IFNAR2 MUTANTS, THEIR PRODUCTION AND USE

FIELD OF THE INVENTION

The present invention relates to mutant polypeptides of the beta chain of the type I IFN receptor (MIFNAR2) with enhanced affinity for interferon-β as compared to the wild type protein for prolonging the effect of IFNβ *in vivo*.

BACKGROUND OF THE INVENTION

Interferons are classified either as the leukocyte and fibroblast derived Type I interferons, or as the mitogen induced or "immune" Type II interferons (Pestka et al, 1987). Through analysis of sequence identities and common biological activities, type I interferons include interferon alpha (IFN-α), interferon beta (IFN-β) and interferon omega (IFNω), while type II interferon includes interferon gamma (IFNγ).

The IFNα, IFNβ and IFNω genes are clustered on the short arm 25 of chromosome 9 (Lengyl, 1982). There are at least 25 non-allelic IFNα genes, 6 non-allelic IFNω genes and a single IFNβ gene. All are believed to have evolved from a single common ancestral gene. Within species, IFNα genes share at least 80% sequence identity with each other. The IFNβ gene shares approximately 50% sequence identity with IFNα; and the IFNω gene shares 70% homology with IFNα (Weissman et al, 1986; Dron et al, 1992). IFNα has a molecular weight range of 17-23 kDa (165-166 amino acids), IFNβ, about 23 kDa (166 amino acids) and IFNω, about 24 kDa (172 amino acids).

Type I interferons are pleiotropic cytokines having activity such as host defense against viral and parasitic infections, anti-cancer properties and as immune modulators (Baron et al, 1994; Baron et al, 1991). Type I interferon physiological responses include anti-proliferative activity on normal and transformed cells, stimulation of cytotoxic activity in lymphocytes, natural killer cells and phagocytic cells, modulation of cellular differentiation, stimulation of expression of class I MHC antigens,

inhibition of class II MHC, and modulation of a variety of cell surface receptors. Under normal physiological conditions, IFNα and IFNβ (IFNα/β) are secreted constitutively by most human cells at low levels with expression being up-regulated by addition of a variety of inducers, comprising infectious agents (viruses, bacteria, mycoplasma and protozoa), dsRNA, and cytokines (M-CSF, IL-1α, IL-2, TNFα). The actions of Type I interferon *in vivo* can be monitored using the surrogate markers, neopterin, 2', 5' oligoadenylate synthetase, and β2 microglobulin (Alam et al, 1997; Fierlbeck et al, 1996; Salmon et al, 1996).

Type I interferons (IFNα/β/ω) act through a cell surface receptor complex to induce specific biologic effects, such as anti-viral, anti-tumor, and immune modulators. The type I IFN receptor (IFNAR) is a hetero- multimeric receptor complex composed of at least two different polypeptide chains (Colamonici et al, 1992; Colamonici et al, 1993; Platanias et al, 1993). The genes coding for these chains are found on chromosome 21, and their proteins are expressed on the surface of most cells (Tan et al, 1973). The receptor chains were originally designated alpha and beta and have been renamed IFNAR1 for the alpha subunit and IFNAR2 for the beta subunit. In most cells, IFNAR1 (alpha chain, Uze subunit) (Uze et al, 1990) has a molecular weight of 100-130 kDa, while IFNAR2 (beta chain, β_L, IFNα/βR) has a molecular weight of 100 kDa. In certain cell types (monocytic cell lines and normal bone marrow cells) an alternate receptor complex has been identified, where the IFNAR2 subunit (β_S) is expressed as a truncated receptor with a molecular weight of 51 kDa. The IFNAR1 and IFNAR2 β_S and β_L subunits have been cloned (Novick et al, 1994; Domanski et al, 1995). The IFNAR2 β_S and β_L subunits have identical extracellular and transmembrane domains; however, in the cytoplasmic domain they only share identity in the first 15 amino acids. The IFNAR2 subunit alone is able to bind IFNα/β, while the IFNAR1 subunit is unable to bind IFNα/β. When the human IFNAR1 receptor subunit alone was transfected into murine L-929 fibroblasts, no human IFNas except IFNα8/IFNαB were able to bind to the cells (Uze et al, 1990). The human IFNAR2 subunit, transfected into L cells in the absence of the human IFNAR1 subunit, bind human IFNα, binding with a Kd of approximately 0.45 nM. When human IFNAR2 subunits were transfected in the presence of the human IFNAR1 subunit, high affinity binding could be shown with a Kd of 0.026-0.114 nM (Novick et al, 1994; Domanski et al, 1995). It is estimated that from 500- 20,000 high affinity and 2,000-100,000 low affinity IFN binding sites exist

on most cells. Although the IFNAR1/2 complex (α/β_S or α/β_L) subunits bind IFNα with high affinity, only the α/β_L pair appears to be a functional signaling receptor.

Transfection of the IFNAR1 and the IFNAR2 β_L subunits into mouse L-929 cells, followed by incubation with IFNα2, induces an anti-viral state, initiates intracellular protein phosphorylation, and causes the activation of intracellular kinases (Jak1 and Tyk2) and transcription factors (STAT 1, 2, and 3) (Novick et al, 1994; Domanski et al, 1995). In a corresponding experiment, transfection of the IFNAR2 βs subunit was unable to initiate a similar response. Thus, the IFNAR2 β_L subunit is required for functional activity (anti-viral response) with maximal induction occurring in association with the IFNAR1 subunit.

In addition to membrane bound cell surface IFNAR forms, a soluble IFNAR has been identified in both human urine and serum (Novick et al, 1994; Novick et al, 1995; Novick et al, 1992; Lutfalla et al, 1995). The soluble IFNAR isolated from serum has an apparent molecular weight of 55 kDa on SDS- PAGE, while the soluble IFNAR from urine has an apparent molecular weight of 40-45 kDa (p40). Transcripts for the soluble p40 IFNAR2 are present at the mRNA level and encompass almost the entire extracellular domain of the IFNAR2 subunit with two additional amino acids at the carboxy terminal end. There are five potential glycosylation sites on the soluble IFNAR2 receptor. The soluble p40 IFNAR2 has been shown to bind IFNα2 and IFNβ and to inhibit *in vitro* the anti-viral activity of a mixture of IFNα species ("leukocyte IFN") and individual Type I IFNs (Novick et al, 1995). A recombinant IFNAR2 subunit Ig fusion protein was shown to inhibit the binding of a variety of Type I IFN species (IFNαA, IFNαB, IFNαD, IFNβ, IFNα Con1 and IFNω) to Daudi cells and α/β_S subunit double transfected COS cells.

Type I IFN signaling pathways have been identified (Platanias et al, 1996; Yan et al., 1996; Qureshi et al., 1996; Duncan et al., 1996; Sharf et al, 1995; Yang et al, 1996). Initial events leading to signaling are thought to occur by the binding of IFNα/β/ω to the IFNAR2 subunit, followed by the IFNAR1 subunit associating to form an IFNAR1/2 complex (Platanias et al, 1994). The binding of IFNα/β/ω to the IFNAR1/2 complex results in the activation of two Janus kinases (Jak1 and Tyk2), which are believed to phosphorylate specific tyrosines on the IFNAR1 and IFNAR2 subunits. Once these subunits are phosphorylated, STAT molecules (STAT 1, 2 and 3) are

phosphorylated, which results in dimerization of STAT transcription complexes followed by nuclear localization of the transcription complex and the activation of specific IFN inducible genes.

A randomized, double-blinded, placebo-controlled, two-year multicenter study demonstrated that natural human fibroblast interferon (interferon beta) administered intrathecally (IT) is effective in reducing the exacerbations of exacerbating-remitting multiple sclerosis (MS). The mean reduction in exacerbation rate of 34 patients with MS who received interferon beta administered IT was significantly greater during the study than that of 35 control patients who received placebo (Jacobs et al. 1987).

The pharmacokinetics and pharmacodynamics of Type I IFNs have been assessed in humans (Alan et al, 1997; Fierlbeck et al, 1996; Salmon et al, 1996). The clearance of IFNβ is fairly rapid with the bioavailability of IFNβ lower than expected for most cytokines. Although the pharmacodynamics of IFNβ has been assessed in humans, no clear correlation has been established between the bioavailability of IFNβ and clinical efficacy. In normal healthy human volunteers, administration of a single intravenous (iv) bolus dose (6 MIU) of recombinant CHO derived IFNβ resulted in a rapid distribution phase of 5 minutes and a terminal half-life of about 5 hours (Alam et al, 1997). Following subcutaneous (sc) or intramuscular (im) administration of IFNβ, serum levels are flat with only about 15% of the dose systemically available. The pharmacodynamics of IFNβ following iv, im or sc administration (as measured by changes in 2'5/-oligoadenylate synthetase (2', 5'-AS) activity in PBMCs) were elevated within the first 24 hours and slowly decreased to baseline levels over the next 4 days. The magnitude and duration of the biologic effect was the same regardless of the route of administration.

A multiple dose pharmacodynamic study of IFNβ has been conducted in human melanoma patients (Fierlbeck et al, 1996) with IFNβ being administrated by sc route, three times per week at 3 MIU/dose over a six-month period. The pharmacodynamic markers, 2', 5' -AS synthetase, β_2- microglobulin, neopterin, and NK cell activation peaked by the second injection (day 4) and dropped off by 28 days, remaining only slightly elevated out to six months.

Purification and refolding of the extracellular part of human IFNAR2 (IFNAR2-EC) expressed in *Escherichia coli* and its characterization with respect to its interaction

with interferon alpha2 (IFNα2) has been reported (Piehler and Schreiber 199A). The 25 kDa, non-glycosylated IFNAR2-EC was shown to be a stable, fully active protein, which inhibits antiviral activity of IFNα2. The stoichiometry of binding IFNα2 is 1:1, as determined by gel filtration, chemical cross-linking and solid-phase detection. The affinity of this interaction was found to be about 3 nM (Piehler and Schreiber 2001). The rate of complex formation is relatively high compared to other cytokine-receptor interactions. The salt dependence of the association kinetics suggests a limited but significant contribution of electrostatic forces towards the rate of complex formation. The dissociation constant increases with decreasing pH according to the protonation of a base with a pKa of 6.7. The affinity of IFNβ to IFNAR2 is about two-fold higher than that of IFNα2 to IFNAR2 (Piehler and Schreiber 1999B).

Single mutations in the binding site of IFNAR2 allowed mapping of differences in binding of IFα2 and IFNβ (Piehler and Schreiber 1999B). For example, a mutation H78A was found to stabilize the complex with IFNβ nearly by two fold, while destabilized the complex with IFα2 more than two fold. A mutation N100A was found to hardly affect the rates for binding IFα2, whereas it decreased the dissociation rate constant for IFNβ by almost four folds.

EP1037658 discloses that the in vivo effect of Type I interferon (IFN) can be prolonged by administering the interferon in the form of a complex with an IFN binding chain of the human interferon alpha / beta receptor (IFNAR) i.e. IFNAR behaves as a carrier protein for IFN. Such a complex also improves the stability of the IFN and enhances the potency of the IFN. The complex may be a non-covalent complex or one in which the IFN and the IFNAR are bound by a covalent bond or a peptide. EP1037658 also discloses that storing IFN in the form of such a complex improves the storage life of the IFN and permits storage under milder conditions than would otherwise be possible.

There exists a need for an IFNAR2 with improved affinity towards IFNβ, but not to IFNα2, making IFNAR2 a better and specific carrier for IFNβ.

SUMMARY OF THE INVENTION

The invention provides an IFNAR2 mutant polypeptide (MIFNAR2) mutated at amino acid residues histidine 78 and asparagine 100, having higher affinity for interferon-β (IFN-β) than the wild type polypeptide, or an analog, functional derivative, fusion protein or salt thereof. The mutations are substitutions of amino acids, preferable conservative amino acids, more preferable alanine, aspartic acid and histidine. The IFNAR2 mutant has about 25, preferably 50 and more preferably 100-fold higher affinity than the wild type protein and a preferred of value is about 30 pM.

More particularly the invention provides an IFNAR2 mutant polypeptide fragment comprising the extracellular domain.

In addition the invention provides a DNA encoding the IFNAR2 mutant polypeptide of the invention, a vector comprising said DNA, host cells comprising said vector and methods for producing a polypeptide mutant of the invention by cultivating said host cells and isolating the produced polypeptide mutant.

In another aspect the invention provides the use of an IFNAR2 mutant polypeptide for the manufacture of a medicament for modulating the effects of IFN, preferably IFNβ, in vivo.

The invention also provides a pharmaceutical composition comprising a therapeutically effective amount of an IFNAR2 mutant or its extracellular domain fragment, to be administrated alone or co-administrated with IFN, more preferably IFNβ, separately or covalently bound. More specifically the invention provides pharmaceutical compositions for augmenting the anti-viral, anti-cancer and immune modulating properties of IFNβ and for treatment autoimmune diseases, such as multiple sclerosis, rheumatoid arthritis, myasthenia gravis, diabetes, ulcerative colitis and lupus.

Furthermore, the invention provides methods of treatment of autoimmune diseases, viral disease and cancer, comprising administration of an IFNAR2 mutant polypeptide of the invention.

In addition the invention provides the use of IFNAR2 mutant polypeptide, preferably co-administered with an IFN antagonist, for inhibition of IFN activity in a disease which is aggravated or caused by IFN.

The invention also provides the use of the IFNAR2 mutant polypeptide of the invention in a formulation to prevent IFN oligomerization

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 (left) depicts a simulation of the concentrations of bound and free IFNβ using a constant concentration of IFN (50 pM) and increasing concentrations of with type IFNAR2 EC (left) and mutant IFNAR2 EC (right), with a Kd of 3 nM and 50 pM respectively, calculated in accordance with the law of mass action.

Figure 2 shows the amino acid sequence of the extracellular domain IFNAR2 protein (not including the leader sequence) and the modified amino acid residues (marked with an asterisk).

Figure 3 shows the binding of IFNβ and IFNα2 to the IFNAR2 EC H78A/N100A mutant. Association and disassociation of IFNβ and IFNα2 to the Wild type IFNAR2 EC (upper panel), to the IFNAR2 EC H78A/N100A mutant (middle panel) and the binding of the wild type and mutant IFNAR2 EC H78A/N100A mutant to IFNβ (Lower panel) was measured using reflectometric interference spectroscopy (RifS),

with IFNAR2 immobilized to the surface (described in Piehler and Schreiber 2001). Y-axis = signal (nanometer) and the X-axis = time (seconds).

Figure 4 shows occlusion of IFNβ by IFNAR2 wild type and mutants. A constant amount of IFNβ (10 pM) was mixed with different concentrations of IFNAR2 (R2) wild type and mutants (single mutants R2N100A and R2H78A, double mutants R2 H78A/N100A, R2 H78A/N100H and R2 H78A/N100D), and the residual antiviral activity at equilibrium was determined in WISH cells. In the upper box, a plot of the antiviral activity of IFNβ as a function of its concentration in the absence of IFNAR2 is shown (Y-axis = survival index). This plot is used as a standard to determine how much of the IFNβ is free (active) in the anti-viral assay.

DETAILED DESCRIPTION OF THE INVENTION

The invention relates to a mutant of the beta chain of the type I IFN receptor (IFNAR2), mutated at amino acid residues H78 and N100 (see DNA sequence of wild type IFNAR2 in Figure 2, SEQ ID N: 1) having increased affinity to IFNβ, but not to IFNα2 (MIFNAR2). The invention relates also to a drug carrier system to enhance activity of IFN comprising the extracellular domain (EC) of MIFNAR2. The invention relates to MIFNAR2, or an analog, functional derivative, fusion protein, fragment thereof or salts thereof.

Carriers are usually administered to prolong the intra-vascular retention time of proteins having molecular weight below 50,000 daltons (e.g. interferon). Particularly beneficial are such carriers that bind non-covalently and permit constant release of the drug. Using such a carrier is desirable in order to have at any time some portion of the free drug available for curative activity (about 20%) and some amount of drug bound to the carrier and protected (about 80%).

Figure 1 (left panel) depicts a simulation of the concentration of bound and free IFNβ in the presence of different concentrations of IFNAR2 based on the law of mass action and on a Kd of 3nM (tested by reflectometric interference spectroscopy [RifS]). This

simulation shows that in order to achieve 20 % of free IFNβ (10 pM, which equals about 100 Units), and 80 % bound, a very high concentration of IFNAR2 protein such as 12.5 nM (which is equivalent to 300 μg/Kg of non-glycosilated IFNAR2) is needed.

Thus, using an IFNAR2 mutant with 50 fold and higher affinities to IFNβ as a carrier (see simulation Figure 1, right panel), would be advantageous since with such a mutant theoretically only about 0.24 nM will be required to get 20 % IFNβ free (which is equivalent to 6 μg/Kg).

A mutant of the IFNAR2 with increased affinity to IFN (MIFNAR2) was generated. To get MIFNAR2 EC, the wild type IFNAR2 EC (Figure 1, SEQIDNO1) was modified in two amino acid residues, residue 78 histidine and residue 100 asparagine (see Figures 2, 3 and 4, SEQ ID sNO: 2, 3, and 4). This mutant IFNAR2 EC proteins turned out to be a better carrier specifically for IFN-β i.e. has improved affinity for IFN-β while its affinity towards IFNα2 remains unchanged. The affinity of the mutants for IFNβ was found to be 26, 40 and above 50 fold higher than that of the wild type (Table 4). The results obtained show that despite the increased affinity of this mutated soluble receptor (Kd of the H78A/N100A IFNAR2 mutant ~ 30pM versus Kd of WT protein = 3nM), enough IFNβ remains unbound and therapeutically active, as evidenced by the anti-viral protective activity of VSV challenged WISH cells (Figure 4). The results show also that the levels of IFN occlusion (bound IFN at equilibrium conditions) obtainable with wild type IFNAR2 EC could be accomplished using lower concentrations of IFNAR mutants EC. The best results are obtained with mutants modified in both residues, particularly when both amino acids are mutated to alanine, H78A/N100A IFNAR2, e.g. in order to get 80% of IFNβ bound (8 pM occluded and 2 pM free IFNβ) about 30 fold less H78A/N100A IFNAR mutant is required over the wild type IFNAR2 protein.

This result show that the double mutated IFNAR2 occludes more effectively IFNβ and administration of considerably lower amounts is required to fulfill its carrier activity towards IFNβ.

The advantages of using M IFNAR2 EC are that (I) it is possible to administrate lower quantities (thus technically feasible) of the receptor as a carrier (II) because of the stabilizing activity of the mutant it is possible to reduce the amount of IFNβ

administrated, and consequently to reduce some of the unwanted side effects of interferon treatment (III) the increase in the activity by the mutant is specific to IFNβ, and (IV) that in some inflammatory disorders, where it may be required to lower the IFN concentrations, it is possible under certain conditions to use this mutant as an effective antagonist specifically towards IFNβ, but not IFNα2.

MIFNAR2-EC may be administered alone to stabilize and enhance the activity of endogenous IFNβ, this is particularly useful for the treatment of patients having a disease or condition which naturally causes the elevation of native IFN, so that the IFN will already be circulating in the body for its intended natural effect of fighting such disease or condition. MIFNAR2-EC will act specifically on endogenous IFNβ, but less towards IFNα2. Alternatively, MIFNAR2-EC may be co-administrated together with IFN, preferably IFNβ or may be administrated covalently bound to IFNβ to modulate the activity of IFN-β. Preferably, MIFNAR2 and IFNβ used to generate the complex are recombinant molecules.

The technology required to produce the fusion protein of the mutant EC IFNAR2 and IFN is similar to the technology described for wild type IFNAR/IFN complex production which is described in detail in WO9932141, wherein the IFNAR2 mutated in H78 and N100 (MIFNAR2) is used instead of the wild type version.

The implications of using a MIFNAR2/ IFNβ non covalently bound complex according to the invention is that lower concentrations of the IFNAR2 EC are required and may be used for a variety of therapeutic indications in which IFN by itself is therapeutically active.

These indications include those in which free IFNs have shown some therapeutic activity, such as anti-viral, anti-cancer and immune modulatory activity. It is expected that the mutant IFNAR2/IFN complex, by virtue of its greater potency, enhanced activity and/or improved pharmacokinetics (i.e. half-life), will be more efficacious in treating viral, oncologic and autoimmune disorders.

When administered in vivo, the interferon receptor complex enhances the bioavailability, pharmacokinetics, and/or pharmacodynamics of the IFN, thus augmenting the anti-viral, anti-cancer and immune modulating properties of the IFN.

The preferred molecules for use in the complexes of the present invention comprise the amino acid sequence of native IFN-β and MIFNAR2 (SEQ ID NOs: 2, 3, and 4). The native sequence is that of a naturally occurring human IFN-β. Such sequences are known and can be readily found in the literature. Naturally occurring allelic variations are also considered to be native sequences.

The present invention also concerns analogs of the above MIFNAR2 EC. Such analogs may be ones in which up to about 30, preferably up to 20 and most preferably 10 amino acid residues may be deleted, added or substituted by others in the proteins, except mutations in residues 78 and 100 which results in a decrease in the affinity of MIFNAR2 for IFN-β to the wild type IFNAR2 affinity for IFN-β. These analogs are prepared by known synthesis and/or by site-directed mutagenesis techniques or any other known technique suitable therefore.

Any such analog preferably has a sequence of amino acids sufficiently duplicative of that of the basic MIFNAR2 such as to have substantially similar activity thereto. Thus, it can be determined whether any given analog has substantially the same activity and/or stability as the protein and complex of the invention by means of routine experimentation, comprising subjecting each such analog to binding and biological activity tests. MIFNAR2 EC analogs may bind IFNβ with at least 15 folds and about 50 to 100 fold higher affinity over the wild type protein wherein the affinity towards IFNα2 is not significantly changed. The MIFNAR2 EC analogs my exhibit a Kd of about 30 pM and lower towards IFNβ. The binding tests for MIFNAR2 and IFN interaction may involve analytical gel filtration, optical heterogeneous phase detection (such as surface plasmon resonance [SPR], or reflectometric interference spectroscopy [RifS] which resembles the widely used BIACORE technique) and fluorescent spectroscopy (Piehler and Schreiber 1999A Piheler and Schreiber 2001).

Analogs of the complex which can be used in accordance with the present invention, or nucleic acid sequence coding therefore, include a finite set of substantially corresponding sequences as substitution peptides or polynucleotides which can be routinely obtained by one of ordinary skill in the art, without undue experimentation, based on the teachings and guidance presented herein. For a detailed description of protein chemistry and structure, see Schulz et al, Principles of Protein Structure, Springer Verlag, New York (1978); and Creighton, T.E., proteins: Structure and Molecular Properties, W.H. Freeman & Co, San Francisco (1983), which are hereby incorporated by reference.

For a presentation of nucleotide sequence substitutions, such as codon preferences, see Ausubel et al (1987, 1992), A.1. I-A. 1.24, and Sambrook et al (1987, 1992), 6.3 and 6.4, at Appendices C and D.

Preferred changes for analogs in accordance with the present invention are what are known as "conservative" substitutions. Conservative amino acid substitutions of those in the sequence of the proteins in the invention may include synonymous amino acids within a group, which have sufficient similar physicochemical properties that substitution between members of the group will preserve the biological function of the molecule (Grantham, 1974). It is clear that insertions and deletions of amino acids may also be made in the above-defined sequences without altering their function, particularly if the insertions or deletions only involve a few amino acids, e.g., under thirty, and preferably under ten, and do not remove or displace amino acids which are critical to a functional conformation, e.g., cysteine residues (Anfinsen, 1973). Analogs produced by such deletions and or insertions come within the purview of the present invention. Preferably, the synonymous amino acid groups are those defined in Table I. More preferably, the synonymous amino acid groups are those defined in Table II; and most preferably the synonymous amino acid groups are those defined 5 in Table III.

TABLE I

Preferred Groups of Synonymous Amino Acids

Amino Acid	Synonymous Group
Ser	Ser, Thr, Gly, Asn
Arg	Arg, Gln, Lys, Glu, His
Leu	Ile, Phe, Tyr, Met, Val, Leu
Pro	Gly, Ala, Thr, Pro
Thr	Pro, Ser, Ala, Gly, His, Gln, Thr
Ala	Gly, Thr, Pro, Ala
Val	Met, Tyr, Phe, Ile, Leu, Val
Gly	Ala, Thr, Pro, Ser, Gly
Ile	Met, Tyr, Phe, Val, Leu, Ile
Phe	Trp, Met, Tyr, Ile, Val, Leu, Phe
Tyr	Trp, Met, Phe, Ile, Val, Leu, Tyr
Cys	Ser, Thr, Cys
His	Glu, Lys, Gln, Thr, Arg, His
Gln	Glu, Lys, Asn, His, Thr, Arg, Gln
Asn	Gln, Asp, Ser, Asn
Lys	Glu, Gln, His, Arg, Lys
Asp	Glu, Asn, Asp
Glu	Asp, Lys, Asn, Gln, His, Arg, Glu
Met	Phe, Ile, Val, Leu, Met
Trp	Trp

TABLE 2

More Preferred Groups of Synonymous Amino Acids

Amino Acid	Synonymous Group
Ser	Ser
Arg	His, Lys, Arg
Leu	Leu, Ile, Phe, Met
Pro	Ala, Pro
Thr	Thr
Ala	Pro, Ala
Val	Val, Met, Ile
Gly	Gly
Ile	Ile, Met, Phe, Val, Leu
Phe	Met, Tyr, Ile, Leu, Phe
Tyr	Phe, Tyr
Cys	Cys, Ser
His	His, Gln, Arg
Gln	Glu, Gln, His
Asn	Asp, Asn
Lys	Lys, Arg
Asp	Asp, Asn
Glu	Glu, Gln
Met	Met, Phe, Ile, Val, Leu
Trp	Trp

TABLE 3

Most Preferred Groups of Synonymous Amino Acids

Amino Acid	Synonymous Group
Ser	Ser
Arg	Arg
Leu	Leu, Ile, Met
Pro	Pro
Thr	Thr
Ala	Ala
Val	Val
Gly	Gly
Ile	Ile, Met, Leu
Phe	Phe
Tyr	Tyr
Cys	Cys, Ser
His	His
Gln	Gln
Asn	Asn
Lys	Lys
Asp	Asp
Glu	Glu
Met	Met, Ile, Leu
Trp	Met

Examples of production of amino acid substitutions in proteins which can be used for obtaining analogs of MIFNAR2 or MIFNAR2 EC for use in the present invention include any known method steps, such as presented in U.S. patents RE 33,653; 4,959,314; 4,588,585 and 4,737,462, to Mark et al; 5,116,943 to Koths et al; 4,965,195

to Namen et al; and 5,017,691 to Lee, et al, and lysine substituted proteins presented in US patent 4,904,584 (Shaw et al).

The term "essentially corresponding to" is intended to comprehend analogs with minor changes to the sequence of the basic MIFNAR2 or MIFNAR2 EC which do not affect the basic characteristics thereof e.g. its specific enhanced binding and affinity to IFNβ. The type of changes which are generally considered to fall within the "essentially corresponding to" language are those which would result from conventional mutagenesis techniques of the DNA encoding the complex of the invention, resulting in a few minor modifications, and screening for the desired activity in the manner discussed above.

Preferably, the MIFNAR2 portion of the complex will have a core sequence which is the same as that of the native sequence or biologically active fragment thereof, or a variant thereof which has an amino acid sequence having at least 70% identity to the native amino acid sequence and retains the biological activity thereof. More preferably, such a sequence has at least 85% identity, at least 90% identity, or most preferably at least 95% identity to the native sequence.

With respect to the IFN portion of the complex, the core sequence which may be used is the native sequence, or a biologically active fragment thereof, or a variant thereof which has an amino acid sequence having at least 70% identity thereto, more preferably, at least 85% or at least 90% identity, and most preferably at least 95% identity. Such analogs must retain the biological activity of the native IFN sequence or fragment thereof, or have antagonist activity as discussed herein below.

The term "sequence identity" as used herein means that the sequences are compared as follows. The sequences are aligned using Version 9 of the Genetic Computing Group's GAP (global alignment program), using the default (BLOSUM62) matrix (values -4 to +11) with a gap open penalty of -12 (for the first null of a gap) and a gap extension penalty of -4 (per each additional consecutive null in the gap). After alignment,

percentage identity is calculated by expressing the number of matches as a percentage of the number of amino acids in the claimed sequence.

Analogs in accordance with the present invention may also be determined in accordance with the following procedure. With respect to either the MIFNAR2 portion of the complex or the IFN portion of the complex, the DNA of the IFNAR and IFN sequence are known to the prior art and is either found in the literature cited in the background section of the present specification or can be readily located by those of ordinary skill in the art. Polypeptides encoded by any nucleic acid, such as DNA or RNA, which hybridize to the complement of the native DNA or RNA under highly stringent or moderately stringent conditions, as long as that polypeptide maintains the biological activity of the native sequence or, in the case of IFN, either maintains the biological activity of MIFNAR2 or MIFNAR2 EC or possesses antagonistic activity, are also considered to be within the scope of the present invention. "stringent conditions" refers to hybridization and subsequent washing conditions, which those of ordinary skill in the art conventionally refer to as "stringent". See Ausubel et al., Current Protocols in Molecular Biology, supra, Interscience, N.Y., §§6.3 and 6.4 (1987, 1992), and Sambrook et al. (Sambrook, J. C., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY).

Without limitation, examples of stringent conditions include washing conditions 12-20°C below the calculated Tm of the hybrid under study in, *e.g.*, 2 x SSC and 0.5% SDS for 5 minutes, 2 x SSC and 0.1% SDS for 15 minutes; 0.1 x SSC and 0.5% SDS at 37°C for 30-60 minutes and then, a 0.1 x SSC and 0.5% SDS at 68°C for 30-60 minutes. Those of ordinary skill in this art understand that stringency conditions also depend on the length of the DNA sequences, oligonucleotide probes (such as 10-40 bases) or mixed oligonucleotide probes. If mixed probes are used, it is preferable to use tetramethyl ammonium chloride (TMAC) instead of SSC. See Ausubel, supra.

"Functional derivatives" as used herein covers derivatives which may be prepared from the functional groups which occur as side chains on the residues or the N- or C-terminal groups, by means known in the art, and are included in the invention as long as they remain pharmaceutically acceptable, i.e., they do

not destroy the biological activity of the corresponding protein of the complex as described herein and do not confer toxic properties on compositions containing it or the complex made therefore. Derivatives may have chemical moieties, such as carbohydrate or phosphate residues, provided such a fraction has the same biological activity and remains pharmaceutically acceptable.

For example, derivatives may include aliphatic esters of the carboxyl of the carboxyl groups, amides of the carboxyl groups by reaction with ammonia or with primary or secondary amines, N-acyl derivatives or free amino groups of the amino acid residues formed with acyl moieties (e.g., alkanoyl or carbocyclic aroyl groups) or O-acyl derivatives of free hydroxyl group (e.g., that of seryl or threonyl residues) formed with acyl moieties. Such derivatives may also include for example, polyethylene glycol side-chains, which may mask antigenic sites and extend the residence of the complex or the portions thereof in body fluids.

The term "fused protein" refers to a polypeptide comprising an MIFNAR2, or MIFNAR2 EC or a analog or fragment thereof, fused with another protein, which, e.g., has an extended residence time in body fluids. An MIFNAR2 or MIFNAR2 EC may thus be fused to another protein, polypeptide or the like, e.g., an immunoglobulin or a fragment thereof.

A "fragment" according to the present invention may e.g. be a fragment of MIFNAR2 or MIFNAR2 EC. The term fragment refers to any subset of the molecule, that is, a shorter peptide that retains the desired biological activity. Fragments may readily be prepared by removing amino acids from either end of the MIFNAR2 molecule and testing the resultant fragment for its properties to bind to IFN-β. Proteases can be used for removing one amino acid at a time from either the N-terminal or the C- terminal of a polypeptide are known, and so determining fragments, which retain the desired biological activity, involves only routine experimentation.

As active fragment of an MIFNAR2, analogs and fused proteins thereof, the present invention further covers any fragment or precursors of the polypeptide

chain of the protein molecule alone or together with associated molecules or residues linked thereto, e.g., sugar or phosphate residues, or aggregates of the protein molecule or the sugar residues by themselves, provided said fragment has substantially similar activity.

The term "salts" herein refers to both salts of carboxyl groups and to acid addition salts of amino groups of the complex of the invention or analogs thereof. Salts of a carboxyl group may be formed by means known in the art and include inorganic salts, for example, sodium, calcium, ammonium, ferric or zinc salts, and the like, and salts with organic bases as those formed, for example, with amines, such as triethanolamine, arginine or lysine, piperidine, procaine and the like. Acid addition salts include, for example, salts with mineral acids, such as, for example, hydrochloric acid or sulfuric acid, and salts with organic acids, such as, for example, acetic acid or oxalic acid. Of course, any such salts must have substantially similar biological activity to the complex of the invention or its analogs.

The term "biological activity" as used herein is interpreted as follows. Insofar as the MIFNAR2 is concerned, the important biological activity is its ability to bind to IFNβ with increased affinity. Thus, analogs or variants, salts and functional derivatives must be those chosen so as to maintain this interferon-binding ability. This can be tested by routine binding assay experiments. In addition, fragments of the MIFNAR2, or analogs thereof, can also be used as long as they retain their interferon-enhanced binding activity. Fragments may readily be prepared by removing amino acids from either end of the interferon-binding polypeptide and testing the resultant for interferon-binding properties.

Additionally, the polypeptide which has such interferon-binding activity, be it MIFNAR2, MINFAR2 EC, an analog , functional derivative, fragment, can also contain additional amino acid residues flanking the interferon-binding polypeptide. As long as the resultant molecule retains the increased interferon-binding ability of the core polypeptide, one can determine whether any such flanking residues affect the

basic and novel characteristics of the core peptide, i.e., its interferon-binding characteristics, by routine experimentation. The term "consisting essentially of", when referring to a specified sequence, means that additional flanking residues can be present which do not affect the basic and novel characteristic of the specified sequence. This term does not comprehend substitutions, deletions or additions within the specified sequence.

While MIFNAR2 or MIFNAR2 EC have been used throughout this description and in the examples, it should be understood that this is merely the preferred example and that the IFNAR1 subunit, and particularly its extracellular domain, may be used together with MIFNAR2 or MIFNAR2 EC.

With respect to the interferon part of the complex of the present invention, the biological activity which must be maintained in any analog, functional derivative, fusion protein or fragment is the activity of the interferon relied upon for the intended utility. In most instances, this will be the ability to bind to a native cell surface receptor and thereby mediate signal production by the receptor. Thus, any such analog, derivative or fragment should maintain such receptor agonist activity to be useful in the present invention for such a utility. On the other hand, it is sometimes useful to have a molecule with antagonist activity on the receptor so as to prevent the biological activity of native interferon. Such an antagonist can also be used for prolonged beneficial effect by means of the complex of the present invention. For such utilities in which it is desired to eliminate an undesired effect of interferon, analogs which are still bound by the receptor and by the IFNAR portion of the complex but which do not mediate a signal and block signal generation by the native interferon on that receptor (i.e interferon antagonist), may also be considered to be biologically active for the purpose of this invention and to be encompassed by the term interferon when used with respect to the complexes of the present invention. Straightforward assays can determine whether any such analog maintains such receptor agonist activity or has receptor antagonist activity and would, thus, be useful for one of the utilities of the present invention.

The present invention also relates to DNA sequences encoding MIFNAR2 EC e.g. DNA encoding the amino acid sequences in SEQIDNOs: 2, 3 and 4 or analogs, and fragments thereof, as well as DNA vectors carrying such DNA sequences for expression in suitable prokaryotic or eukaryotic host cells.

The ability to generate large quantities of heterologous proteins using a recombinant protein expression system has led to the development of various therapeutic agents, e.g., t-PA and EPO (Edington, 1995). The various expression hosts from which recombinant proteins can be generated range from prokaryotic in origin (e.g., bacteria) (Olins, 1993), through lower eukaryotes (e.g., yeast) (Ratner, 1989) to higher eukaryotic species (e.g., insect and mammalian cells (Reuveny, 1993; Reff, 1993). All of these systems rely upon the same principle - introducing the DNA sequence of the protein of interest into the chosen cell type (in a transient or stable fashion, as an integrated or episomal element) and using the host transcription, translation and transportation machinery to over-express the introduced DNA sequence as a heterologous protein (Keown, 1990).

Various protocols for the production of recombinant heterologous proteins are described (Ausubel et al., Current Protocols in Molecular Biology, Greene Publications and Wiley Interscience, New York, NY, 1987-1995; Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989).

In addition to the expression of native gene sequences, the ability to manipulate DNA at the nucleotide level has expedited the development of novel engineered sequences which, although based on natural proteins, possess novel activities as a result of the alteration in primary protein structure (Grazia, 1997).

Moreover, chosen sequences of DNA can be physically linked to generate transcripts which develop into novel fusion proteins where once independent proteins are now expressed as one polypeptide unit (Ibanez, 1991). The activity of such fusion proteins can be different, e.g., more potent, than either of the individual proteins (Curtis, 1991).

For co-administration of MIFNAR2 EC with IFN, human IFNβ may be derived from a production process, which uses the mammalian Chinese hamster ovary cell (CHO) as disclosed in EP220574. Type 1 interferons can be expressed in a variety of host cells including bacteria (Utsumi, 1987), insect (Smith, 1983) and human (Christofinis, 1981). Also human MIFNAR2 or a fragment thereof may be expressed using the CHO host cell. For secretion of MIFNAR2 EC from CHO cells, MIFNAR2 EC DNA sequence may be ligated to the sequence of the human growth hormone signal peptide as described in the patent application WO0022146. Alternatively, soluble receptors, such as MIFNAR2 EC, may be expressed successfully in bacterial expression systems (Terlizzese, 1996).

The invention also relates to a pharmaceutical composition comprising as active ingredient an, MIFNAR2, MIFNAR2 EC, MIFNAR2 EC/IFN complex or analogs, fusion proteins, functional derivatives, fragments thereof or mixtures thereof or salts thereof and a pharmaceutical acceptable carrier, diluent or excipient. An embodiment of the pharmaceutical composition of the invention includes a pharmaceutical composition for enhanced IFN type action, in the treatment of viral diseases, in anti-cancer therapy, in immune modulation therapy e.g. in autoimmune diseases and other applications of interferons and cytokines related thereto.

The pharmaceutical compositions of the invention are prepared for administration by mixing the an, MIFNAR2, MIFNAR2 EC, MIFNAR2 EC/IFN complex or analogs, fusion proteins, functional derivatives, fragments thereof or mixtures thereof or salts thereof with physiologically acceptable stabilizers and/or excipients, and prepared in dosage form, e.g., by lyophilization in dosage vials. The method of administration can be via any of the accepted modes of administration for similar agents and will depend on the condition to be treated, e.g., intravenously, intramuscularly, and subcutaneously, by local injection or topical application, or continuously by infusion, etc. The amount of active compound to be administered will depend on the route of administration, the disease to be treated and the condition of the patient.

The invention relates to method for treatment of autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, myasthenia gravis, diabetes, lupus and ulcerative colitis, comprising aministration of a theurapeutically effective amount of an, MIFNAR2, MIFNAR2 EC, MIFNAR2 EC/IFN complex or analogs, fusion proteins, functional derivatives, fragments thereof or mixtures thereof or salts thereof.

The invention relates to method for treatment of a viral disease such as granulomatous disease, condyloma acuminatum, juvenile laryngeal papillomatosis, hepatitis A or chronic infection with hepatitis B and C viruses, comprising aministration of a theurapeutically effective amount of an, MIFNAR2, MIFNAR2 EC, MIFNAR2 EC/IFN complex or analogs, fusion proteins, functional derivatives, fragments thereof or mixtures thereof or salts thereof.

The invention relates to method for treatment of various types of cancer such as such as hairy cell leukemia, Kaposi's sarcoma, multiple myeloma, chronic myelogenous leukemia, non-Hodgkins's lymphoma or melanoma, comprising aministration of a theurapeutically effective amount of an, MIFNAR2, MIFNAR2 EC, MIFNAR2 EC/IFN complex or analogs, fusion proteins, functional derivatives, fragments thereof or mixtures thereof or salts thereof.

In the above methods the , MIFNAR2, MIFNAR2 EC, MIFNAR2 EC/IFN complex or analogs, fusion proteins, functional derivatives, fragments thereof or mixtures thereof or salts thereof may be administered together with IFN, preferably IFN-β.

A "therapeutically effective amount" is such that when administered, an, MIFNAR2, MIFNAR2 EC, MIFNAR2 EC/IFN complex or analogs, fusion proteins, functional derivatives, fragments thereof or mixtures thereof or salts thereof results in modulation of the biological activity of IFN-β. The dosage administered, as single or multiple doses, to an individual may vary depending upon a variety of factors, including the route of administration, patient conditions and characteristics (sex, age, body weight, health, size), extent of symptoms, concurrent treatments, frequency of treatment and the effect desired. Adjustment and manipulation of established dosage ranges are well within the ability of those skilled in the art, as well as in vitro and in vivo methods of determining the activity of an, MIFNAR2, MIFNAR2 EC,

MIFNAR2 EC/IFN complex or analogs, fusion proteins, functional derivatives, fragments thereof or mixtures thereof or salts thereof.

Local injection, for instance, will require a lower amount of the protein on a body weight basis than will intravenous infusion.

Free IFNβ has a tendency to oligomerize. To suppress this tendency, present day formulations of IFNβ have an acidic pH, which may cause some localized irritation when administered. As an, MIFNAR2, MIFNAR2 EC, or analogs, fusion proteins, functional derivatives, fragments thereof or mixtures thereof or salts thereof can serve as a superior stabilizing over the wild type version factor for IFNβ and thereby prevent oligomerization, its use in IFNβ formulations can serve to stabilize the IFNβ and thereby obviate the necessity of acidic formulations. Accordingly, a non-acidic pharmaceutical composition containing an, MIFNAR2, MIFNAR2 EC, or analogs, fusion proteins, functional derivatives, fragments thereof or mixtures thereof or salts thereof, along with other conventional pharmaceutically acceptable excipients, is also a part of the present invention.

The present invention also concerns uses of an, MIFNAR2, MIFNAR2 EC, MIFNAR2 EC/IFN complex or analogs, fusion proteins, functional derivatives, fragments thereof or mixtures thereof or salts thereof for anti-viral, anti-cancer and immune modulation therapy. Specifically, the mutant interferon receptor and - mutant interferon receptor and interferon complexes of this invention are useful for anti-viral therapy in such therapeutic indications as chronic granulomatous disease, condyloma acuminatum, juvenile laryngeal papillomatosis, hepatitis A and chronic infection with hepatitis B and C viruses.

In particular, the mutant interferon receptor and - mutant interferon receptor and interferon complexes of this invention are useful for anti-cancer therapy in such therapeutic indications as hairy cell leukemia, Kaposi's sarcoma, multiple myeloma, chronic myelogenous leukemia, non-Hodgkins's lymphoma and melanoma.

The mutant interferon receptor and - mutant interferon receptor and interferon complexes of this invention are also useful for immune modulation therapy, in autoimmune diseases e.g. multiple sclerosis, rheumatoid arthritis, myasthenia gravis, diabetes, lupus, ulcerative colitis etc.

"An autoimmune disorder" is a disease in which a person's immune system begins to attack his or her own body. The immune system creates antibodies against its own tissues. Virtually every part of the body is susceptible to an autoimmune disorder.

The mutant interferon receptor and - mutant interferon receptor and interferon complexes are also useful for treating neurodegenerative diseases, preferably multiple sclerosis.

The invention further relates to a pharmaceutical composition comprising an, MIFNAR2, MIFNAR2 EC, MIFNAR2 EC/IFN complex or analogs, fusion proteins, functional derivatives, fragments thereof or mixtures thereof or salts thereof , to a pharmaceutical composition comprising an expression vector, in particular a lentiviral gene therapy vector expressing an, MIFNAR2, MIFNAR2 EC, MIFNAR2 EC/IFN complex or analogs, fusion proteins, fragments thereof.

The terms "treating" as used herein should be understood as preventing, inhibiting, attenuating, ameliorating or reversing any or all symptoms or cause(s) of the disease.

Having now described the invention, it will be more readily understood by reference to the following examples that are provided by way of illustration and are not intended to be limiting of the present invention.

EXAMPLES

Example 1: Protein expression and purification.

IFNAR2-EC (extracellular domain) and IFNα was expressed in *E. coli* purified by ion exchange and size-exclusion chromatography as described (Piehler & Schreiber, 1999A). The levels of expression of IFNAR2-EC mutants were as high as the wild type. Wild type, glycosylated IFNβ produced in CHO (disclosed in EP220574). Protein concentrations were determined from absorbance at 280 nm (Piehler & Schreiber, 1999A) with 1:280 = 18,070 M-1 for IFNa2, 1:280 = 30,050 M-1 for 1FNβ and 1:280 = 26,500 M-1 for IFNAR2-EC (corrected to 1:280 = 21,100 M-1 for the tryptophan mutants of IFNAR2- EC W102A and W74F). Protein purity was analyzed by SD5-PAGE under non-reducing conditions.

Example 2: Generation of IFNAR EC mutants.

Site-directed mutagenesis was carried out by PCR with the template pT72CR2 (Piehler and Schreiber 1999) and with 18-21 nucleotide primers containing the mutated codon using high fidelity polymerases *pwo* (Boehringer Mannheim) and *Pfu* (Stratagene) as described in detail (Albeck & Schreiber, 1999). After phosphorylation and ligation, the mutated plasmids were used to transform *E. coli* TG1 cells. The sequence of the whole expressed gene containing the mutation was verified by DNA sequencing (Ausubel *et al.,* Current Protocols in Molecular Biology, Greene Publications *and Wiley Interscience, New York, NY*, 1987-1995; Sambrook *et al.,* Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989).

Mutants were generated in which two amino acid residues, histidine 78 (H78) and Asparagine 100 (N100), were mutated: A- both to alanine residue (H78A/N100A

mutant), B- to alanine and aspartic acid respectively (H78A/N100D) and C- to alanine and histidine respectively (H78A/N100H).

Example 3: Thermodynamic and kinetic analysis.

All thermodynamic and kinetic data were obtained from label-free heterogeneous phase detection. The interaction between IFNβ2 and IFNAR2-EC was monitored by reflectometric interference spectroscopy (RIfS) under flow-through conditions as described (Piehler & Schreiber, 1999A). This technique is similar to Biacore and is used to accurately measure affinity of binding between two proteins. IFNAR2-EC (wild-type or mutant) was immobilized trough immobilized specific antibodies (as described by Piehler and Schreiber 2001). All measurements with IFNβ, IFNα2 and IFNAR2-EC were carried out in 50 mM Hepes with 500 mM NaCl and 0.01 % Triton X100 at pH 7.4. The interaction was measured at 500 mM NaCl in order to eliminate non-specific interactions with the surface, which was observed with IFNβ at 150 mM NaCl.

Association and dissociation kinetics were measured by standard injection protocols and corrected by blank runs. Dissociation rate constants were measured at IFN concentration in the range of 1-1000 nM in order to saturate the surface. The total range of dissociation was used for fitting a 1:1 kinetic model (Piehler & Schreiber, 2001).

Example 4: Anti viral activity assay.

Anti-viral activity of IFNβ was assayed as the inhibition of the cytophatic effect of vesicular stomatitis virus (VSV) on human WISH cells (Rubinstein *et al.*, 1981).

Example 5: Measurement of IFN binding to mutant IFNAR2 .

Binding of the IFNβ and IFNα2 to the H78A/N100A mutant (example 2) was measured and compared to the wild type EC receptor by RifS (example 3). While the association rate of IFNβ to the H78A/N100A mutant was found to be similar to that of the wild type (Figure 3) the disassociation rate was found to be significantly lower. The calculated affinity of IFNβ to H78A/N100A mutant is about 30 pM versus the affinity to the WT protein of about 3 nM. In contrast to IFNβ, both the association and disassociation rate of IFNα2 to the H78A/N100A mutant, were found to be similar to the rates obtained with the wild type protein (Figure 3). These results show that the affinity of the IFNAR2 mutant mutant was found to be approximately 100 times higher than the wild type towards IFNβ and unchanged towards IFNα2.

Example 6: Relative affinities of interferon towards the mutant IFNAR2.

The binding and affinities of IFNAR EC receptor and mutant receptor EC (example 2) to IFNβ and IFNα2 were measured using RIfS, with IFNAR2 wild type or mutant immobilized to the surface trough specific antibodies (example 3). After measuring the affinities, the relative affinities were obtained by comparing the Kd of the mutant receptor over the Kd of the wild type receptor (Table 4).

The Kd of binding of interferon to IFNAR2 extracellular domain (EC) was measured by RIfS and was found to be about 3nM (example 5). The Kd of IFNβ binding to H78A/N100A (EC) mutant was about 30 pM. The exact measurement of Kd for this mutant was not possible, because binding was to tight to get good data from RIfS. The Kd of IFNα2 to the H78A/N100A EC mutant was found to be similar to the wild type receptor. The results in Table 4 show the relative affinities of the IFNAR EC mutants compared to the wild type IFNAR2 receptor EC. The mutants were the following: mutated in one amino acid residue, H78A or N100A, and mutated in two amino acids H78A/N100A, H78A/N100D and H78A/N100H, wherein the amino acid N100 is mutated into alanine, aspartic acid or histidine respectively (example 2). The results demonstrate that the single mutations in IFNAR2 increase the affinity of the complex from 4.6 up to 7.3 fold, while the double mutation causes a synergistic effect, increasing the affinity of the complex by 26 and to above 50-fold. The best mutant in terms of affinity was found to be the double mutant with the N100 modified to alanine, exhibiting over 50 fold increased affinity versus the wild type version.

ifnar2	IFNa2	IFNb
wt	1.0	1.0
H78A	0.4	4.6
N100A	2.0	7.3
H78A/N100A	0.7	>50
H78A/N100D	1.0	40.0
H78A/N100H	0.9	26.0

Table 4

Example 7: Occlusion of interferon beta by the IFNAR2 mutant.

The capability of IFNAR2 EC wild type and mutants EC to serve as carriers of IFNβ was compared. For that purpose antiviral activity of IFNβ left (free) in samples comprising a constant concentration of IFNβ (10 pM) mixed with varying concentrations of recombinant soluble IFNAR2 EC or IFNAR2 mutants EC (example 6) was monitored. In the antiviral assay, the mixture (IFNAR2/IFN complex) was added to WISH cells (human amniotic cells). These WISH cells were then challenged with vesicular stomatitis virus (VSV), and the residual (free) anti-viral activity of IFNβ was monitored as the degree of cell survival following 24-hour incubation (example 4). The free IFNβ present in samples having different amount of WT or mutant IFNAR2 EC (R2) concentration was determined from a survival dose curve of antiviral activity as a function of IFNβ concentration carried out in the absence of IFNAR2 (Figure 2 upper plot).

The mutants tested were the following: IFNAR2 EC mutated in one amino acid residue, H78A or N100A, and mutated in two amino acids H78A/N100A, H78A/N100D and H78A/N100H wherein the amino acid N100 is mutated into alanine, aspartic acid and histidine respectively example 2). The double mutant of IFNAR2 H78A/N100A (example 2) showed the highest affinity of all the generated mutants (Kd of about 30 pM and lower, see examples 5 and 6).

Figure 4 shows that in the presence of 2.5 nM of wild type IFNAR2 EC about 20% IFNβ is bound to the soluble receptor (occluded), while in the presence of only 0.2 nM of the double mutant EC H78A/N100A 50 % of IFNβ is bound and using only 0.4nM of H78A/N100A mutant EC 80 % of the IFNβ is bound. The biological assay demonstrated also, that the same extent of occluded IFNβ (bound of IFNβ under equilibrium conditions) and the residual antiviral activity (free IFNβ) obtainable with wild type IFNAR2 could be accomplished using about 30 fold lower concentration of the H78A/N100A IFNAR2 mutant EC. The results show also that the double modified mutant yield the best results, particularly the one in which both amino acids were mutated to alanine, H78A/N100A IFNAR2.

This result shows that the double mutated IFNAR2 occlude more effectively IFNβ and therefore administration of considerably lower amounts will be required to accomplish its carrier activity.

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CLAIMS:

1. An IFNAR2 mutant polypeptide (MIFNAR2) mutated at amino acid residues histidine 78 and asparagine 100, having higher affinity for interferon-β (IFN-β) than the wild type polypeptide, or an analog, functional derivative, fusion protein or salt thereof.

2. An IFNAR2 mutant polypeptide according to claim 1, wherein the mutations are substitutions.

3. An IFNAR2 mutant polypeptide according to claim 2, wherein the substitutions are non-conservative.

4. An IFNAR2 mutant polypeptide according to anyone of claims 1 to 3, wherein the histidine residue 78 is substituted by alanine.

5. An IFNAR2 mutant polypeptide according to anyone of claims 1 to 4 wherein the asparagine residue 100 is substituted by alanine, aspartic acid or histidine.

6. An IFNAR2 mutant polypeptide according to claims 4 and 5, wherein both residues 78 and 100 are substituted by alanine.

7. An IFNAR2 mutant polypeptide according to claim 1 comprising the sequence in SEQ ID NO: 2.

8. An IFNAR2 mutant polypeptide according to claim 1 comprising the sequence in SEQ ID NO: 3.

9. An IFNAR2 mutant polypeptide according to claim 1 comprising the sequence in SEQ ID NO: 4.

10. An IFNAR2 mutant polypeptide according to anyone of claims 1 to 9, wherein its affinity to IFN-β is about 30 pM.

11. An IFNAR2 mutant polypeptide according to anyone of claims 1 to 9, wherein its affinity to IFN-β is about 25, preferably up to 50 and more preferably up to 100-fold higher than the affinity of the wild type IFNAR2.

12. An IFNAR2 mutant polypeptide according to anyone of claims 1 to 9, wherein the fragment comprises the extracellular domain (MIFNAR2 EC).

13. An IFNAR2 mutant polypeptide according to anyone of claims 1 to 12 being covalently bound to IFN.

14. An IFNAR2 mutant polypeptide according to claim 13, wherein the IFN is IFN-β.

15. An IFNAR2 mutant polypeptide according to anyone of claims 1-14, wherein the IFNAR mutant is PEGylated.

16. A DNA encoding a polypeptide according to anyone of claims 1-14.

17. A DNA according to claim 16, wherein the DNA is fused to a signal peptide sequence.

18. A DNA according to claim 17, wherein the signal peptide sequence is that of the human growth hormone.

19. A vector comprising a DNA according to anyone of claims 16 to 18, capable of expressing the polypeptide encoded by said DNA in a prokaryotic host cell.

20. A vector comprising a DNA according to anyone of claims 16 to 18, capable of expressing the polypeptide encoded by said DNA in a eukaryotic host cell.

21. A prokaryotic host cell comprising the vector according to claim 19.

22. A eukaryotic cell comprising the vector according to claim 20.

23. A method for producing an IFNAR2 mutant polypeptide according to anyone of claims 1 to 14, comprising cultivating a cell according to claim 21 and isolating the IFNAR mutant polypeptide produced.

24. A method for producing an IFNAR2 mutant polypeptide according to anyone of claims 1 to 14, comprising cultivating the cell according to claim 22 and isolating the IFNAR mutant polypeptide produced.

25. The use of the IFNAR2 mutant according to anyone of claims 1 to 15 in the manufacture of a medicament.

26. The use according to claim 25, wherein the medicament further comprises IFN.

27. The use according to claim 26, wherein the IFN is IFN-β.

28. The use according to claim 25, wherein the medicament further comprises an IFN antagonist.

29. The use according to anyone of claims 25 to 28, for modulating the effects of IFN.

30. The use according to claim 29, for enhancing the activities of IFN.

31. The use according to calim 30, for enhancing the anti-cancer activities of IFN.

32. The use according to claim 30, for enhancing the immune modulatory therapeutic properties of IFN.

33. The use accoprding to claim 30, for enhancing the immune modulatory activities of IFN in autoimmune diseases selected from multiple sclerosis, rheumatoid arthritis, myasthenia gravis, diabetes, lupus and ulcerative colitis.

34. The use according to claim 28 or 29, for inhibiting the activity of IFN.

35. A pharmaceutical composition comprising a therapeutically effective amount of the IFNAR2 mutant according to anyone of claims 1 to 15.

36. A pharmaceutical composition comprising a gene therapy expression vector, expressing a therapeutically effective amount of the IFNAR2 mutant according to anyone of claims 1 to 15.

37. A pharmaceutical composition according to claim 35 or 36, further comprising IFN.

38. A pharmaceutical composition according to claim 37, wherein the IFN is IFN-β.

39. A pharmaceutical composition according to claim 35 or 36, further comprising an IFN antagonist.

40. A pharmaceutical composition according to claim 38, wherein the IFNAR2 mutant and IFN-β are covalently bound.

41. A pharmaceutical composition according to claims 35 and 40, wherein the fragment of IFNAR2 mutant comprises the extracellular domain.

42. A pharmaceutical composition according to anyone of claims 35 to 41, for augmenting the anti-viral properties of IFN.

43. A pharmaceutical composition according to claim 42, for the treatment of chronic granulomatous disease, condyloma acuminatum, juvenile laryngeal papillomatosis, hepatitis A or chronic infection with hepatitis B and C viruses.

44. A pharmaceutical composition according to anyone of claims 35 to 41, for augmenting the anti-cancer properties of IFN.

45. A pharmaceutical composition according to claim 44, for the treatment of hairy cell leukemia, Kaposi's sarcoma, multiple myeloma, chronic myelogenous leukemia, non-Hodgkins's lymphoma or melanoma.

46. A pharmaceutical composition according to anyone of claims 35 to 41, for augmenting the immune modulating properties of IFN.

47. A pharmaceutical composition according to claim 46, for treatment of diseases selected from multiple sclerosis, rheumatoid arthritis, myasthenia gravis, diabetes, ulcerative colitis and lupus.

48. A pharmaceutical composition according to claim 39, for the inhibition of immune modulating properties of IFN.

49. A method of treatment of an autoimmune disease comprising aministration of a theurapeutically effective amount of an IFNAR2 mutant polypeptide according to anyone of claims 1 to 15.

50. A method according to claim 49, wherein the autoimmune disease is selected from multiple sclerosis, rheumatoid arthritis, myasthenia gravis, diabetes, lupus and ulcerative colitis.

51. A method of treatment of a viral disease comprising aministration of a theurapeutically effective amount of an IFNAR2 mutant polypeptide according to anyone of claims 1 to 15.

52. A method of treatment according to claim 51, for the treatment of chronic granulomatous disease, condyloma acuminatum, juvenile laryngeal papillomatosis, hepatitis A or chronic infection with hepatitis B and C viruses.

53. A method of treatment of cancer comprising aministration of a theurapeutically effective amount of an IFNAR2 mutant polypeptide according to anyone of claims 1 to 15.

54. A method according to claim 53, for the treatment of hairy cell leukemia, Kaposi's sarcoma, multiple myeloma, chronic myelogenous leukemia, non-Hodgkins's lymphoma or melanoma.

55. A method of treatment according to anyone of claims 49 to 54, further comprising a therapeutically effective amount of IFN-β

56. A method of treatment of a disease caused or aggravated by IFN-β comprising the inhibition of immune modulating properties of IFN by administration of an IFNAR2 mutant polypeptide according to anyone of claims 1-15.

57. A method of treatment according to claim 56, further comprising the administration of an IFN-β antagonist.

58. The use of the IFNAR2 mutant polypeptide according to anyone of claims 1 to 14 in a formulation to prevent IFN oligomerization.

59. The use according to claim 58, to prevent IFN-β oligomerization.

60. An IFN formulation comprising the IFNAR2 mutant polypeptide according to anyone of claims 1 to 14.

61. A formulation according to claim 60, wherein the formulation comprises IFN-β.