

DESCRIPTION

Vector for Gene Therapy and Method of Quantifying Target Protein in Mammal or Cultured Cells with the Administration of the Vector for Gene Therapy

Technical Field

5 The present invention relates to a vector for gene therapy for the production of a desired protein in the body, which vector enables to simply quantify the desired protein produced in the body or in cultured cells.

Background Art

10 When a gene therapy by which a desired protein is made to be produced in the body of a patient, the blood level of the desired protein cannot be measured unless a method for measuring the protein, such as ELISA, has been established. To counter this problem, an assay method in which a label protein is used and the level of the protein is measured has been practically used and sold. However, the measurement sensitivity of the method is low, and so there is no established method for measuring blood level in gene therapy. In Treatment of Murine Lupus with cDNA encoding IFN- γ R/Fc, The Journal of Clinical Investigation, July 2000, volume 106, Number 2 p207-215, the desired protein is not measured but a protein influenced by the desired protein is quantified so as to indirectly prove the expression of the desired protein. This is thought to show the difficulty in the measurement of the blood level.

20 Disclosure of the Invention

 An object of the present invention is to provide a vector for gene therapy, by which the blood level of a desired protein can be monitored at high sensitivity when a gene therapy is conducted, and by which undesired physiological action or antigen-antibody reaction due to the label scarcely occurs.

25 The present inventor intensively studied to discover that by carrying out the gene therapy with a vector in which a fusion protein of the desired protein which should be produced in the body by the gene therapy and a glucagon C-terminal side

19-29 amino acid peptide, the blood level of the desired protein may be measured with high sensitivity using the glucagon peptide as a label, and that undesired physiological action or induction of immunological reaction due to the label peptide scarcely occurs, thereby completing the present invention.

5 That is, the present invention provides a vector for gene therapy comprising an expression vector for mammalian cells and a nucleic acid coding for a fusion protein of glucagon C-terminal side 19-29 amino acid peptide region and a desired protein region which should be produced in the body, which vector can produce the fusion protein in the mammalian cells. The present invention also provides a
10 method for gene therapy comprising administering an effective amount of the vector for gene therapy according to the present invention to a mammal or cultured mammalian cells, in which expression of the desired protein in the body or in the cultured mammalian cells is desired. The present invention further provides a use of the vector for gene therapy according to the present invention for the production of
15 a drug for gene therapy. The present invention still further provides a method for quantifying a desired protein produced in the body or in cultured cells by expression of the vector for gene therapy, comprising quantifying, by immunoassay, the glucagon C-terminal side 19-29 amino acid peptide region in a test sample collected from a mammal or cultured mammalian cells to which the vector for gene therapy
20 according to the present invention was administered. The present invention still further provides a label for labeling a desired protein produced by expression of an externally administered expression vector in the body of a mammal or in cultured mammalian cells, consisting essentially of glucagon C-terminal side 19-29 amino acid peptide. The present invention still further provides a method for labeling a
25 protein produced in the body or in cultured cells, comprising labeling a desired protein produced in the body or in cultured cells with glucagon C-terminal side 19-29 amino acid peptide by expressing the desired protein produced by expression of an

externally administered expression vector in the body of a mammal or in cultured mammalian cells, as a fusion protein with the glucagon C-terminal side 19-29 amino acid peptide as a label. The present invention still further provides a use of glucagon C-terminal side 19-29 amino acid peptide as a label for a desired protein produced by expression of an externally administered expression vector in the body of a mammal or in cultured mammalian cells.

By the present invention, a vector for gene therapy by which the blood level of the desired protein can be measured with high sensitivity, wherein the label peptide does not have a physiological action, was first provided. Since the glucagon C-terminal side 19-29 itself does not have a physiological action and is well conserved in various mammals, it does not substantially induce an immunological reaction while it can be quantified by immunoassay with high sensitivity using a commercially available immunoassay kit.

Brief Description of the Drawings

Fig. 1 shows the nucleotide sequence of the nucleic acid fragment inserted into the vector for gene therapy prepared in Example 1, together with the amino acid sequence encoded thereby.

Fig. 2 shows the continuation of Fig. 1.

Fig. 3 shows the continuation of Fig. 2.

Fig. 4 shows a gene map of pCAGGS which is an expression vector for mammals used in Examples 1 to 5.

Fig. 5 shows the relationship between the days after administration of the vector for gene therapy and the blood level of the desired protein measured by measuring the glucagon-originated label peptide, in Example 1.

Fig. 6 shows the change with time of the blood level of the desired protein when gene therapy was carried out using the vector of the present invention, and shows the change with time of the blood glucose level when gene therapy was carried

out using the vector of the present invention or using a vector in which a nucleic acid coding for the desired protein not fused with the glucagon-originated label peptide was inserted.

Fig. 7 shows the nucleotide sequence of the nucleic acid fragment inserted
5 into the vector for gene therapy prepared in Example 2, together with the amino acid sequence encoded thereby.

Fig. 8 shows the continuation of Fig. 7.

Fig. 9 shows the continuation of Fig. 8.

Fig. 10 shows the relationship between the days after administration of the
10 vector for gene therapy and the blood level of the desired protein measured by measuring the glucagon-originated label peptide, in Example 2.

Fig. 11 shows the days for which the transplanted hearts were taken by the rats in Example 2 and Comparative Example 2.

Fig. 12 shows the nucleotide sequence of the nucleic acid fragment inserted
15 into the vector for gene therapy prepared in Example 3, together with the amino acid sequence encoded thereby.

Fig. 13 shows the continuation of Fig. 12.

Fig. 14 shows the continuation of Fig. 13.

Fig. 15 shows the nucleotide sequence of the nucleic acid fragment inserted
20 into the vector for gene therapy prepared in Comparative Example 3, together with the amino acid sequence encoded thereby.

Fig. 16 shows the continuation of Fig. 15.

Fig. 17 shows the relationship between the days after administration of the
25 vector for gene therapy and the blood level of the desired protein measured by measuring the glucagon-originated label peptide, in Example 3.

Fig. 18 shows the percentage of the lesion area of myocarditis in rats in Example 3 and Comparative Example 3.

Fig. 19 shows the nucleotide sequence of the nucleic acid fragment inserted into the vector for gene therapy prepared in Example 4, together with the amino acid sequence encoded thereby.

Fig. 20 shows the continuation of Fig. 19.

5 Fig. 21 shows the continuation of Fig. 20.

Fig. 22 shows the relationship between the days after administration of the vector for gene therapy and the blood level of the desired protein measured by measuring the glucagon-originated label peptide, in Example 4.

10 Fig. 23 shows the percentage of the lesion area of rat myocarditis in Example 4 and Comparative Example 4.

Fig. 24 shows the nucleotide sequence of the nucleic acid fragment inserted into the vector for gene therapy prepared in Example 5, together with the amino acid sequence encoded thereby.

15 Fig. 25 shows the relationship between the blood level of the desired protein measured by measuring the glucagon-originated label peptide and the blood level of the desired protein measured by measuring human interleukin 8, in Example 5.

Best Mode for Carrying Out the Invention

The "glucagon C-terminal side 19-29 amino acid peptide" which is expressed by the vector according to the present invention in the form of a fusion protein,
20 means the peptide consisting essentially of totally 11 amino acids located from the 19th to 29th amino acid counted from the C-terminal of glucagon. That is, the peptide has the amino acid sequence shown in SEQ ID NO: 1 in SEQUENCE LISTING. Since the "glucagon C-terminal side 19-29 amino acid peptide" is used as a label of the desired protein, the peptide may be hereinafter referred to as
25 "glucagon-originated label peptide" for convenience.

In the vector according to the present invention, a nucleic acid coding for a fusion protein of the glucagon-originated label peptide region and a desired protein

region is incorporated.

The expression vector for mammalian cells used in the present invention is well-known in the field of gene therapy, and is not restricted as long as it is an expression vector for mammalian cells. The characteristic feature of the present invention resides in that the glucagon-originated label peptide region as a label of the desired protein is expressed in the form of a fusion protein with the desired protein, so that the expression vector for mammalian cells is not restricted at all, and any of the known expression vectors for mammalian cells used in the field of gene therapy may be employed. Although the vector may be a plasmid vector or a virus vector, a plasmid vector is preferred from the view point of safety. Various expression vectors for mammalian cells are well-known and are commercially available, and the well-known or commercially available vectors may preferably be employed.

Examples of the well-known or commercially available vectors include pCAGGS (Efficient selection for high expression transfectants with a novel eukaryotic vector, Gene 1991 Dec.15,108(2)p193-P199., whose gene map is shown in Fig. 4, and whose nucleotide sequence is shown in SEQ ID NO: 3 in SEQUENCE LISTING), pCI vectors, pSI vectors and pTARGET vectors from PROMEGA, and pcDNA5/TO and the like from INVITROGEN, but the vectors are not restricted thereto.

The desired protein to be produced in the body by the gene therapy is not restricted at all, and examples thereof include various cytokines such as interferons, interleukins and CTLA4; hormones such as growth factor and insulin; and cell adhesion factors; as well as receptors thereof. An arbitrary antigenic protein may also be produced in the body by a gene vaccine. The desired protein itself may also be a fusion protein. For example, a terminal of a desired cytokine which is desired to be bound to a Fc receptor may be fused with an immunoglobulin, preferably IgG, especially the constant region (Fc) of IgG1 so as to promote the binding ability with the Fc receptor, and the resulting fusion protein may be employed as the desired

protein (see Examples 1 to 4 below). The nucleotide sequence coding for the Fc region of IgG is well-known. For example, the nucleotide sequence of the nucleic acid coding for the Fc region of human IgG is described in, for example, GenBank Accession No. BC020823 and so on, and the nucleotide sequence of the nucleic acid coding for the Fc region of rat IgG is shown in Figs. 1 to 3 of the present application, and so on.

The vector according to the present invention may be obtained by inserting the nucleic acid coding for the fusion protein of the desired protein and the above-described label peptide into a cloning site of the expression vector for mammalian cells. It is preferred to fuse the label peptide to a terminal, especially to the C-terminal of the desired protein.

Gene therapy may be carried out by administering the vector for gene therapy according to the present invention to a mammal. As the administration route, a parenteral route such as intravenous injection or intramuscular injection is preferred. The dose of the vector may be appropriately selected depending on the properties of the desired protein, the type and the severity of the disease to be treated, and the dose of the vector per 1 kg of body weight may be usually about 1 mg to 10 mg, preferably about 2 mg to 4 mg. As for the formulation, the vector for gene therapy may be dissolved in Ringer solution and the obtained solution may be used as an injection solution. Additives for injection solution, well-known in the field of drug formulation may also be added. Alternatively, the vector for gene therapy according to the present invention may be administered to mammalian cells cultured *in vitro*. More particularly, there is a gene therapy in which the cells such as lymphocytes and bone marrow cells from a patient are collected from the body and cultured, a gene vector is administered to the cultured cells, and the cells acquired the ability to produce the desired protein are returned again to the patient. The vector for gene therapy according to the present invention may also be administered to such cultured

mammalian cells. Alternatively, the vector for gene therapy may be administered to cultured mammalian cells in experiments for examining the therapeutic effect, *in vitro*, of the vector for gene therapy or the like.

In the gene therapy, the fusion protein of the above-described desired protein and the glucagon-originated label peptide is produced by the vector introduced into the body. Alternatively, in cases where the vector for gene therapy is administered to mammalian cells cultured *in vitro*, the fusion protein of the above-described desired protein and the glucagon-originated label peptide is produced in the cultured cells. Since the desired protein is fused with the label peptide, the level of the desired protein can be measured by measuring the level of the glucagon-originated label peptide. Since kits (including an antibody obtained by using the glucagon-originated label peptide as an antigen) for quantifying, by immunoassay, the glucagon-originated label peptide used in the present invention are commercially available (Pancreas Glucagon RIA kit commercially available from DAIICHI RADIOISOTOPE LABORATORIES, LTD and the like), the glucagon-originated label peptide may easily be measured using such a commercially available immunoassay kit.

The test sample for which the glucagon-originated label peptide is quantified may be various body fluids or tissues or dilutions thereof, collected from the individual to which the vector for gene therapy according to the present invention was administered, and preferably blood samples such as whole blood, serum or plasma as well as dilutions thereof. Alternatively, in cases where the vector for gene therapy is administered to the mammalian cells cultured *in vitro*, the test sample may be the homogenate of the cultured cell, culture supernatant or the like.

Example 1, Comparative Example 1

PCR was carried out using as a template the cDNA of cultured rat spleen cells stimulated by lection, and using as primers 5'-

gagaattcatttaaatgagagcgccgcccgtgccagaaactgtg-3' and 5'-

tcaaccactgcacaaaatcttgggctttacccggagagtgggagagact-3'. Thereafter, PCR was performed using as the template the 300-fold diluted PCR product obtained above, and using as primers 5'-gagaattcatttaaatgagagcgccgcccgtgccagaaactgtg-3' and 5'-

5 gagagagagaattctcaggtattcatcaaccactgcacaaaatcttgggc-3'. The amplified product was inserted into a cloning site of the above-described expression vector pCAGGS for mammalian cells. By this, pCAGGS-IgG-glu19-29 (pCAGGS in which a nucleic acid fragment having a region coding for the Fc region of immunoglobulin G1 (IgG1) and a region coding for the glucagon-originated label peptide, ligated to the downstream of the Fc-coding region, was inserted at the EcoRI site of pCAGGS),
10 having restriction sites of SwaI and NotI, was obtained.

Thereafter, PCR was performed using as a template the cDNA of heart of rat suffering from myocarditis, and using as primers 5'-

gagaattcatttaaatgattctgctggtgctcctgatg-3' and 5'-

15 gcagcatcgccgcccgttcttctgtcatcatggagaaa-3'. The obtained PCR product was inserted into the pCAGGS-IgG-glu19-29 prepared above using SwaI and NotI.

By this, a vector according to the present invention, in which the DNA fragment having a nucleotide sequence shown in SEQ ID NO:2 in SEQUENCE LISTING (shown together with the restriction sites) was inserted into the EcoRI site
20 of the above-described expression vector pCAGGS for mammalian cells was prepared (Example 1). SEQ ID NO:2, together with other information, is shown in Figs. 1 to 3. As shown in Figs. 1 to 3, the inserted nucleic acid fragment (IFN γ R-IgG-glucagon¹⁹⁻²⁹) had EcoRI sites at both ends, in which a region coding for the glucagon-originated label peptide was ligated to the downstream of the region coding
25 for the fusion protein of interferon γ receptor (IFN γ R) protein and Fc region of immunoglobulin G1 (IgG1).

A plasmid vector not containing the glucagon-originated label peptide

(Comparative Example 1) and the plasmid vector (Example 1) according to the present invention prepared as described above, which contained the glucagon-originated label peptide, were intravenously administered by jet injection to 7 rats, respectively, from the tail vein, thereby performing gene therapy. The injection solution was one obtained by dissolving 800 µg per rat of the plasmid dissolved in 20 ml of Ringer solution. After the injection, blood was sampled with time, the sampled 1 to 10 µl of plasma was 100-fold to 1000-fold diluted, and the level of the glucagon-originated label peptide, and in turn, the level of the desired protein (in this Example, the IFN γ R/IgG1Fc fusion protein) was measured using a commercially available RIA kit (Pancreas Glucagon RIA kit commercially available from DAIICHI RADIOISOTOPE LABORATORIES, LTD and the like) in accordance with the instructions attached to the kit. That is, more concretely, RIA was performed as follows: To 400 µl of buffer for assay, 200 µl of standard glucagon solution or diluted test plasma was added, and 100 µl of glucagon-¹²⁵I solution and 100 µl of glucagon anti-serum solution were added, followed by leaving the resultant to stand at 4°C for 48 hours. Thereafter, 100 µl of a second antibody and 400 µl of a precipitation stabilizer for glucagon RIA were added, and the resulting mixture was left to stand at 4°C for 30 minutes. The resultant was then centrifuged (2000 x g, 30 minutes, 4°C), and the count rate was measured to determine the level after removing the supernatant.

Fig. 5 shows the results of the measurement of the blood level. In Example 1, on Day 1 after intravenous injection, the level was 2870 ± 1062 ng/ml (mean $\pm$ standard deviation), on Day 3, the level was 1440 ± 334 ng/ml, on Day 7, the level was 1120 ± 433 ng/ml, and on Day 16, the level was 281 ± 162 ng/ml, and the level was able to be measured in all cases. On the other hand, in the gene therapy with the plasmid vector not containing the glucagon peptide (Comparative Example 1), the level was less than the detection limit in all cases by the same assay.

Fig. 6 shows blood glucose level and the blood protein level measured by the RIA as described above, at 4, 8 and 12 hours after the intravenous injection of the plasmid. In Example 1, the blood level was 2815 ± 2318 ng/ml at 4 hours later, 6061 ± 2789 ng/ml at 8 hours later, and 5752 ± 2270 ng/ml at 12 hours later. The blood glucose levels at 8 to 12 hours later in which the blood protein level was the maximum, were 89.3 ± 15.1 mg/dl (Example 1) vs. 81.8 ± 7.5 mg/dl (Comparative Example 1), and 63.5 ± 5.7 mg/dl (Example 1) vs. 71.4 ± 6.9 mg/dl (Comparative Example 1), so that there were no differences.

From the results described above, it was shown that the blood level of the desired protein can be well measured until several tens days after the administration of the vector, using a very small quantity of plasma sample.

Example 2, Comparative Example 2

PCR was carried out using as a template the cDNA of cultured rat spleen cells stimulated by lectin, and using as primers 5'-gagaattcatttaaatggcttgccttggaactccagagg-3' and 5'-gcagcatcgcgccgcgtctgaatctgggcatggttctgg -3'. The obtained PCR product was incorporated into pCAGGS-IgG-glu19-29 prepared by the method described in Example 1, using *Swa*I and *Not*I.

By this, a recombinant vector containing rat CTLA4-IgG-glucagon¹⁹⁻²⁹ (a nucleic acid fragment in which the rat IgG Fc-coding region was ligated to the downstream of rat CTLA4-coding region, and the glucagon-originated label peptide-coding region was ligated to the downstream of the rat IgG Fc-coding region) having the nucleotide sequence shown in Figs. 7 to 9 and in SEQ ID NO:4 (the restriction sites are also shown), inserted into the *Eco*RI site of the above-described expression vector pCAGGS for mammalian cells, which recombinant vector expresses CTLA4-IgG-glucagon¹⁹⁻²⁹ in rat cells was prepared (Example 2). For comparison, a recombinant vector in which IgG-glucagon¹⁹⁻²⁹-coding region alone not containing the CTLA4-coding region was inserted was also prepared (Comparative Example 2).

To each rat after heart transplantation, the recombinant vector was administered in the same manner as in Example 1, and the blood level was measured. The days during which the heart was taken were also checked.

The results are shown in Figs. 10 and 11. As shown in Fig. 10, in Example 2, the CTLA4-IgG-glucagon¹⁹⁻²⁹ protein level changed as shown in Fig. 7. That is, although the glucagon in a 100-fold diluted sample could not be measured before the administration, the level sharply increased on Day 1 to a protein level of exceeding 5000 ng/ml. Although the protein level gradually decreased thereafter, the protein level was kept at more than 1000 ng/ml until the rats were sacrificed for evaluation on Day 105. As shown in Fig. 11, in Example 2, the heart was rejected in one animal among 10 animals on Day 14, in all of the remaining 9 animals, the heart was taken until Day 105 on which evaluation was performed. In the group treated with pCAGGS-SP-IgG-glucagon¹⁹⁻²⁹ not containing CTLA4, the heart of one animal among 5 animals was rejected on Day 5, the heart of one animal was rejected on Day 6, and the hearts of 3 animals were rejected on Day 7. These results show significant effectiveness of the therapy by pCAGGS-CTLA4-IgG-glucagon¹⁹⁻²⁹.

Example 3, Comparative Example 3

PCR was carried out using as a template the cDNA of cultured rat spleen cells stimulated by lectin, and using as primers 5'-gagaattcatttaaatggcactctgggtgactgcagtc-3' and 5'-gcagcatcgccgccgctggccatagcggaaaagttgctt-3'. The obtained PCR product was incorporated into pCAGGS-IgG-glu19-29 prepared by the method described in Example 1, using *Swa*I and *Not*I.

By this, a recombinant vector containing rat IL13-IgG-glucagon¹⁹⁻²⁹ (a nucleic acid fragment in which the rat IgG Fc-coding region was ligated to the downstream of rat interleukin 13 (IL13)-coding region, and the glucagon-originated label peptide-coding region was ligated to the downstream of the rat IgG Fc-coding region) having the nucleotide sequence shown in Figs. 12 to 14 and in SEQ ID NO:5

(the restriction sites are also shown), inserted into the EcoRI site of the above-described expression vector pCAGGS for mammalian cells, which recombinant vector expresses IL13-IgG-glucagon¹⁹⁻²⁹ in rat cells was prepared (Example 3). For comparison, a recombinant vector in which SP-IgG-glucagon¹⁹⁻²⁹-coding region alone (SEQ ID NO:6 and Figs. 15 and 16) not containing the IL13-coding region was
 5 inserted was also prepared (Comparative Example 3).

To rats suffering from autoimmune myocarditis (A novel experimental model of giant cell myocarditis induced in rats by immunization with cardiac myosin fraction, Clinical Immunology and Immunopathology, November 1990, volume 57,
 10 p250-262), the recombinant vector was administered in the same manner as in Example 1, and the blood level was measured. On Day 16 from the administration, the animals were sacrificed and anatomized. The percentage of the area of the myocarditis lesion was also checked.

The results are shown in Figs. 17 and 18. The IL13-IgG-glucagon¹⁹⁻²⁹ protein level changed as shown in Fig. 17. That is, on Day 1, a level exceeding
 15 2000 ng/ml was observed. Although the protein level gradually decreased thereafter, the protein level was kept at more than about 8 ng/ml until the rats were sacrificed for evaluation on Day 16. As shown in Fig. 18, in the group to which pCAGGS-IL13-IgG-glucagon¹⁹⁻²⁹ (the vector pCAGGS in which IL13-IgG-glucagon¹⁹⁻²⁹ was
 20 inserted) that is a vector for gene therapy according to the present invention, the area of the myocarditis lesion was significantly smaller than in the group to which pCAGGS-SP-IgG-glucagon¹⁹⁻²⁹ not containing IL13 was administered. Thus, the effectiveness of the therapy by pCAGGS-IL13-IgG-glucagon¹⁹⁻²⁹ was shown.

Example 4, Comparative Example 4

25 PCR was carried out using as a template the cDNA of cultured mouse spleen cells stimulated by lectin, and using as primers 5'-gagaattcatttaaatggaaatctgctggggaccctac-3' and 5'-

gcagcatcgccggccgttggtcttctggaagtagaactt-3'. The obtained PCR product was incorporated into pCAGGS-IgG-glu19-29 prepared by the method described in Example 1, using *Swa*I and *Not*I.

By this, a recombinant vector containing rat IL1RA-IgG-glucagon¹⁹⁻²⁹ (a nucleic acid fragment in which the rat IgG Fc-coding region was ligated to the downstream of interleukin 1 receptor antagonist-coding region, and the glucagon-originated label peptide-coding region was ligated to the downstream of the rat IgG Fc-coding region) having the nucleotide sequence shown in Figs. 19 to 21 and in SEQ ID NO:7 (the restriction sites are also shown), inserted into the *Eco*RI site of the above-described expression vector pCAGGS for mammalian cells, which recombinant vector expresses IL1RA-IgG-glucagon¹⁹⁻²⁹ in rat cells was prepared (Example 4).

To rats suffering from autoimmune myocarditis (A novel experimental model of giant cell myocarditis induced in rats by immunization with cardiac myosin fraction, *Clinical Immunology and Immunopathology*, November 1990, volume 57, p250-262), the recombinant vector was administered in the same manner as in Example 1, and the blood level was measured. On Day 16 from the administration, the animals were sacrificed and anatomized. The percentage of the area of the myocarditis lesion was also checked. For comparison, the above-described vector of Comparative Example 3 was also administered (Comparative Example 4).

The results are shown in Figs. 22 and 23. The IL1RA-IgG-glucagon¹⁹⁻²⁹ protein level changed as shown in Fig. 22. That is, on Day 1, a level exceeding 2000 ng/ml was observed. Although the protein level gradually decreased thereafter, the protein level was kept at more than about 20 ng/ml until the rats were sacrificed for evaluation on Day 16. As shown in Fig. 23, in Example 4, the area of the myocarditis lesion was significantly smaller than in Comparative Example 4, so that the effectiveness of the therapy by pCAGGS-IL1RA-IgG-glucagon¹⁹⁻²⁹ was shown.

Example 5

To prepare pCAGGS-glu19-29 having *Swa*I and *Not*I restriction sites, PCR was performed using as primers 5'-

gagaattcatttaaatgagagcggccgccccgggtaaagcccaagattttgtgcagtgggtg-3' and 5'-

5 gagagagagaattctcaggtattcatcaaccactgcacaaaatcttgggc-3' alone, and the PCR product was inserted into the cloning site of pCAGGS using *Eco*RI.

Thereafter, PCR was performed using the cDNA of Cos7 cells as a template and using as primers 5'-gagaattcatttaaatgacttccaagctggccgtggct-3' and 5'-

10 gcagcatcgccggcgctgaattctcagccctcttcaaaaa-3'. The PCR product was incorporated into the pCAGGS-glu19-29 prepared above using *Swa*I and *Not*I.

By this, a recombinant vector containing human IL8-glucagon¹⁹⁻²⁹ (a nucleic acid fragment in which the glucagon-originated label peptide-coding region was ligated to the downstream of the human interleukin 8-coding region) having the nucleotide sequence shown in Figs. 24 and in SEQ ID NO:8, inserted into the *Eco*RI site of the above-described expression vector pCAGGS for mammalian cells, which
15 recombinant vector expresses IL8-glucagon¹⁹⁻²⁹ in rat cells was prepared (Example 5).

The recombinant vector was administered to rats in the same manner as in Example 1. One day after, the blood level was measured. The human IL-8 level in
20 the same sample was also quantified. The quantification of human IL-8 was carried out using IL-8 ELISA kit commercially available from BIOSOURCE (Nivelles, Belgium) in accordance with the Protocols of the product.

The results are shown in Fig. 25. As shown in Fig. 25, the molar concentrations determined by the both measurements were substantially identical, so
25 that the accuracy of the method using the label peptide of glucagon¹⁹⁻²⁹ was proved.

CLAIMS

1. A vector for gene therapy comprising an expression vector for mammalian cells and a nucleic acid coding for a fusion protein of glucagon C-terminal side 19-29 amino acid peptide region and a desired protein region which should be produced in the body, which vector can produce said fusion protein in the mammalian cells.
2. The vector according to claim 1, wherein said glucagon C-terminal side 19-29 amino acid peptide region is ligated to the C-terminal of said desired protein region.
3. The vector according to claim 1 or 2, wherein said desired protein is a cytokine, a fusion protein comprising a cytokine and a constant region of immunoglobulin ligated to said cytokine, a growth factor, a hormone or a cell adhesion factor, or a receptor thereof.
4. The vector according to claim 3, wherein said cytokine or the receptor thereof is selected from the group consisting of interferons and receptors thereof, CTLA4, interleukins and receptors thereof.
5. A method for gene therapy comprising administering an effective amount of said vector for gene therapy according to any one of claims 1 to 4 to a mammal or cultured mammalian cells, in which expression of said desired protein in the body or in the cultured mammalian cells is desired.
6. The method according to claim 5, wherein said vector for gene therapy is administered to a mammal.
7. Use of the vector for gene therapy according to any one of claims 1 to 4 for the production of a drug for gene therapy.
8. A method for quantifying a desired protein produced in the body or in cultured cells by expression of said vector for gene therapy, comprising quantifying, by immunoassay, said glucagon C-terminal side 19-29 amino acid peptide region in a test sample collected from a mammal or cultured mammalian cells to which said vector for gene therapy according to any one of claims 1 to 4 was administered.

9. The method according to claim 8, wherein said test sample is collected from said mammal to which said vector for gene therapy was administered.

10. The method according to claim 9, wherein said test sample is a blood sample.

11. A label for labeling a desired protein produced by expression of an externally administered expression vector in the body of a mammal or in cultured mammalian cells, consisting essentially of glucagon C-terminal side 19-29 amino acid peptide.

12. A label for labeling a desired protein produced by expression of an externally administered expression vector in the body of a mammal, consisting essentially of glucagon C-terminal side 19-29 amino acid peptide.

13. A method for labeling a protein produced in the body or in cultured cells, comprising labeling a desired protein produced in the body or in cultured cells with glucagon C-terminal side 19-29 amino acid peptide by expressing said desired protein produced by expression of an externally administered expression vector in the body of a mammal or in cultured mammalian cells, as a fusion protein with said glucagon C-terminal side 19-29 amino acid peptide as a label.

14. The method according to claim 13, comprising labeling said desired protein produced in the body with glucagon C-terminal side 19-29 amino acid peptide by expressing said desired protein produced by expression of an externally administered expression vector in the body of a mammal, as a fusion protein with said glucagon C-terminal side 19-29 amino acid peptide as a label.

15. Use of glucagon C-terminal side 19-29 amino acid peptide as a label for a desired protein produced by expression of an externally administered expression vector in the body of a mammal or in cultured mammalian cells.

16. Use of glucagon C-terminal side 19-29 amino acid peptide as a label for a desired protein produced by expression of an externally administered expression vector in the body of a mammal.

SEQUENCE LISTING

<110> NIIGATA TLO CORPORATION

<120> Vector for gene therapy and method for quantifying target protein in mammal or cultured cell to which the vector for gene therapy was administered

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<150> JP 2003-3967

<151> 2003-01-10

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<211> 11

<212> PRT

<213> Artificial Sequence

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1

5

10

<210> 2

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<222> (13).. (1461)

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1

5

10

ggg agt gga gct ttg atg agc acc gag gat cct aag ccg ccc tcg gtg 99

Gly Ser Gly Ala Leu Met Ser Thr Glu Asp Pro Lys Pro Pro Ser Val

15

20

25

cct gcg cca aca aat gtt cta att acg tcc tat gac ttg aac cct gtc 147

Pro Ala Pro Thr Asn Val Leu Ile Thr Ser Tyr Asp Leu Asn Pro Val

30

35

40

45

gta cat tgg aag cac cag aac gtg tcg cag gct gcc gtc ttc act gta 195

Val His Trp Lys His Gln Asn Val Ser Gln Ala Ala Val Phe Thr Val

50

55

60

cag gta aag atg tat cca gaa tac tgg act gat gcc tgc acc aac att 243

Gln Val Lys Met Tyr Pro Glu Tyr Trp Thr Asp Ala Cys Thr Asn Ile

65

70

75

gcc cat cat tat tgt aat atc tac aaa cac att tcc tat cct gac tca 291

Ala His His Tyr Cys Asn Ile Tyr Lys His Ile Ser Tyr Pro Asp Ser

80

85

90

tct gcc tgg gcc aga gtt aag gcc aag gtt gga caa aga gaa tct gcc 339

Ser Ala Trp Ala Arg Val Lys Ala Lys Val Gly Gln Arg Glu Ser Ala

95

100

105

tat gcg cag tca gaa gag ttt att atg tgc cga aag ggg aag gtt gga 387

Tyr Ala Gln Ser Glu Glu Phe Ile Met Cys Arg Lys Gly Lys Val Gly

110

115

120

125

ccg cct ggc ctg gac atc gga agg aag gaa gat cag ctg att gtc cac 435

Pro Pro Gly Leu Asp Ile Gly Arg Lys Glu Asp Gln Leu Ile Val His

130	135	140	
ata ttt cac cct aag gtc aat gtg agt cag gaa acc atg ttt ggt gac			483
Ile Phe His Pro Lys Val Asn Val Ser Gln Glu Thr Met Phe Gly Asp			
145	150	155	
gga aat acc tgt tac aca ttc gac tac act gtg ttt gtg aaa cat tac			531
Gly Asn Thr Cys Tyr Thr Phe Asp Tyr Thr Val Phe Val Lys His Tyr			
160	165	170	
agg agt ggg gag atc cta cat aca gaa cat agc gtc cta aaa gaa gat			579
Arg Ser Gly Glu Ile Leu His Thr Glu His Ser Val Leu Lys Glu Asp			
175	180	185	
tgt agc gaa act ctg tgt gag tta aac atc tca gtg tcc acg ctg aat			627
Cys Ser Glu Thr Leu Cys Glu Leu Asn Ile Ser Val Ser Thr Leu Asn			
190	195	200	205
tcc aat tac tgt gtt tca gta gtt gga aag tcg tct ttc tgg caa gtt			675
Ser Asn Tyr Cys Val Ser Val Val Gly Lys Ser Ser Phe Trp Gln Val			
210	215	220	
aat aca gaa aca tca aaa gac gcc tgt atc ccc ttt ctc cat gat gac			723
Asn Thr Glu Thr Ser Lys Asp Ala Cys Ile Pro Phe Leu His Asp Asp			
225	230	235	
aga gaa gaa gcg gcc gcc gtg ccc aga aac tgt gga ggt gat tgc aag			771
Arg Glu Glu Ala Ala Ala Val Pro Arg Asn Cys Gly Gly Asp Cys Lys			
240	245	250	
cct tgt ata tgt aca ggc tca gaa gta tca tct gtc ttc atc ttc ccc			819
Pro Cys Ile Cys Thr Gly Ser Glu Val Ser Ser Val Phe Ile Phe Pro			
255	260	265	
cca aag ccc aaa gat gtg ctc acc atc act ctg act cct aag gtc acg			867
Pro Lys Pro Lys Asp Val Leu Thr Ile Thr Leu Thr Pro Lys Val Thr			

270	275	280	285	
tgt gtt gtg gta gac att agc cag gac gat ccc gag gtc cat ttc agc				915
Cys Val Val Val Asp Ile Ser Gln Asp Asp Pro Glu Val His Phe Ser				
290	295	300		
tgg ttt gta gat gac gtg gaa gtc cac aca gct cag act cga cca cca				963
Trp Phe Val Asp Asp Val Glu Val His Thr Ala Gln Thr Arg Pro Pro				
305	310	315		
gag gag cag ttc aac agc act ttc cgc tca gtc agt gaa ctc ccc atc				1011
Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val Ser Glu Leu Pro Ile				
320	325	330		
ctg cac cag gac tgg ctc aat ggc agg acg ttc aga tgc aag gtc acc				1059
Leu His Gln Asp Trp Leu Asn Gly Arg Thr Phe Arg Cys Lys Val Thr				
335	340	345		
agt gca gct ttc cca tcc ccc atc gag aaa acc atc tcc aaa ccc gaa				1107
Ser Ala Ala Phe Pro Ser Pro Ile Glu Lys Thr Ile Ser Lys Pro Glu				
350	355	360	365	
ggc aga aca caa gtt ccg cat gta tac acc atg tca cct acc aag gaa				1155
Gly Arg Thr Gln Val Pro His Val Tyr Thr Met Ser Pro Thr Lys Glu				
370	375	380		
gag atg acc cag aat gaa gtc agt atc acc tgc atg gta aaa ggc ttc				1203
Glu Met Thr Gln Asn Glu Val Ser Ile Thr Cys Met Val Lys Gly Phe				
385	390	395		
tat ccc cca gac att tat gtg gag tgg cag atg aac ggg cag cca cag				1251
Tyr Pro Pro Asp Ile Tyr Val Glu Trp Gln Met Asn Gly Gln Pro Gln				
400	405	410		
gaa aac tac aag aac act cca cct acg atg gac aca gat ggg agt tac				1299
Glu Asn Tyr Lys Asn Thr Pro Pro Thr Met Asp Thr Asp Gly Ser Tyr				

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415	420	425	
ttc ctc tac agc aag ctc aat gtg aag aag gaa aaa tgg cag cag gga			1347
Phe Leu Tyr Ser Lys Leu Asn Val Lys Lys Glu Lys Trp Gln Gln Gly			
430	435	440	445
aac acg ttc acg tgt tct gtg ctg cat gaa ggc ctg cac aac cac cat			1395
Asn Thr Phe Thr Cys Ser Val Leu His Glu Gly Leu His Asn His His			
450	455	460	
act gag aag agt ctc tcc cac tct ccg ggt aaa gcc caa gat ttt gtg			1443
Thr Glu Lys Ser Leu Ser His Ser Pro Gly Lys Ala Gln Asp Phe Val			
465	470	475	
cag tgg ttg atg aat acc tgagaattct			1471
Gln Trp Leu Met Asn Thr			
480			

<210> 3

<211> 4790

<212> DNA

<213> Artificial Sequence

<220>

<223> DNA sequence of artificial expression vector pCAGGS

<400> 3

gtogacattg attattgact agttattaat agtaatcaat tacgggggtca ttagttcata	60
gcccataatat ggagttccgc gttacataac ttacggtaaa tggcccgccct ggctgaccgc	120
ccaacgaccc ccgcccattg acgtcaataa tgacgtatgt tcccatagta acgccaatag	180
ggactttcca ttgacgtcaa tgggtggact atttacggta aactgcccac ttggcagtac	240
atcaagtgtat tcatatgccca agtaogcccc ctattgacgt caatgacggt aaatggcccc	300

cctggcatta tgcccagtac atgaccttat gggactttcc tacttggcag tacatctacg 360
 tattagtcat cgtattacc atgggtcgag gtgagcccca cgttctgctt cactctcccc 420
 atctcccccc cctccccacc cccaattttg tatttatita tttttaatt attttgtga 480
 gcgatggggg cggggggggg gggggcgcg cccaggcggg gcggggcggg gcgagggcg 540
 gggcggggcg aggcggagag gtgcggcggc agccaatcag agcggcgcg tccgaaagtt 600
 tcottttatg gcgagggcg gcggcgggc gccctataaa aagcgaagcg cgcggcgggc 660
 gggagtcgct gcgttgctt cggccgtgc ccgctccgc gccgcctgc gccgccggc 720
 cggctctga ctgaccgct tactcccaca ggtgagcggg cgggacggc cttctctcc 780
 gggctgtaat tagcgcttg tttaatgacg gctcgtttct tttctgtggc tgcgtgaaag 840
 ccttaaaggg ctccgggagg gccctttgtg cgggggggag cggctcggg ggtgcgtgcg 900
 tgtgtgtgtg cgtggggagc gccgcgtgc gccgcgctg ccggcggt gtgagcgtg 960
 cgggcgggc gcggggcttt gtgcgtccg cgtgtgcgc aggggagcgc gccgggggc 1020
 ggtgccccgc ggtgcggggg ggtgcgagg ggaacaaagg ctgcgtcgg ggtgtgtgcg 1080
 tgggggggtg agcagggggt gtgggcggc cggtcgggt gtaaccccc cctgcacccc 1140
 cctccccgag ttgtgagca cggcccggt tcgggtcgg ggtccgtgc gggcggtggc 1200
 gcggggctcg ccgtccggg cggggggtg oggcaggtg ggtgcccgg cggggcggg 1260
 ccgcctcgg ccggggagg ctgggggag gggcgcgcg gccccggag gcggcggt 1320
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gacttccttt gtoccaaato tggcggagcc gaaatctggg aggcgcgcgc gcacccctc 1440
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 agtatttggt ttagagtttg gcaacatatg ccatatgctg gctgccatga acaaaggctg 2040
 ctataaagag gtcacagta tatgaaacag cccctgctg tccattcctt attccataga 2100
 aaagccttga cttgaggta gatttttttt atattttgtt ttgtgttatt tttttctta 2160
 acatccctaa aattttcctt acatgtttta ctagccagat ttttcctcct ctctgacta 2220
 ctcccagtca tagctgtccc tcttotctta tgaagatccc tcgacctgca gcccaagctt 2280
 ggcgtaatca tggcatagc tgtttcctgt gtgaaattgt tatccgotca caattccaca 2340
 caacatacga gccggaagca taaagtgtaa agcctggggg gcctaataag tgagctaact 2400
 cacattaatt gcgttgccgt cactgcccgc tttccagtcg ggaaacctgt cgtgccagcg 2460

gatccgcac tcaattagtc agcaaccata gtcccgcccc taactccgcc catcccgccc 2520
 ctaactccgc ccagttccgc ccattctcgc ccccatggct gactaatttt tttattttat 2580
 gcagaggcgc aggcgcctc ggctctgag ctattccaga agtagtgagg aggctttttt 2640
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 aaagcaatag catcacaaat ttacaaaata aagcattttt ttactgcat tctagtgtg 2760
 gttgtccaa actcatcaat gtatcttato atgtctggat ccgctgcatt aatgaatcgg 2820
 ccaacgcgcg gggagaggcg gtttgcgtat tgggcgtct tccgcttcc cgtcactga 2880
 ctgctgcgc togtctgtc ggctgcggcg agcggatatca gctcactcaa aggcggtaat 2940
 acggttatcc acagaatcag gggataacgc aggaagaac atgtgagcaa aaggccagca 3000
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 aagataccag gcgtttcccc ctggaagctc cctcgtgcgc tctcctgttc cgacctgcc 3180
 gcttacogga tacctgtcgc cctttctccc ttogggaagc gtggcggtt ctcaatgctc 3240
 acgctgtagg tatctcagtt cgggttaggt cgttcgctcc aagctgggct gtgtgcacga 3300
 acccccggt cagccgacc gctgcgcctt atccggtaac tatcgtcttg agtccaacco 3360
 ggtaagacac gacttatcgc cactggcagc agccactggt aacaggatta gcagagcgag 3420
 gtatgtaggc ggtgtacag agttcttgaa gtgggtggct aactacggct aactagaag 3480
 gacagtattt ggtatctgcg ctctctgaa gccagttacc ttcggaataa gagttggtag 3540

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ctcttgatcc ggcaaacaaa ccaccgctgg tagcgggtgt tttttgttt gcaagcagca 3600
gattacgcgc agaaaaaag gatctcaaga agatccttg atottttcta cggggtctga 3660
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gtaaacttgg tctgacagtt accaatgctt aatcagttag gcacctatct cagcgatctg 3840
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gggcttiacca tctggcccca gtgtgcaat gataccgcga gaccacgct caccggctcc 3960
agatttatca gcaataaacc agccagccgg aaggccgag cgcagaagtg gtcctgcaac 4020
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gtttggtatg gcttcattca gctccggttc ccaacgatca aggcgagtta catgatcccc 4200
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ggccgcagtg ttatcaacta tggttatggc agcactgcat aattctotta ctgtcatgcc 4320
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ottacogctg ttgagatoca gttogatga acccactcgt gcaccaact gatottcagc 4560
atcttttact ttcaccagcg tttctgggtg agcaaaaaca ggaaggcaaa atgccgcaaa 4620

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aaaggaata agggcgacac ggaaatgttg aatactcata ctcttccttt ttcaatatta 4680

ttgaagcatt tatcagggtt attgtctcat gagcggatac atatttgaat gtatttagaa 4740

aaataaacia ataggggttc cgcgcacatt tccccgaaaa gtgccacctg 4790

<210> 4

<211> 1233

<212> DNA

<213> Artificial Sequence

<220>

<221> CDS

<222> (13)..(1224)

<223> DNA insert encoding rat CTLA4, rat IgG Fc region and glucagon C19-29 region

<400> 4

gaattcattt aa atg gct tgt ctt gga ctc cag agg tac aaa act cac ctg 51

Met Ala Cys Leu Gly Leu Gln Arg Tyr Lys Thr His Leu

1

5

10

cag ctg cct tct agg act tgg cct ttt gga gtc ctg ott tct ott ctc 99

Gln Leu Pro Ser Arg Thr Trp Pro Phe Gly Val Leu Leu Ser Leu Leu

15

20

25

ttc atc oca atc ttc tct gaa gcc ata caa gtg acc caa cct toa gtg 147

Phe Ile Pro Ile Phe Ser Glu Ala Ile Gln Val Thr Gln Pro Ser Val

30

35

40

45

gtg ttg gcc agc agc cac ggt gtc gcc agc ttt oca tgt gaa tat gca 195

Val Leu Ala Ser Ser His Gly Val Ala Ser Phe Pro Cys Glu Tyr Ala

50

55

60

tct tca cac aac act gat gag gtc ogg gtg acg gtg ctg ogg cag aca 243

Ser Ser His Asn Thr Asp Glu Val Arg Val Thr Val Leu Arg Gln Thr

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65	70	75	
aat gac caa gtg aca gag gtc tgt gcc acg aca ttc aca gtg aag aac			291
Asn Asp Gln Val Thr Glu Val Cys Ala Thr Thr Phe Thr Val Lys Asn			
80	85	90	
acg ttg ggc ttc cta gat gac ccc ttc tgc agt ggt acc ttt aat gaa			339
Thr Leu Gly Phe Leu Asp Asp Pro Phe Cys Ser Gly Thr Phe Asn Glu			
95	100	105	
agc aga gtg aac ctc acc atc caa gga ctg agg got gct gac acc gga			387
Ser Arg Val Asn Leu Thr Ile Gln Gly Leu Arg Ala Ala Asp Thr Gly			
110	115	120	125
ctg tac ttc tgc aag gtg gaa ctc atg tac cca ccg cca tac ttt gtg			435
Leu Tyr Phe Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr Phe Val			
130	135	140	
ggc atg ggc aac ggg acc cag att tat gtc atc gat cca gaa cca tgc			483
Gly Met Gly Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu Pro Cys			
145	150	155	
cca gat tca gac gcg gcc gcc gtg ccc aga aac tgt gga ggt gat tgc			531
Pro Asp Ser Asp Ala Ala Ala Val Pro Arg Asn Cys Gly Gly Asp Cys			
160	165	170	
aag cct tgt ata tgt aca ggc tca gaa gta tca tot gtc ttc atc ttc			579
Lys Pro Cys Ile Cys Thr Gly Ser Glu Val Ser Ser Val Phe Ile Phe			
175	180	185	
ccc cca aag ccc aaa gat gtg ctc acc atc act ctg act cct aag gtc			627
Pro Pro Lys Pro Lys Asp Val Leu Thr Ile Thr Leu Thr Pro Lys Val			
190	195	200	205
acg tgt gtt gtg gta gac att agc cag gac gat ccc gag gtc cat ttc			675
Thr Cys Val Val Val Asp Ile Ser Gln Asp Asp Pro Glu Val His Phe			

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210	215	220	
agc tgg ttt gta gat gac gtg gaa gtc cac aca gct cag act cga cca			723
Ser Trp Phe Val Asp Asp Val Glu Val His Thr Ala Gln Thr Arg Pro			
225	230	235	
cca gag gag cag ttc aac agc act ttc cgc tca gtc agt gaa ctc ccc			771
Pro Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val Ser Glu Leu Pro			
240	245	250	
atc ctg cac cag gac tgg ctc aat ggc agg acg ttc aga tgc aag gtc			819
Ile Leu His Gln Asp Trp Leu Asn Gly Arg Thr Phe Arg Cys Lys Val			
255	260	265	
acc agt gca gct ttc cca tcc ccc atc gag aaa acc atc tcc aaa ccc			867
Thr Ser Ala Ala Phe Pro Ser Pro Ile Glu Lys Thr Ile Ser Lys Pro			
270	275	280	285
gaa ggc aga aca caa gtt ccg cat gta tac acc atg tca cct acc aag			915
Glu Gly Arg Thr Gln Val Pro His Val Tyr Thr Met Ser Pro Thr Lys			
290	295	300	
gaa gag atg acc cag aat gaa gtc agt atc acc tgc atg gta aaa ggc			963
Glu Glu Met Thr Gln Asn Glu Val Ser Ile Thr Cys Met Val Lys Gly			
305	310	315	
ttc tat ccc cca gac att tat gtg gag tgg cag atg aac ggg cag cca			1011
Phe Tyr Pro Pro Asp Ile Tyr Val Glu Trp Gln Met Asn Gly Gln Pro			
320	325	330	
cag gaa aac tac aag aac act cca cct acg atg gac aca gat ggg agt			1059
Gln Glu Asn Tyr Lys Asn Thr Pro Pro Thr Met Asp Thr Asp Gly Ser			
335	340	345	
tac ttc ctc tac agc aag ctc aat gtg aag aag gaa aaa tgg cag cag			1107
Tyr Phe Leu Tyr Ser Lys Leu Asn Val Lys Lys Glu Lys Trp Gln Gln			

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350	355	360	365	
gga aac acg ttc acg tgt tct gtg ctg cat gaa ggc ctg cac aac cac				1155
Gly Asn Thr Phe Thr Cys Ser Val Leu His Glu Gly Leu His Asn His				
	370	375	380	
cat act gag aag agt ctc tcc cac tct ccg ggt aaa gcc caa gat ttt				1203
His Thr Glu Lys Ser Leu Ser His Ser Pro Gly Lys Ala Gln Asp Phe				
	385	390	395	
gtg cag tgg ttg atg aat acc tgagaattc				1233
Val Gln Trp Leu Met Asn Thr				
	400			

<210> 5

<211> 1143

<212> DNA

<213> Artificial Sequence

<220>

<221> CDS

<222> (13).. (1134)

<223> DNA insert encoding rat IL13, rat IgG Fc region and glucagon C19-29 region

<400> 5

gaattcattt aa atg gca ctc tgg gtg act gca gtc ctg gct ctc gct tgc	51
Met Ala Leu Trp Val Thr Ala Val Leu Ala Leu Ala Cys	
1 5 10	

ott ggt ggt ott gcc acc cca ggg cca gtg cgg aga tcc aca tct ccc	99
Leu Gly Gly Leu Ala Thr Pro Gly Pro Val Arg Arg Ser Thr Ser Pro	
15 20 25	

cct gtg gcc ctc agg gag ctt atc gag gag ctg agc aac atc aca caa	147
Pro Val Ala Leu Arg Glu Leu Ile Glu Glu Leu Ser Asn Ile Thr Gln	
30 35 40 45	

gac cag aag act tcc ctg tgc aac agc agc atg gta tgg agc gtg gac 195
 Asp Gln Lys Thr Ser Leu Cys Asn Ser Ser Met Val Trp Ser Val Asp
 50 55 60

ctg aca gct ggc ggg ttc tgt gca gcc ctg gaa tcc ctg acc aac atc 243
 Leu Thr Ala Gly Gly Phe Cys Ala Ala Leu Glu Ser Leu Thr Asn Ile
 65 70 75

tcc agt tgc aat gcc atc cac agg acc cag agg ata ttg aat ggc ctc 291
 Ser Ser Cys Asn Ala Ile His Arg Thr Gln Arg Ile Leu Asn Gly Leu
 80 85 90

tgt aac caa aag gcc tcg gat gtg gct tcc agc ccc cca gat acc aaa 339
 Cys Asn Gln Lys Ala Ser Asp Val Ala Ser Ser Pro Pro Asp Thr Lys
 95 100 105

atc gaa gta gcc cag ttt ata tca aaa ctg ctc aat tac tcc aag caa 387
 Ile Glu Val Ala Gln Phe Ile Ser Lys Leu Leu Asn Tyr Ser Lys Gln
 110 115 120 125

ott ttc cgc tat ggc cac gcg gcc gcc gtg ccc aga aac tgt gga ggt 435
 Leu Phe Arg Tyr Gly His Ala Ala Ala Val Pro Arg Asn Cys Gly Gly
 130 135 140

gat tgc aag cct tgt ata tgt aca ggc tca gaa gta tca tct gtc ttc 483
 Asp Cys Lys Pro Cys Ile Cys Thr Gly Ser Glu Val Ser Ser Val Phe
 145 150 155

atc ttc ccc cca aag ccc aaa gat gtg ctc acc atc act ctg act cct 531
 Ile Phe Pro Pro Lys Pro Lys Asp Val Leu Thr Ile Thr Leu Thr Pro
 160 165 170

aag gtc acg tgt gtt gtg gta gac att agc cag gac gat ccc gag gtc 579
 Lys Val Thr Cys Val Val Val Asp Ile Ser Gln Asp Asp Pro Glu Val
 175 180 185

cat ttc agc tgg ttt gta gat gac gtg gaa gtc cac aca gct cag act 627
 His Phe Ser Trp Phe Val Asp Asp Val Glu Val His Thr Ala Gln Thr
 190 195 200 205

cga cca cca gag gag cag ttc aac agc act ttc cgc tca gtc agt gaa 675
 Arg Pro Pro Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val Ser Glu
 210 215 220

ctc ccc atc ctg cac cag gac tgg ctc aat ggc agg acg ttc aga tgc 723
 Leu Pro Ile Leu His Gln Asp Trp Leu Asn Gly Arg Thr Phe Arg Cys
 225 230 235

aag gtc acc agt gca gct ttc cca tcc ccc atc gag aaa acc atc tcc 771
 Lys Val Thr Ser Ala Ala Phe Pro Ser Pro Ile Glu Lys Thr Ile Ser
 240 245 250

aaa ccc gaa ggc aga aca caa gtt cgc cat gta tac acc atg tca cct 819
 Lys Pro Glu Gly Arg Thr Gln Val Pro His Val Tyr Thr Met Ser Pro
 255 260 265

acc aag gaa gag atg acc cag aat gaa gtc agt atc acc tgc atg gta 867
 Thr Lys Glu Glu Met Thr Gln Asn Glu Val Ser Ile Thr Cys Met Val
 270 275 280 285

aaa ggc ttc tat ccc cca gac att tat gtg gag tgg cag atg aac ggg 915
 Lys Gly Phe Tyr Pro Pro Asp Ile Tyr Val Glu Trp Gln Met Asn Gly
 290 295 300

cag cca cag gaa aac tac aag aac act cca cct acg atg gac aca gat 963
 Gln Pro Gln Glu Asn Tyr Lys Asn Thr Pro Pro Thr Met Asp Thr Asp
 305 310 315

ggg agt tac ttc ctc tac agc aag ctc aat gtg aag aag gaa aaa tgg 1011
 Gly Ser Tyr Phe Leu Tyr Ser Lys Leu Asn Val Lys Lys Glu Lys Trp
 320 325 330

cag cag gga aac acg ttc acg tgt tct gtg ctg cat gaa ggc ctg cac 1059
 Gln Gln Gly Asn Thr Phe Thr Cys Ser Val Leu His Glu Gly Leu His
 335 340 345

aac cac cat act gag aag agt ctc tcc cac tct ccg ggt aaa gcc caa 1107
 Asn His His Thr Glu Lys Ser Leu Ser His Ser Pro Gly Lys Ala Gln
 350 355 360 365

gat ttt gtg cag tgg ttg atg aat acc tgagaattc 1143
 Asp Phe Val Gln Trp Leu Met Asn Thr
 370

<210> 6

<211> 825

<212> DNA

<213> Artificial Sequence

<220>

<221> CDS

<222> (13)..(816)

<223> DNA insert encoding rat signal peptide, rat IgG Fc region and glucagon C19-29 region

<400> 6

gaattcattt aa atg aag tcc tgc ggc ctg ttc cct ctc atg gtg ctc ctt 51
 Met Lys Ser Cys Gly Leu Phe Pro Leu Met Val Leu Leu
 1 5 10

gct ctg ggt gta ctg gca ccc tgg agt gtg gaa gga gcg gcc gcc gtg 99
 Ala Leu Gly Val Leu Ala Pro Trp Ser Val Glu Gly Ala Ala Ala Val
 15 20 25

ccc aga aac tgt gga ggt gat tgc aag cct tgt ata tgt aca ggc tca 147
 Pro Arg Asn Cys Gly Gly Asp Cys Lys Pro Cys Ile Cys Thr Gly Ser
 30 35 40 45

gaa gta tca tct gtc ttc atc ttc ccc cca aag ccc aaa gat gtg ctc	195
Glu Val Ser Ser Val Phe Ile Phe Pro Pro Lys Pro Lys Asp Val Leu	
50 55 60	
acc atc act ctg act cct aag gtc acg tgt gtt gtg gta gac att agc	243
Thr Ile Thr Leu Thr Pro Lys Val Thr Cys Val Val Val Asp Ile Ser	
65 70 75	
cag gac gat ccc gag gtc cat ttc agc tgg ttt gta gat gac gtg gaa	291
Gln Asp Asp Pro Glu Val His Phe Ser Trp Phe Val Asp Asp Val Glu	
80 85 90	
gtc cac aca gct cag act cga cca cca gag gag cag ttc aac agc act	339
Val His Thr Ala Gln Thr Arg Pro Pro Glu Glu Gln Phe Asn Ser Thr	
95 100 105	
ttc cgc tca gtc agt gaa ctc ccc atc ctg cac cag gac tgg ctc aat	387
Phe Arg Ser Val Ser Glu Leu Pro Ile Leu His Gln Asp Trp Leu Asn	
110 115 120 125	
ggc agg acg ttc aga tgc aag gtc acc agt gca gct ttc cca tcc ccc	435
Gly Arg Thr Phe Arg Cys Lys Val Thr Ser Ala Ala Phe Pro Ser Pro	
130 135 140	
atc gag aaa acc atc tcc aaa ccc gaa ggc aga aca caa gtt ccg cat	483
Ile Glu Lys Thr Ile Ser Lys Pro Glu Gly Arg Thr Gln Val Pro His	
145 150 155	
gta tac acc atg tca cct acc aag gaa gag atg acc cag aat gaa gtc	531
Val Tyr Thr Met Ser Pro Thr Lys Glu Glu Met Thr Gln Asn Glu Val	
160 165 170	
agt atc acc tgc atg gta aaa ggc ttc tat ccc cca gac att tat gtg	579
Ser Ile Thr Cys Met Val Lys Gly Phe Tyr Pro Pro Asp Ile Tyr Val	
175 180 185	

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gag tgg cag atg aac ggg cag cca cag gaa aac tac aag aac act cca 627
 Glu Trp Gln Met Asn Gly Gln Pro Gln Glu Asn Tyr Lys Asn Thr Pro
 190 195 200 205

cct acg atg gac aca gat ggg agt tac ttc ctc tac agc aag ctc aat 675
 Pro Thr Met Asp Thr Asp Gly Ser Tyr Phe Leu Tyr Ser Lys Leu Asn
 210 215 220

gtg aag aag gaa aaa tgg cag cag gga aac acg ttc acg tgt tct gtg 723
 Val Lys Lys Glu Lys Trp Gln Gln Gly Asn Thr Phe Thr Cys Ser Val
 225 230 235

ctg cat gaa ggc ctg cac aac cac cat act gag aag agt ctc tcc cac 771
 Leu His Glu Gly Leu His Asn His His Thr Glu Lys Ser Leu Ser His
 240 245 250

tct cgg ggt aaa gcc caa gat ttt gtg cag tgg ttg atg aat acc 816
 Ser Pro Gly Lys Ala Gln Asp Phe Val Gln Trp Leu Met Asn Thr
 255 260 265

tgagaattc 825

<210> 7

<211> 1284

<212> DNA

<213> Artificial Sequence

<220>

<221> CDS

<222> (13).. (1275)

<223> DNA insert encoding rat IL1 receptor antagonist, rat IgG Fc region and glucagon
 C19-29 region

<400> 7

gaattcattt aa atg gaa atc tgc tgg gga ccc tac agt cac cta atc tct 51

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Met Glu Ile Cys Trp Gly Pro Tyr Ser His Leu Ile Ser

1

5

10

ctc ctt ctc atc ctt ctg ttt cat tca gag gca gcc tgc cgc cct tct 99
Leu Leu Leu Ile Leu Leu Phe His Ser Glu Ala Ala Cys Arg Pro Ser
15 20 25

ggg aaa aga ccc tgc aag atg caa gcc ttc aga atc tgg gat act aac 147
Gly Lys Arg Pro Cys Lys Met Gln Ala Phe Arg Ile Trp Asp Thr Asn
30 35 40 45

cag aag acc ttt tac ctg aga aac aac cag ctc att gct ggg tac tta 195
Gln Lys Thr Phe Tyr Leu Arg Asn Asn Gln Leu Ile Ala Gly Tyr Leu
50 55 60

caa gga cca aat atc aaa cta gaa gaa aag ata gac atg gtg cct att 243
Gln Gly Pro Asn Ile Lys Leu Glu Glu Lys Ile Asp Met Val Pro Ile
65 70 75

gac ctt cat agt gtg ttc ttg ggc atc cac ggg ggc aag ctg tgc ctg 291
Asp Leu His Ser Val Phe Leu Gly Ile His Gly Gly Lys Leu Cys Leu
80 85 90

tct tgt gcc aag tct gga gat gat atc aag ctc cag ctg gag gaa gtt 339
Ser Cys Ala Lys Ser Gly Asp Asp Ile Lys Leu Gln Leu Glu Glu Val
95 100 105

aac atc act gat ctg agc aag aac aaa gaa gaa gac aag cgc ttt acc 387
Asn Ile Thr Asp Leu Ser Lys Asn Lys Glu Glu Asp Lys Arg Phe Thr
110 115 120 125

ttc atc cgc tct gag aaa ggc ccc acc acc agc ttt gag tca gct gcc 435
Phe Ile Arg Ser Glu Lys Gly Pro Thr Thr Ser Phe Glu Ser Ala Ala
130 135 140

tgt cca gga tgg ttc ctc tgc aca aca cta gag gct gac cgt cct gtg 483

Cys Pro Gly Trp Phe Leu Cys Thr Thr Leu Glu Ala Asp Arg Pro Val
 145 150 155

ago ctc acc aac aca ccg gaa gag ccc ctt ata gtc acg aag ttc tac 531
 Ser Leu Thr Asn Thr Pro Glu Glu Pro Leu Ile Val Thr Lys Phe Tyr
 160 165 170

ttc cag gaa gac caa gcg gcc gcc gtg ccc aga aac tgt gga ggt gat 579
 Phe Gln Glu Asp Gln Ala Ala Ala Val Pro Arg Asn Cys Gly Gly Asp
 175 180 185

tgc aag cct tgt ata tgt aca ggc tca gaa gta tca tct gtc ttc atc 627
 Cys Lys Pro Cys Ile Cys Thr Gly Ser Glu Val Ser Ser Val Phe Ile
 190 195 200 205

ttc ccc cca aag ccc aaa gat gtg ctc acc atc act ctg act cct aag 675
 Phe Pro Pro Lys Pro Lys Asp Val Leu Thr Ile Thr Leu Thr Pro Lys
 210 215 220

gtc acg tgt gtt gtg gta gac att agc cag gac gat ccc gag gtc cat 723
 Val Thr Cys Val Val Val Asp Ile Ser Gln Asp Asp Pro Glu Val His
 225 230 235

ttc agc tgg ttt gta gat gac gtg gaa gtc cac aca gct cag act cga 771
 Phe Ser Trp Phe Val Asp Asp Val Glu Val His Thr Ala Gln Thr Arg
 240 245 250

cca cca gag gag cag ttc aac agc act ttc cgc tca gtc agt gaa ctc 819
 Pro Pro Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val Ser Glu Leu
 255 260 265

ccc atc ctg cac cag gac tgg ctc aat ggc agg acg ttc aga tgc aag 867
 Pro Ile Leu His Gln Asp Trp Leu Asn Gly Arg Thr Phe Arg Cys Lys
 270 275 280 285

gtc acc agt gca gct ttc cca tcc ccc atc gag aaa acc atc tcc aaa 915

Val Thr Ser Ala Ala Phe Pro Ser Pro Ile Glu Lys Thr Ile Ser Lys
 290 295 300

ccc gaa ggc aga aca caa gtt ccg cat gta tac acc atg tca cct acc 963
 Pro Glu Gly Arg Thr Gln Val Pro His Val Tyr Thr Met Ser Pro Thr
 305 310 315

aag gaa gag atg acc cag aat gaa gtc agt atc acc tgc atg gta aaa 1011
 Lys Glu Glu Met Thr Gln Asn Glu Val Ser Ile Thr Cys Met Val Lys
 320 325 330

ggc ttc tat ccc cca gac att tat gtg gag tgg cag atg aac ggg cag 1059
 Gly Phe Tyr Pro Pro Asp Ile Tyr Val Glu Trp Gln Met Asn Gly Gln
 335 340 345

cca cag gaa aac tac aag aac act cca cct acg atg gac aca gat ggg 1107
 Pro Gln Glu Asn Tyr Lys Asn Thr Pro Pro Thr Met Asp Thr Asp Gly
 350 355 360 365

agt tac ttc ctc tac agc aag ctc aat gtg aag aag gaa aaa tgg cag 1155
 Ser Tyr Phe Leu Tyr Ser Lys Leu Asn Val Lys Lys Glu Lys Trp Gln
 370 375 380

cag gga aac acg ttc acg tgt tct gtg ctg cat gaa ggc ctg cac aac 1203
 Gln Gly Asn Thr Phe Thr Cys Ser Val Leu His Glu Gly Leu His Asn
 385 390 395

cac cat act gag aag agt ctc tcc cac tct ccg ggt aaa gcc caa gat 1251
 His His Thr Glu Lys Ser Leu Ser His Ser Pro Gly Lys Ala Gln Asp
 400 405 410

ttt gtg cag tgg ttg atg aat acc tgagaatto 1284
 Phe Val Gln Trp Leu Met Asn Thr
 415 420

<210> 8
 <211> 369
 <212> DNA
 <213> Artificial Sequence

<220>
 <221> CDS
 <222> (13)..(360)
 <223> DNA insert encoding human IL8 and glucagon C19-29 region

<400> 8
 gaattcattt aa atg act tcc aag ctg gcc gtg gct ctc ttg gca gcc ttc 51
 Met Thr Ser Lys Leu Ala Val Ala Leu Leu Ala Ala Phe
 1 5 10
 ctg att tct gca gct ctg tgt gaa ggt gca gtt ttg cca agg agt got 99
 Leu Ile Ser Ala Ala Leu Cys Glu Gly Ala Val Leu Pro Arg Ser Ala
 15 20 25
 aaa gaa ctt aga tgt cag tgc ata aag aca tac tcc aaa cct ttc cac 147
 Lys Glu Leu Arg Cys Gln Cys Ile Lys Thr Tyr Ser Lys Pro Phe His
 30 35 40 45
 ccc aaa ttt atc aaa gaa ctg aga gtg att gag agt gga cca cac tgc 195
 Pro Lys Phe Ile Lys Glu Leu Arg Val Ile Glu Ser Gly Pro His Cys
 50 55 60
 gcc aac aca gaa att att gta aag ctt tct gat gga aga gag ctc tgt 243
 Ala Asn Thr Glu Ile Ile Val Lys Leu Ser Asp Gly Arg Glu Leu Cys
 65 70 75
 ctg gac ccc aag gaa aac tgg gtg cag agg gtt gtg gag aag ttt ttg 291
 Leu Asp Pro Lys Glu Asn Trp Val Gln Arg Val Val Glu Lys Phe Leu
 80 85 90
 aag agg gct gag aat tca gog gcc gcc ccg ggt aaa gcc caa gat ttt 339

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Lys Arg Ala Glu Asn Ser Ala Ala Ala Pro Gly Lys Ala Gln Asp Phe
95 100 105

gtg cag tgg ttg atg aat acc tgagaattc 369
Val Gln Trp Leu Met Asn Thr
110 115

<210> 9

<211> 45

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer used for constructing a vector pCAGGS-IgG-glu19-29

<400> 9

gagaattcat ttaaatgaga gggcgccgcg tgcccagaaa ctgtg 45

<210> 10

<211> 49

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer used for constructing a vector pCAGGS-IgG-glu19-29

<400> 10

tcaaccactg cacaaaatct tgggotttac cggagagtg ggagagact 49

<210> 11

<211> 45

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer used for constructing a vector pCAGGS-IgG-glu19-29

<400> 11

gagaattcat ttaaatgaga gggcgccg tgcccagaaa ctgtg

45

<210> 12

<211> 50

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer used for constructing a vector pCAGGS-IgG-glu19-29

<400> 12

gagagagaga attctcaggt attcatcaac cactgcacaa aatcttgggc

50

<210> 13

<211> 38

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer used for constructing a vector
pCAGGS-IFN-rR-IgG-glu19-29

<400> 13

gagaattcat ttaaatgatt ctgctggtgg toctgatg

38

<210> 14

<211> 40

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer used for constructing a vector
pCAGGS-IFN-rR-IgG-glu19-29

<400> 14

gcagcatgc ggccgcttct tctctgtcat catggagaaa

40

<210> 15

<211> 38

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer used for constructing a vector
pCAGGS-CTLA4-IgG-glu19-29

<400> 15

gagaattcat ttaaattgct tgtcttggac tccagagg

38

<210> 16

<211> 40

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer used for constructing a vector
pCAGGS-CTLA4-IgG-glu19-29

<400> 16

gcagcatgc ggccgctct gaattctggc atggttctgg

40

<210> 17

<211> 38

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer used for constructing a vector
pCAGGS-IL13-IgG-glu19-29

<400> 17

gagaattcat ttaaattggca ctctgggtga ctgcagtc

38

<210> 18

<211> 40

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer used for constructing a vector
pCAGGS-IL13-IgG-glu19-29

<400> 18

gcagcatcgc ggcgcgtgg ccatacogga aaagttgctt

40

<210> 19

<211> 38

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer used for constructing a vector
pCAGGS-IL1RA-IgG-glu19-29

<400> 19

gagaattcat ttaaattggaa atctgctggg gaccctac

38

<210> 20

<211> 40

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer used for constructing a vector
pCAGGS-IL1RA-IgG-glu19-29

<400> 20

gcagcatogc ggccgcttgg tcttcttgga agtagaactt

40

<210> 21

<211> 62

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer used for constructing a vector pCAGGS-glu19-29

<400> 21

gagaattcat ttaaagaga gcggccgccc cgggtaaagc ccaagatttt gtgcagtgt 60

tg 62

<210> 22

<211> 50

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer used for constructing a vector pCAGGS-glu19-29

<400> 22

gagagagaga attctcaggt attcatcaac cactgcacaa aatcttgggc 50

<210> 23

<211> 38

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer used for constructing a vector pCAGGS-IL8-glu19-29

<400> 23

gagaattcat ttaaagact tccaagctgg ccgtggct 38

<210> 24

<211> 40

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer used for constructing a vector pCAGGS-IL8-glu19-29

<400> 24

gcagcatogc ggccgctgaa ttctcagccc tottcaaaaa

40