Title: SUPPRESSOR OF THE ENDOGENOUS INTERFERON- GAMMA

Abstract: The invention relates to suppressor of the endogenous human interferon-gamma (hIFN-γ) applicable in treatment of diseases associated with impaired activity of endogenous hIFN-γ, especially autoimmune diseases and for prevention of graft arteriosclerosis and rejection of organs in allograft transplanted patients. It is based on inactive analogues of the hIFN-γ with preserved affinity to the hIFN-γ receptor, genetically modified in the domain responsible for triggering the signal transduction pathway.
SUPPRESSOR OF THE ENDOGENOUS HUMAN INTERFERON-GAWIMA

Field of Invention

The present invention relates to suppressors of the endogenous human interferon-gamma (hIFN-γ) applicable for treatment of autoimmune diseases and for prevention graft arteriosclerosis and rejection of organs in allograft transplanted patients.

Background of Invention

Immune system protects organism from pathogenic microorganisms and foreign macromolecular substances. It identifies exogenous (foreign) bodies of molecular mass exceeding 5000 Da and produces specific antibodies for their neutralization. Immune response is regulated by numerous protein factors (cytokines) produced by specialized cells, in case of dysfunction (due to genetic disorders or infection diseases) the immune system misidentifies certain body proteins as exogenous products and produces specific antibodies for their neutralization. This process lies in the etiology of a great number of autoimmune diseases such as asthma, rheumatoid arthritis, infertility, alopecia areata, multiple sclerosis (MS) and other neurodegenerative pathologies leading to disability and early death of about 2% of the human population. There is substantial evidence that immune responses resulting in IFN-γ production are associated also with the development of graft arteriosclerosis (GA) in allograft transplanted patients. The chronic rejection of allografts (including heart) is preceded by a luminal stenosis of the blood vessels and is denoted as "graft arteriosclerosis". As many as 50% of heart transplant recipients develop angiographically detectable GA three to five years following transplantation. The only treatment currently available for GA is retransplantation, which is costly and not always possible because of shortage of suitable donors. In that sense, the demand of therapeutics for treatment autoimmune diseases and GA is a major priority of the experimental medicine and pharmacy.
Inflammation reaction accompanying the autoimmune process is related with a lavish infiltration of the target tissue with T-lymphocytes and macrophages. They are represented by CD4+ cells producing Th1 proinflammatory cytokines such as interleukin 12 (IL-12) and IFN-γ. The latter activates mononuclear cells to produce destructive substances like lymphotoxins and tumor necrosis factor alpha (TNF-α). It is shown that the pathogenesis of most autoimmune diseases is related with an abnormal production of IFN-γ [1-6].

The overproduction of IFN-γ (as in the case of MS) is inhibited by parenteral application of IFN-β (see patents US082138, WO9530435, CA2361081). In other patents (RU2073522, RU2187332, RU02166959) mixtures of the three different interferons IFN-α, IFN-β and IFN-γ are recommended. It is reported that high dosage (8,000,000 IU/day) of IFN-β provoke unfavorable effects such as: a) T-cells proliferation blockade; b) neutralization of IL-12 thus enhancing the IFN-γ effect; c) decreased CD4+ (Th1, Th2) and CD8+ (Tc1) cell content without changing the Th1/Th2 cell ratio [7]; d) decreased levels of both pro- and anti-inflammatory cytokines [8], etc.

Another approach for the overproduced IFN-γ in autoimmune disease is based on the application of humanized anti-IFN-γ antibodies (patent application WO0145747 and [9-11]). The anti-IFN-γ antibodies, however, deprive the organism from IFN-γ and their long-term application worsens the patients' conditions.

An alternative way for decreasing the abnormal production of IFN-γ in autoimmune diseases is based on the application of the so called "consensus interferons" IFN-con1, IFN-con2 and IFN-con3, derivatives of the Type I IFN-α, IFN-β and IFN-γ (US0086534 and CA2299361). They show various side effects including toxicity.

Proteins with aminoacid sequence partly coinciding with that of the human IFN-γ have been applied as antiviral, antiproliferative and immunomodulating agents (US4832959, WO02081507, AT393690). Their effects, however, can not be presently assessed since the cited patents are not supported by clinics! dst3.

In a recent patent application, published as WO2006 1099701, it is described a new approach for inhibition of the endogenous IFN-γ using inactive recombinant analogues of the IFN-γ with preserved affinity to the IFN-γ receptor. Subject of the
patent application are three different inactive variants of hIFN-γ (a truncated hIFN-γ lacking 27 C-terminal aminoacids, a fusion hIFN-γ-hIFN-α1 protein and a UV inactivated hIFN-γ) which compete with the natural (endogenous) hIFN-γ for the hIFN-γ receptor. Thus, competing with the hIFN-γ receptor, the inactive variants of IFN-γ suppress its activity. Since that effect is dose dependant, the effect of endogenous IFN-γ could be modulated by varying blood concentration of the hIFN-γ derivative proteins. This approach is applicable in the cases when the overproduction of endogenous hIFN-γ causes health problems as in the case of autoimmune diseases, including MS. Although these proteins are good competitors of hIFN-γ for its receptor, their tertiary structure is quite different in comparison with the native wild-type hIFN-γ, which in turn is a potential risk of formation of conformational antibodies. Related with this there is a need of new inactive variants of the hIFN-γ containing negligible changes in domains responsible for triggering the signal transduction pathway.

Description

The invention relates to a suppressor of the endogenous human interferon-gamma (endogenous) which is capable for treatment of autoimmune diseases and graft artery sclerosis. More precisely, the suppressor relates to inactive analogues of the hIFN-γ containing negligible changes in domains responsible for triggering the signal transduction pathway. The suppressor has preserved affinity to the hIFN-γ receptor but is incapable of activating the signal transduction pathway.

According to the present invention, the inactive analogues of the hIFN-γ are obtained by site directed mutagenesis of the hIFN-γ gene to substitute amino acids at positions 86, 87 and 88, which are known to play a key role in the hIFN-γ mediated signal transduction. Taking into consideration that the receptor binding sites are located at the N-terminal part of the molecule, this part remains unchanged.

With the aim of constructing hIFN-γ variants with aminoacid substitutions at positions 86, 87 and 88, a set of PCR primers is synthesized. The forward primer \( 5'\text{SFo}_{-1}\text{rx NCo}_{-1}\)-carrIβ C JHFN diW-site followed by 3 CSRQsNCC I dsnticoS with the 5' end of the hIFN-γ gene and the reverse primer bears a randomized region of 9 nucleotides (A, G, C or T) followed by an AsoW restriction site. Both restriction sites (HindIII and AsoW) are designed for cloning of the PCR products into the expression
vector PJP₁₃-hIFN-γ as described in WO20061099701. The PCR reaction is carried out with a synthetic hIFN-γ gene coding for 143 aa protein (EP 0446582) as a template. Thus a combinatorial library is constructed containing a randomized region between nucleotides 218 and 227 in the hIFN-γ gene, corresponding to the aminoacid area 86-88.

The PCR fragments are purified by agarose gel electrophoresis, digested with both *Hin*I and *Asu*I and cloned into the expression vector pJP₃-hIFN-γ. A set of 162 clones is selected, plasmid DNA is isolated from each clone and the exact nucleotide sequence of the randomized region is determined by DNA sequencing. Thus 101 individual clones are chosen for testing of both antiviral and antiproliferative activities as well as for measuring their ability to compete with the wild-type hIFN-γ for the hIFN-γ receptor.

To construct a hIFN-γ variant, according to the invention, where Glu₈₈ is substituted for Lys₈₈ (Lys/Gln88), the reverse primer SEQ ID NO: 3 is synthesized and the PCR amplification, purification and cloning is performed as described above.

According to another embodiment of the present invention, it is constructed a hIFN-γ variant containing both Lys/Gln88 substitution and deletion of 21 C-terminal aminoacids using the reverse primer SEQ ID NO: 4. It carries a SamHI restriction site and eliminates 21 3'-terminal codons from the hIFN-γ gene during the PCR amplification. The PCR product thus obtained is a gene coding for 122 aminoacids. It is cloned at the site of the wild type hIFN-γ gene in the expression vector PUP₃-hIFN-γ.

Two type of biological activity was measured: a) Antiviral activity (measuring the protective effect against the cytopathic action of the vesicular stomatitis virus (VSV) on the amniotic cell line WISH [20]) and b) Antiproliferative activity (as determined by the kynurenine bioassay [21]). The results presented in Table 1 show that both activities vary between 4.3x10⁴ and 1.2x10⁴ IU/mg for the constructs 19, 46-1 and from 7.25x10⁵ to 6.7x10³ IU/mg for Lys/Gln88 and Lys/Gln88/T7 respectively. This is much lower in comparison with the activity of intact hIFN-γ (10⁷-10⁸ IU/mg). No biological activity was registered for the constructs 27, 36, 134, 135 and 144.
Table 1: Mutant hIFN-γ gene variants

<table>
<thead>
<tr>
<th>Clone signature</th>
<th>Nucleotide sequence between nucleotides 218 and 227</th>
<th>Aminoacids at positions 86, 87 and 88</th>
<th>Specific activity of the mutant hIFN-γ proteins (IU/mg)</th>
<th>Competition with the wild type hIFN-γ</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>TGG ACT TTT</td>
<td>Ser Thr Phe</td>
<td>*7.2x10^5 **2x10^4</td>
<td>+</td>
</tr>
<tr>
<td>27</td>
<td>GAA ATG CCC</td>
<td>Glu Met Pro</td>
<td>0</td>
<td>+++</td>
</tr>
<tr>
<td>36</td>
<td>CTG TGT CCC</td>
<td>Leu Cys Pro</td>
<td>0</td>
<td>++</td>
</tr>
<tr>
<td>46-1</td>
<td>ACC CTC CTC</td>
<td>Thr Leu Leu</td>
<td>*3.0x10^6</td>
<td>+</td>
</tr>
<tr>
<td>134</td>
<td>ACC AAT GGT</td>
<td>Thr Asn Gly</td>
<td>0</td>
<td>+++</td>
</tr>
<tr>
<td>135</td>
<td>GTT TCC CCC</td>
<td>Val Ser Pro</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>144</td>
<td>TGG CGC CGC</td>
<td>Cys Ala Pro</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Lys/Gln88</td>
<td>CGG TAC CTC</td>
<td>Lys Lys Gln</td>
<td>*1.7x10^5 **1.2x10^4</td>
<td>+</td>
</tr>
<tr>
<td>Lys/Gln88/T7</td>
<td>CCC AAT TAT</td>
<td>Lys Lys Gln and C-terminus deleted</td>
<td>*8.7x10^5 **4.3x10^4</td>
<td>+++</td>
</tr>
</tbody>
</table>

* Antiproliferative activity; ** Antiviral activity; + Competition with the wild type hIFN-γ; +++ High competition, ++ Intermediate competition, + Low competition

All constructs without or with decreased biological activity are further assayed for their ability to compete with intact (wild-type) hIFN-γ for its receptor using WISH cells as a test system. To this end the mutant proteins are mixed in equimolar amounts in sterile bacterial lysates with purified hIFN-γ and the antiproliferative activity of the mixtures is measured by the kynurenine bioassay using wild-type hIFN-γ as a standard. The results must be interpreted as follows: In case that the mutant protein has the same affinity to the hIFN-γ receptor as that of the wild type hIFN-γ and zero antiproliferative activity, the activity of the equimolar mixture of both substances should be 50% of that of the control (pure wild type hIFN-γ). As it is shown in Table 1, the strongest competitive effect is expressed by the constructs showing zero antiproliferative activity (constructs 27 and 134) and also by the C-terminally truncated construct Lys/Gln88/T7.

The advantages of the hIFN-γ suppressor according to the invention cover several aspects: a) The hIFN-γ mutants represent negligibly minor modified (1-3 aminoacid substitutions) human proteins. They resemble allelic variants of the hIFN-γ and therefore they should not be immunogenic; b) The inactive hIFN-γ derivatives occupy the hIFN-γ receptor via a reversible manner and can be replaced by higher concentrations of intact hIFN-γ; c) The equal opportunity of both molecules (mutant and wild-type hIFN-γ) to bind the hIFN-γ receptor makes it possible to control the extent of inhibition of the activity of the endogenous hIFN-γ by varying the
concentration of the suppressors in the bloodstream; d) Unlike other approaches for suppressing hlFN-γ activity (inhibition of its biosynthesis or irreversible neutralization, e.g. by specific monoclonal antibodies) the inactive derivatives of the hlFN-γ do not deprive the organism of the vitally important endogenous hlFN-γ.

The suppressor, according to the present invention, is designed for manufacturing of medicaments for inhibition the activity of the endogenous hlFN-γ, produced in high concentrations in patients with autoimmune diseases such as Multiple sclerosis, Alopecia areata, Myastenia gravis as well as for graft arteriosclerosis in post-transplanted patients.

The following examples illustrate the present invention without limiting its scope and spirit:

**Example 1:** Construction of hlFN-γ derivative proteins with aminoacid substitutions at positions 86, 87 and 88.

Recombinant proteins derivative of the hlFN-γ with aminoacid substitutions at positions 86, 87 and 88 are prepared by PCR mutagenesis of a synthetic hlFN-γ gene using appropriate primers. The latter are synthesized on a Cyclon Plus (MilliGene) synthesizer by the pridsphoπmioxite meinoαs and purified on a πδιδ polyacrylamide gel [22]. Two primers (forward and reverse) are synthesized and their primary structure is presented on the sequence listing. The forward primer (SEQ ID NO: 1) is designed to introduce a H/ndlll site and the reverse primer contains a randomized 9 nucleotide long region plus an AsuW site (SEQ ID NO: 2). The two (H/ndlll and AsuW) restriction sites are to be used for cloning into the expression vector pJPiR 3-hlFN-γ. The hlFN-γ is mutagenized under PCR conditions presented in Tables 2 and 4.

Table 2: PCR conditions for primers SEQ ID NO: 1 and SEQ ID NO: 2

<table>
<thead>
<tr>
<th>Programme</th>
<th>Number of cycles</th>
<th>Time (min)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1</td>
<td>5</td>
<td>94</td>
</tr>
<tr>
<td>II</td>
<td>5</td>
<td>0.3</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.6</td>
<td>74</td>
</tr>
<tr>
<td>III</td>
<td>35</td>
<td>0.5</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5</td>
<td>74</td>
</tr>
<tr>
<td>IV</td>
<td>1</td>
<td>10</td>
<td>74</td>
</tr>
</tbody>
</table>
The PCR fragments are purified by electrophoresis in 1.5% Agarose Type II (Sigma) gel, hydrolyzed by Hndlll and AsuW and cloned into the expression vector PJP1R3-hlFN-γ pre-digested with the same enzymes (see WO20061099701). To this end 20 µg plasmid (vector) DNA is dissolved in 150 µl HindII buffer and digested with 20 U Hndlll for 3 h at 37 °C. Reaction mixture is treated consecutively with phenol and chloroform and DNA is precipitated with 3 v/v of ethanol at -20 °C. The precipitate is dissolved in 150 µl AsuW buffer and digested with 20 U AsuW for 3 h at 37 °C. The linear vector is dephosphorylated by calf intestinal alkaline phosphatase (CIAP, Boehringer Mannheim), purified by agarose gel electrophoresis and mixed in T4 DNA ligase buffer with the PCR fragments at a ratio 3:1. The ligase reaction is carried out overnight at 4 °C and used for transformation of competent E.coli LE392 cells.

The transformed cells are grown in standard Luria-Bertani (LB) broth and/or LB-agar containing 50 µg/ml ampicillin and 10 µg/ml tetracycline. A set of 162 clones were selected, plasmid DNA was isolated from each clone and the exact nucleotide sequence of the randomized region was determined by DNA sequence analysis. Thus the number of individual clones was reduced to 101 and they were all tested for production of hlFN-γ derivative proteins. The level of expression of the latter was determined by ELISA using hlFN-γ specific monoclonal antibodies.

The hlFN-γ derivative proteins are purified in two steps by Octyl-Sepharose and CM-Sepharose (Pharmacia) chromatography as already described (EP0446582).

Two biological activities, antiviral and antiproliferative, were determined for the hlFN-Y derivative proteins. The antiviral activity (expressed in International Units) is measured by the protective effect of hlFN-γ against the cytopathic action of the vesicular stomatitis virus (VSV) on the amniotic cell line WISH [12] and the antiproliferative activity was determined by the kynurenine bioassay [13].

The data presented in Table 1 show that most of the mutant hlFN-γ proteins have either decreased or completely loss of antiviral and antiproliferative effect.

**EXAMPLE 2:** Construction of hlFN-γ derivative protein with Gln at position 88.

Recombinant protein derivative of the hlFN-γ containing Gln instead of Lys at position 88 (Gln/Lys88) is prepared by PCR mutagenesis using a synthetic hlFN-γ
gene as a template and the primers SEQ ID NO: 1 and SEQ ID NO: 3. The forward primer (SEQ ID NO: 1) is described above and the latter contains a single nucleotide transition (A->G) to substitute Gln for Lys at position 88. It carries also an AsuUS site for cloning into the expression vector PJP1R3-lIFN-Y. PCR conditions are presented in Tables 3 and 4 and all subsequent procedures are performed as in Example 1.

**Table 3:** PCR conditions for primers SEQ ID NO: 1 (forward) and the reverse primers

<table>
<thead>
<tr>
<th>Program</th>
<th>Number of cycles</th>
<th>Time (min)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1</td>
<td>5</td>
<td>94</td>
</tr>
<tr>
<td>II</td>
<td>5</td>
<td>1</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>35</td>
<td>1</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>1</td>
<td>10</td>
<td>74</td>
</tr>
</tbody>
</table>

**Table 4:** Composition of the PCR reaction mixture

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Quantity (μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Template DNA (50 pg/μl)</td>
<td>1</td>
</tr>
<tr>
<td>Forward primer (20 pmol/μl)</td>
<td>1</td>
</tr>
<tr>
<td>Reverse primer (20 pmol/μl)</td>
<td>1</td>
</tr>
<tr>
<td>Taq-polymerase (3 U/μl)</td>
<td>1</td>
</tr>
<tr>
<td>10 x PCR buffer</td>
<td>2</td>
</tr>
<tr>
<td>2 mM dNTP’s</td>
<td>2</td>
</tr>
<tr>
<td>H2O</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
</tr>
</tbody>
</table>

As it is seen on Table 1, the Gln/Lys88 mutant demonstrates almost 1000 fold lower antiviral and antiproliferative activities in comparison with the wild type hIFN-γ.

**EXAMPLE 3:**Construction of hIFN-γ derivative protein with Gln at position 88 and deleted 21 C-terminal aminoacids.

Recombinant protein derivative of the hIFN-γ containing both Lys88->Gln substitution and deletion of 21 C-terminal aminoacids (Lys/Gln88/T7) is prepared by PCR mutagenesis using the already mutated gene described in Example 2 and the primers SEQ ID NO:1 and SEQ ID NO:4. The forward primer (SEQ ID NO:1) is
described in Example 1 and the reverse primer (SEQ ID NO:4) is designed to eliminate 21 3'-terminal codons from the hIFN-γ gene during the PCR amplification. The PCR product thus obtained is a gene coding for 122 amino acids and a substitution of Gln for Lys at position 88. It carries two restriction sites (H/ndIII and SamHI) for cloning into the expression vector pJP1R3-lhIFN-γ after pretreatment with the same restriction enzymes. The PCR reaction conditions are presented in Tables 3 and 4. All subsequent procedures are performed as in Example 1.

As it is seen on Table 1, the Gln/I ys88/T7 mutant demonstrates more than 1000 fold decrease in both antiviral and antiproliferative activities in comparison with the wild type hIFN-γ.

**Example 4: Examination of the suppressor activity of mutant hIFN-γ proteins**

Capability of mutant hIFN-γ derivative proteins of competing with the wild-type hIFN-Y for the hIFN-γ receptor is examined on the amniotic cell line WISl-1 (enriched in hIFN-γ receptors). The test is based on measuring the decrease in antiproliferative activity of standard (wild-type) hIFN-γ in the presence of mutant hIFN-γ derivative proteins. The antiproliferative activity itself is determined by the kynurenine bioassay [13] based on the hIFN-γ induction of indoleamine-2,3-dioxygenase (IDO), which is the first and rate-limiting enzyme in the tryptophan catabolism. IDO catalyzes oxidative cleavage of tryptophan to N-formylkynurenine. Following a hydrolysis step, the latter is transformed into kynurenine which gives with the Ehrlich’s reagent a yellow-colored compound absorbing at 490 nm. It is known that the amount of produced kynurenine is directly proportional to the concentration of hIFN-γ used for cell activation.

To measure the suppressor activity, mutant proteins are mixed in equimolar amounts in sterile bacterial lysates with purified hIFN-γ and the antiproliferative activity of the obtained mixtures is determined by the kynurenine bioassay using wild-type hIFN-γ as a standard. Experimentally, clear cell lysates of E. coli LE392 cells transformed with plasmids expressing mutant hIFN-γ proteins are prepared after cultivation in LB broth supplemented with 50 µg/ml ampicillin to a cell density of \(A_{590} = 0.7\). Samples of 2 OD 590 cells are centrifuged, the cells are resuspended in 1 ml 0.14 M NaCl, 10 mM Tris pH 8.0, 0.1 mM PMSF and disrupted by sonication. The lysates are cleared by centrifugation at 12000 rpm for 15 min at 4 °C and used for further analyses.
Total protein content is determined by Bradford using bovine serum albumin (fraction V) as a standard and the samples are diluted by PBS (14.7 mM Na₂CO₃ in 34 mM NaHCO₃, pH 9.6) to a final concentration of 27 µg/ml protein. Samples of 50 µl are added (11 times per sample) to PVC 96 well microplates (Costar Ltd., USA), incubated overnight at 4°C and the content of hlFN-γ or hlFN-γ derivative proteins is measured by ELISA using hlFN-γ specific monoclonal antibodies.

To measure the suppressive effect of the hlFN-γ mutant proteins against binding of wild-type hlFN-γ to the cell receptors, clear cell lysates are serially diluted and samples of 50 µl are mixed with 50 µl of standard hlFN-γ and added to PVC 96 well microplates. WISH cell suspension (50 µl) in MEM Eagle medium supplemented with HEPES, L-myταιΗ and 2% BFS is added, mixed with 50 µl L-tryptophan and the kynurenine bioassay is performed as already described [13]. The final concentration of the standard hlFN-γ in the analyzed samples is 25 III/ml, 50 IU/ml and 100 IU/ml, corresponding to 0.027 nmol, 0.055 nmol and 0.11 nmol respectively. Samples containing standard hlFN-γ (only) were used as positive control and clear cell lysates obtained from host (non-transformed E. coli LE392) cells were used as negative controls in this assay.

The data presented in Table 1 show that the constructs with zero antiproliferative activity (constructs 27 and 134) and also the C-terminally truncated construct Lys/Gln88/T7 demonstrate strongest suppressive effect.
REFERENCES


CLAIMS

1. Suppressor of the endogenous human interferon-gamma (hlFN-γ) based on inactive analogues of the hlFN-γ with preserved affinity to the hlFN-γ receptor, characterized with that it includes derivatives of the hlFN-γ, genetically modified in the domain responsible for triggering the signal transduction pathway.

2. Suppressor of the endogenous hlFN-γ according to claim 1, characterized with that the hlFN-γ derivatives are obtained by introducing point mutations in the hlFN-γ gene causing substitution of aminoacids at positions 86, 87 and 88.

3. Suppressor of the endogenous hlFN-γ according to claims 1 and 2 characterized with that the genetically modified derivatives of the hlFN-γ contain Glu, Met and Pro or Thr, Asn and Gly or Lys, Lys and Gln at positions 86, 87 and 88 respectively.

4. Suppressor of the endogenous hlFN-γ according to claims 1-3, characterized with that it contains Gln instead of Lys at position 88 and a deletion of 21 amino acids at the C-terminus.

5. Suppressor of the endogenous hlFN-γ according to claims 1 and 2, characterized with that the point mutations of the hlFN-γ gene are introduced by the forward primer SEQ ID No:1 and the reverse primer SEQ ID No:2 or SEQ ID No:3 or SEQ ID No:4.

6. Use of the suppressor of endogenous hlFN-γ according to claims 1-5 for manufacture of a medicament for treatment of diseases associated with impaired activity of the endogenous hlFN-γ.

7. Use of the suppressor of endogenous hlFN-γ according to claim 6 for manufacture of a medicament for treatment of autoimmune diseases such as Multiple sclerosis, Alopecia areata and Myastenia gravis.

8. Use of the suppressor of endogenous hlFN-γ according to claim 6 for manufacture of a medicament for prevention of graft arteriosclerosis and rejection of organs in allograft transplanted patents.
INTERNATIONAL SEARCH REPORT

International application No. PCT/BG 2008/000025

A. CLASSIFICATION OF SUBJECT MATTER

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>WO 2006/120580 A2 (NAUTILUS BIOTECH) 16.11.2006, abstract, claims 1, 17</td>
<td>1-8</td>
</tr>
<tr>
<td>A</td>
<td>RU 2268749 C2 (MAKCHIDKEH XOHJILHHIHR X 27.01.2006, abstract, p. 13, claims 1, 7</td>
<td>1-8</td>
</tr>
</tbody>
</table>

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

- C07K14/52, 14/57, C12N 15/23, A61K 38/21, A61P 37/06

According to International Patent Classification (IPC) or to both national classification and IPC

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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

Date of the actual completion of the international search
11 March 2009 (11.03.2009)

Date of mailing of the international search report
19 March 2009 (19.03.2009)

Authorized officer
E. Redo

Telephone No. (495)730-7675

Form PCT/ISA/210 (second sheet) (July 2008)