25 (SEQ ID No.15)
F15/C17Rev	5'-TCTTTGTAGGAATCCAAGCAAGTTGTAGC-3'	29 (SEQ ID No.16)
L47/F50Rev	5'-TCTCCTCAGGGATGTCAAAGTTCATCC-3'	27 (SEQ ID No.17)
L106/F111Rev	5'-CAGGACTGTCTTCAGATGGTTTATCTG-3'	27 (SEQ ID No.18)
L116/L120Rev	5'-CCCCTGGTGAAATCTTCTTTCTC-3'	23 (SEQ ID No.19)
F156Rev	5'-TTCTTAGGATTTCCACTCTGACTATGG-3'	27 (SEQ ID No.20)
L5-C17Rev	5'-TCTTTGTAGG <u>GATCCAAGGG</u> AGTTGTAGC-3'	29 (SEQ ID No.21)
L106-L130Rev	5'-CCCCTGGTG <u>GAA</u> TCTTCTTTCTC <u>GGA</u> -3'	26 (SEQ ID No.22)

Fig. 4

RECOMBINANT HUMAN BETA INTERFERON WITH ENHANCED SOLUBILITY

CROSS-REFERENCES TO RELATED APPLICATIONS

This application is a U.S. national application of international application serial No. PCT/EP98/02238 filed Apr. 16, 1998, which claims priority to German serial No. 197 17 864.2 filed Apr. 23, 1997.

The invention pertains to variants of human recombinant beta-interferon with improved solubility.

Beta-interferon is a regulatory protein which leads to activation of genes by binding receptors. As a result, antiviral, antiproliferative and further biological activities are mediated in the cell.

The inteferons, as is also the case with the interleukins, belong to the class of cytokines and are subdivided into different classes:

Type I interferon (alpha, beta, omega, tau) and Type II (gamma)

Human beta-interferon is a protein with a molecular weight of 22 kDa and 166 amino acid residues. It is formed primarily in fibroblasts during attack by a virus and possesses antiviral, antiproliferative and further biological activities. The amino acid sequence of human beta-interferon was first published by Taniguchi et al. (1980), Gene Ed. 10, pages 11 through 15, and is illustrated in FIG. 1.

Beta-interferon, which is produced from bacterial cells or mammalian cells by genetic engineering, is being used successfully in the treatment of multiple sclerosis, a previously incurable disease in a large group of patients. However, the very high hydrophobicity of the protein, which causes very poor solubility of recombinant human beta-interferon, proves to be problematical for the production and processing of recombinant human beta-interferon.

The problem for the present invention is to make available variants of recombinant human beta-interferon whose solubility is improved in polar media, such as e.g. aqueous liquids. In addition an objective of this invention is to indicate processes for manufacturing and possibilities for using variants of recombinant human beta-interferon with higher solubility in polar media such as aqueous liquids.

This problem is solved by the recombinant human beta-interferon in accordance with claim 1, its use in accordance with claim 5 and its manufacture in accordance with claim 6 or 7.

In accordance with claim 1, at least one of the following ten hydrophobic amino acids in known human beta-interferon is exchanged for a hydrophilic amino acid: Leu 5, Phe 8, Phe 15, Leu 47, Phe 50, Leu 106, Phe 111, Leu 120 or Phe 156 (SED ID No.2). Thus the invention pertains to individual mutations as well as to all the possible combinations of these individual amino acid exchanges.

The designated amino acids are essentially located on the surface of human beta-interferon and they take up a relatively large proportion of the surface there. The exchange of these amino acids therefore leads to more than a proportionately large improvement in the hydrophilic character of the surface of recombinant human beta-interferon and it therefore increases the solubility of this protein in polar media, such as e.g. aqueous liquids. As a result of its increased hydrophilicity, the recombinant human beta-

interferon in accordance with the invention is considerably simpler to handle in production as well as during its processing to give an active substance.

The production of the variants of recombinant human beta-interferon in accordance with the invention takes place in a generally known, conventional way with the help of microorganisms, e.g. in an *Escherichia coli* culture which has been provided with the gene for one of the proteins in accordance with the invention. The production of these microorganisms, which have been changed by means of genetic engineering, also takes place in a generally known way with the help of classical genetic engineering mutagenesis procedures for the exchange of the corresponding amino acids for hydrophilic amino acids and their synthesis will therefore be dispensed with at this juncture.

The proteins in accordance with the invention find use for the manufacture of medicinal drugs, e.g. for combatting multiple sclerosis, as well as fine chemicals for in vitro experiments or for measurements of interferon levels. The improved hydrophilicity of these proteins thereby simplifies their manufacture, transportation, storage and application in the form of a medicinal drug or fine chemical.

Advantageous further developments of the proteins in accordance with the invention are given in the dependent claims.

Exchange for the amino acids serine, tyrosine and threonine is especially advantageous and, with one hydroxy group each, these are especially hydrophilic.

As a result of its small size, serine is especially suitable for exchange since especially slight steric changes in the protein are associated with it.

The amino acid sequence of native recombinant human beta-interferon, in which the abovementioned ten amino acids are electively exchanged for serine, is illustrated in FIG. 2. These exchangeable amino acids are represented by Xaa. If these amino acids are exchanged, then the hydrophilicity of the surface of recombinant human beta-interferon is very much improved whereas only slight impairment arises in terms of the functionality and the efficacy of human recombinant beta-interferon.

An especially advantageous further development is illustrated in FIG. 3 in which all of the abovementioned ten amino acids have been exchanged here for serine so that an especially marked increase in the hydrophilicity of the surface of recombinant human beta-interferon results.

An example of a variant of human recombinant beta-interferon in accordance with the invention is given in the following sections:

FIG. 1 shows native human recombinant beta-interferon;

FIG. 2 shows the variants of recombinant human beta-interferon in accordance with the invention;

FIG. 3 shows a recombinant human beta-interferon [variant] in accordance with the invention; and

FIG. 4 shows primers for the mutagenesis for the manufacture of beta-interferon in accordance with the invention.

As already described above, FIG. 1 describes native human recombinant beta-interferon in the form in which it can already be manufactured currently with the help of known molecular biological and bio-technical possibilities.

The sequence of native human recombinant beta-interferon is illustrated in FIG. 2 in which Xaa designates those amino acids which can be exchanged for an amino acid with at least one hydroxy group (advantageously serine, tyrosine and/or threonine) and hence result in a recombinant human beta-interferon [variant] in accordance with the invention which possesses increased surface hydrophilicity.

By way of example, all ten individual variants of beta-interferon have been manufactured in which Leu (5), Phe (8), Phe (15), Leu (47), Phe (50), Leu (106), Phe (111), Leu (116), Leu (120) or Phe (156) (SEQ ID No.2) were exchanged for serine, whereby, in particular, the variants with exchange of the amino acids Leu (5), Phe (8), Leu (47), Phe (50), Leu (106), Phe (111), Leu (116) and Leu (120) (SEQ ID No.26) relative to native beta-interferon, and also these variants of the Cys-17-Ser variant of human beta-interferon, excel by virtue of comparable biological activity.

FIG. 3 shows an example of a human recombinant beta-interferon [variant] in which the following amino acids have been exchanged for serine:

Leu (5), Phe (8), Phe (15), Leu (47), Phe (50), Leu (106), Phe (111), Leu (116), Leu (120) and Phe (156) (SEQ ID No.3). In the case of this variant of recombinant human beta-interferon, especially high hydrophilicity of the surface of the protein results and, hence, especially good solubility in aqueous solutions.

For example, the multiple variant with amino acid exchanges at positions 4, 50, 106, 111, 116 and 120 (SEQ ID No.23) also shows activity which corresponds to the beta-interferon starting protein, with a Cys17Ser exchange (SEQ ID No.24), which was used in this example.

The manufacture of organisms with a gene, which has been changed in accordance with the invention, for human recombinant beta-interferon takes place by means of classical mutagenesis procedures.

Mutagenesis takes place by means of a PCR (polymerase chain reaction). The mutations are introduced via synthetic oligonucleotides. Plasmid DNA with the beta-INF gene serves as the template. The entire plasmid was replicated with the PCR method which was used here. The selection of the PCR fragments takes place by means of restriction digestion of the entire PCR mixture with the enzyme DpnI. This enzyme recognizes only methylated interfaces. Since fragments, which are produced in vitro by means of PCR, are unmethylated, only the template DNA is degraded by

DpnI. After DpnI digestion, in the event of success, a fragment with the length of the linearized template remains behind and this carries the mutations. The PCR fragments that are produced are cloned and sequenced.

PCR mixture (100 µl):		
template DNA	10 µg	
primers	100 pmol each	
nucleotide mix	200 µM per dNTP	
MgSO ₄	2-6 mM	
DNA polymerase	2.5 units	

PCR protocol	Duration	Temperature
step 1.	4 min	95° C.
step 2.	+ enzyme	
step 3.	45 sec	95° C.
step 4.	1 min	55° C.
step 5.	10 min	68° C.
		go to 3.(11x)
step 6.	10 min	68° C.
step 7.		4° C.

The PCR was carried out in a Thermocycler PTC-200 (MJ Research company).

Two primers are necessary for each PCR, namely a "forward" primer and a "reverse" primer. The two primers follow one another directly but, in each case, they bind to different strands with the opposite orientation.

FIG. 4 illustrates primers which were used for the mutagenesis in order that the amino acid(s), that are designated on the left, be exchanged for serine in the gene product, i.e. the beta-interferon in accordance with the invention.

The last two primers alone contain reverse primer mutations. A PCR with these primers additionally introduces mutations without the previously inserted mutations 5/8 or 106/111 being relinquished. If the wild type is taken as a template, then four mutations can be introduced at the same time via the reverse primers (5-17 or as the case may be, 106-120).

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 26

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<211> LENGTH: 166

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

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 1 5 10 15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
 20 25 30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
 35 40 45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
 50 55 60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
 65 70 75 80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
 85 90 95

-continued

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His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
      100                      105                      110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
      115                      120                      125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
      130                      135                      140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
      145                      150                      155                      160

Thr Gly Tyr Leu Arg Asn
      165

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<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa = Phe, Ser, Tyr, or Thr
<220> FEATURE:
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<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa = Phe, Ser, Tyr, or Thr
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<223> OTHER INFORMATION: Xaa = Leu, Ser, Tyr, or Thr
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<222> LOCATION: (50)..(50)
<223> OTHER INFORMATION: Xaa = Phe, Ser, Tyr, or Thr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (106)..(106)
<223> OTHER INFORMATION: Xaa = Leu, Ser, Tyr, or Thr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (111)..(111)
<223> OTHER INFORMATION: Xaa = Phe, Ser, Tyr, or Thr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (116)..(116)
<223> OTHER INFORMATION: Xaa = Leu, Ser, Tyr, or Thr
<220> FEATURE:
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<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (151)..(151)
<223> OTHER INFORMATION: Xaa = Leu, Ser, Tyr, or Thr

<400> SEQUENCE: 2

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Met Ser Tyr Asn Xaa Leu Gly Xaa Leu Gln Arg Ser Ser Asn Xaa Gln
 1      5      10      15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
 20      25      30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Xaa Gln
 35      40      45

Gln Xaa Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
 50      55      60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
 65      70      75      80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn

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-continued

	85								90							95
His	Leu	Lys	Thr	Val	Leu	Glu	Glu	Lys	Xaa	Glu	Lys	Glu	Asp	Xaa	Thr	
				100					105					110		
Arg	Gly	Lys	Xaa	Met	Ser	Ser	Xaa	His	Leu	Lys	Arg	Tyr	Tyr	Gly	Arg	
		115					120						125			
Ile	Leu	His	Tyr	Leu	Lys	Ala	Lys	Glu	Tyr	Ser	His	Cys	Ala	Trp	Thr	
	130					135					140					
Ile	Val	Arg	Val	Glu	Ile	Xaa	Arg	Asn	Phe	Tyr	Phe	Ile	Asn	Arg	Leu	
145					150					155					160	
Thr	Gly	Tyr	Leu	Arg	Asn											
			165													

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 <211> LENGTH: 166
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 3

Met	Ser	Tyr	Asn	Ser	Leu	Gly	Ser	Leu	Gln	Arg	Ser	Ser	Asn	Ser	Gln
1				5					10					15	
Cys	Gln	Lys	Leu	Leu	Trp	Gln	Leu	Asn	Gly	Arg	Leu	Glu	Tyr	Cys	Leu
			20					25					30		
Lys	Asp	Arg	Met	Asn	Phe	Asp	Ile	Pro	Glu	Glu	Ile	Lys	Gln	Ser	Gln
		35					40					45			
Gln	Ser	Gln	Lys	Glu	Asp	Ala	Ala	Leu	Thr	Ile	Tyr	Glu	Met	Leu	Gln
		50				55					60				
Asn	Ile	Phe	Ala	Ile	Phe	Arg	Gln	Asp	Ser	Ser	Thr	Gly	Trp	Asn	
65					70					75				80	
Glu	Thr	Ile	Val	Glu	Asn	Leu	Leu	Ala	Asn	Val	Tyr	His	Gln	Ile	Asn
				85					90					95	
His	Leu	Lys	Thr	Val	Leu	Glu	Glu	Lys	Ser	Glu	Lys	Glu	Asp	Ser	Thr
			100						105					110	
Arg	Gly	Lys	Ser	Met	Ser	Ser	Ser	His	Leu	Lys	Arg	Tyr	Tyr	Gly	Arg
		115					120						125		
Ile	Leu	His	Tyr	Leu	Lys	Ala	Lys	Glu	Tyr	Ser	His	Cys	Ala	Trp	Thr
	130					135					140				
Ile	Val	Arg	Val	Glu	Ile	Leu	Arg	Asn	Phe	Tyr	Ser	Ile	Asn	Arg	Leu
145					150					155					160
Thr	Gly	Tyr	Leu	Arg	Asn										
			165												

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 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Primer for
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 substitute a serine for another amino acid
 <220> FEATURE:
 <221> NAME/KEY: mutation
 <222> LOCATION: (3)..(4)
 <223> OTHER INFORMATION: Leu to Ser mutation

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ctcccttgga ttctacaaa gaagc

25

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<212> TYPE: DNA
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 substitute a serine for another amino acid
 <220> FEATURE:
 <221> NAME/KEY: mutation
 <222> LOCATION: (12)..(12)
 <223> OTHER INFORMATION: Phe to Ser mutation

 <400> SEQUENCE: 5

 cttgcttgga tccctacaaa gaagc 25

 <210> SEQ ID NO 6
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 <212> TYPE: DNA
 <213> ORGANISM: artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Primer for
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 substitute a serine for another amino acid
 <220> FEATURE:
 <221> NAME/KEY: mutation
 <222> LOCATION: (11)..(11)
 <223> OTHER INFORMATION: Phe to Ser mutation
 <220> FEATURE:
 <221> NAME/KEY: mutation
 <222> LOCATION: (17)..(18)
 <223> OTHER INFORMATION: Cys to Ser mutation

 <400> SEQUENCE: 6

 agcagcaatt ctcaagtccca gaagctcc 28

 <210> SEQ ID NO 7
 <211> LENGTH: 28
 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
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 <223> OTHER INFORMATION: Description of Artificial Sequence: Primer for
 Homo sapiens beta -interferon, wherein nucleotides are altered to
 substitute a serine for another amino acid
 <220> FEATURE:
 <221> NAME/KEY: mutation
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 <223> OTHER INFORMATION: Cys to Ser mutation

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 <211> LENGTH: 27
 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Primer for
 Homo sapiens beta-interferon, wherein nucleotides are altered to
 substitute a serine for another amino acid
 <220> FEATURE:
 <221> NAME/KEY: mutation
 <222> LOCATION: (9)..(11)
 <223> OTHER INFORMATION: Leu to Ser mutation

 <400> SEQUENCE: 8

 ttaagcagtc ccagcagttc cagaagg 27

 <210> SEQ ID NO 9
 <211> LENGTH: 27
 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:

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<223> OTHER INFORMATION: Description of Artificial Sequence: Primer for
Homo sapiens beta -interferon, wherein nucleotides are altered to
substitute a serine for another amino acid

<220> FEATURE:
<221> NAME/KEY: mutation
<222> LOCATION: (19)..(19)
<223> OTHER INFORMATION: Phe to Ser mutation

<400> SEQUENCE: 9

ttaagcagct gcagcagtcc cagaagg 27

<210> SEQ ID NO 10
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer for
Homo sapiens beta -interferon, wherein nucleotides are altered to
substitute a serine for another amino acid

<220> FEATURE:
<221> NAME/KEY: mutation
<222> LOCATION: (10)..(12)
<223> OTHER INFORMATION: Leu to Ser mutation

<400> SEQUENCE: 10

gaagaaaaat ccgagaaaga agatttcacc 30

<210> SEQ ID NO 11
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer for
Homo sapiens beta -interferon, wherein nucleotides are altered to
substitute a serine for another amino acid

<220> FEATURE:
<221> NAME/KEY: mutation
<222> LOCATION: (26)..(26)
<223> OTHER INFORMATION: Phe to Ser mutation

<400> SEQUENCE: 11

gaagaaaaac tggagaaaga agattccacc 30

<210> SEQ ID NO 12
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer for
Homo sapiens beta -interferon, wherein nucleotides are altered to
substitute a serine for another amino acid

<220> FEATURE:
<221> NAME/KEY: mutation
<222> LOCATION: (5)..(6)
<223> OTHER INFORMATION: Leu to Ser mutation

<400> SEQUENCE: 12

aaaatccatg agcagtctgc acctg 25

<210> SEQ ID NO 13
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer for
Homo sapiens beta -interferon, wherein nucleotides are altered to
substitute a serine for another amino acid

<220> FEATURE:
<221> NAME/KEY: mutation
<222> LOCATION: (17)..(19)
<223> OTHER INFORMATION: Leu to Ser mutation

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<400> SEQUENCE: 13

aaaactcatg agcagttccc acctg 25

<210> SEQ ID NO 14

<211> LENGTH: 28

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Primer for
Homo sapiens beta -interferon, wherein nucleotides are altered to
substitute a serine for another amino acid

<220> FEATURE:

<221> NAME/KEY: mutation

<222> LOCATION: (10)..(10)

<223> OTHER INFORMATION: Phe to Ser mutation

<400> SEQUENCE: 14

acttttactc cattaacaga cctacagg 28

<210> SEQ ID NO 15

<211> LENGTH: 25

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Reverse
primer for Homo sapiens beta-interferon, wherein nucleotides in
the forward primer are altered to substitute a serine for another
amino acid

<400> SEQUENCE: 15

ttgtagctca tatgtaagta ttccc 25

<210> SEQ ID NO 16

<211> LENGTH: 29

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Reverse
primer for Homo sapiens beta-interferon, wherein nucleotides in
the forward primer are altered to substitute a serine for another
amino acid

<400> SEQUENCE: 16

tctttgtagg aatccaagca agttgtagc 29

<210> SEQ ID NO 17

<211> LENGTH: 27

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Reverse
primer for Homo sapiens beta-interferon, wherein nucleotides in
the forward primer are altered to substitute a serine for another
amino acid

<400> SEQUENCE: 17

tctcctcagg gatgtcaaag ttcattc 27

<210> SEQ ID NO 18

<211> LENGTH: 27

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Reverse
primer for Homo sapiens beta-interferon, wherein nucleotides in
the forward primer are altered to substitute a serine for another
amino acid

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<400> SEQUENCE: 18

caggactgtc ttcagatggt ttatctg

27

<210> SEQ ID NO 19

<211> LENGTH: 23

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Reverse primer for Homo sapiens beta-interferon, wherein nucleotides in the forward primer are altered to substitute a serine for another amino acid

<400> SEQUENCE: 19

cccctggtga aatcttcttt ctc

23

<210> SEQ ID NO 20

<211> LENGTH: 27

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Reverse primer for Homo sapiens beta-interferon, wherein nucleotides in the forward primer are altered to substitute a serine for another amino acid

<400> SEQUENCE: 20

ttcttaggat ttccactctg actatgg

27

<210> SEQ ID NO 21

<211> LENGTH: 29

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Reverse primer for Homo sapiens beta-interferon, wherein nucleotides are altered to substitute a serine for another amino acid

<220> FEATURE:

<221> NAME/KEY: mutation

<222> LOCATION: (11)..(11)

<223> OTHER INFORMATION: Leu to Ser mutation

<220> FEATURE:

<221> NAME/KEY: mutation

<222> LOCATION: (19)..(20)

<223> OTHER INFORMATION: Cys to Ser mutation

<400> SEQUENCE: 21

tctttgtagg gatccaaggg agttgtagc

29

<210> SEQ ID NO 22

<211> LENGTH: 26

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Reverse primer for Homo sapiens beta-interferon, wherein nucleotides are altered to substitute a serine for another amino acid

<220> FEATURE:

<221> NAME/KEY: mutation

<222> LOCATION: (10)..(10)

<223> OTHER INFORMATION: Leu to Ser mutation

<220> FEATURE:

<221> NAME/KEY: mutation

<222> LOCATION: (24)..(26)

<223> OTHER INFORMATION: Leu to Ser mutation

<400> SEQUENCE: 22

cccctggtgg aatcttcttt ctcgga

26

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<210> SEQ ID NO 23
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 23

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
1          5          10          15
Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
          20          25          30
Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Ser Gln
          35          40          45
Gln Ser Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
          50          55          60
Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65          70          75          80
Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
          85          90          95
His Leu Lys Thr Val Leu Glu Glu Lys Ser Glu Lys Glu Asp Ser Thr
          100          105          110
Arg Gly Lys Ser Met Ser Ser Ser His Leu Lys Arg Tyr Tyr Gly Arg
          115          120          125
Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
          130          135          140
Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
          145          150          155          160
Thr Gly Tyr Leu Arg Asn
          165

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<210> SEQ ID NO 24
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 24

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
1          5          10          15
Ser Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
          20          25          30
Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Ser Gln
          35          40          45
Gln Ser Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
          50          55          60
Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65          70          75          80
Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
          85          90          95
His Leu Lys Thr Val Leu Glu Glu Lys Ser Glu Lys Glu Asp Ser Thr
          100          105          110
Arg Gly Lys Ser Met Ser Ser Ser His Leu Lys Arg Tyr Tyr Gly Arg
          115          120          125
Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
          130          135          140
Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
          145          150          155          160
Thr Gly Tyr Leu Arg Asn

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165

<210> SEQ ID NO 25
 <211> LENGTH: 166
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 25

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Met Ser Tyr Asn Ser Leu Gly Ser Leu Gln Arg Ser Ser Asn Phe Gln
1          5          10          15
Ser Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
20          25          30
Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Ser Gln
35          40          45
Gln Ser Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
50          55          60
Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65          70          75          80
Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
85          90          95
His Leu Lys Thr Val Leu Glu Glu Lys Ser Glu Lys Glu Asp Ser Thr
100         105         110
Arg Gly Lys Ser Met Ser Ser Ser His Leu Lys Arg Tyr Tyr Gly Arg
115         120         125
Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
130         135         140
Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145         150         155         160
Thr Gly Tyr Leu Arg Asn
165

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<210> SEQ ID NO 26
 <211> LENGTH: 166
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 26

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Met Ser Tyr Asn Ser Leu Gly Ser Leu Gln Arg Ser Ser Asn Phe Gln
1          5          10          15
Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
20          25          30
Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Ser Gln
35          40          45
Gln Ser Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
50          55          60
Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65          70          75          80
Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
85          90          95
His Leu Lys Thr Val Leu Glu Glu Lys Ser Glu Lys Glu Asp Ser Thr
100         105         110
Arg Gly Lys Ser Met Ser Ser Ser His Leu Lys Arg Tyr Tyr Gly Arg
115         120         125
Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
130         135         140
Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu

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145

150

155

160

Thr Gly Tyr Leu Arg Asn
165

What is claimed is:

1. A human recombinant beta-interferon wherein the amino acids Leu (47), Phe (50), Leu (106), Phe (111), Leu (116), and Leu (120) have been exchanged for serine and comprises the sequence as set forth in (SEQ ID No.23).

2. A human recombinant beta-interferon wherein the amino acids Cys (17), Leu (47), Phe (50), Leu (106), Phe (111), Leu (116), and Leu (120) have been exchanged for serine and comprises the sequence as set forth in (SEQ ID No.24).

3. A human recombinant beta-interferon wherein the amino acids Leu (5), Phe, (8) Cys (17), Leu (47), Phe (50), Leu (106), Phe (111), Leu (116), and Leu (120) have been exchanged for serine and comprises the sequence as set forth in (SEQ ID No.25).

4. A method of manufacturing a medicinal drug comprising the steps of:

- obtaining a quantity of the beta-interferon according to claim 1, and
- dissolving the beta-interferon in an aqueous liquid.

5. A method of manufacturing a medicinal drug comprising the steps of:

- obtaining a quantity of the beta-interferon according to claim 2, and
- dissolving the beta-interferon in an aqueous liquid.

6. A method of manufacturing a medicinal drug comprising the steps of:

- obtaining a quantity of the beta-interferon according to claim 3, and
- dissolving the beta-interferon in an aqueous liquid.

7. A microorganism having a gene coding for the recombinant human beta-interferon of claim 1.

8. A microorganism having a gene coding for the recombinant human beta-interferon of claim 2.

9. A microorganism having a gene coding for the recombinant human beta-interferon of claim 3.

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