ANTI-IL-6 RECEPTOR ANTIBODY

Technical Field

The present invention relates to pharmaceutical compositions comprising an anti-IL-6 receptor antibody as an active ingredient, methods for producing the compositions, and such.

Background Art

Antibodies are drawing attention as pharmaceuticals as they are highly stable in plasma (blood) and have few adverse effects. Of them, a number of IgG-type antibody pharmaceuticals are available on the market and many antibody pharmaceuticals are currently under development (Non-patent Documents 1 and 2).

IL-6 is a cytokine involved in various autoimmune diseases (Non-patent Document 3). It is thought that TOCILIZUMAB, a humanized anti-IL-6 receptor IgG1 antibody, can be useful as a therapeutic agent for IL-6-associated diseases such as rheumatoid arthritis, since it specifically binds to the IL-6 receptor and thereby neutralizes its biological activity (Patent Documents 1 to 3 and Non-patent Document 4). In fact, TOCILIZUMAB has been approved as a therapeutic agent for Castleman's disease in Japan (Non-patent Document 5).

Various technologies applicable to second-generation antibody pharmaceuticals have been developed, including those that enhance effector function, antigen-binding activity, retention in plasma (blood), or stability, and those that reduce the risk of immunogenicity (antigenicity).

For methods of enhancing drug efficacy or reducing dosage, technologies that enhance antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC) through amino acid substitution in the Fc domain of an IgG antibody have been reported (Non-patent Document 6). Furthermore, affinity maturation has been reported as a technology for enhancing antigen-binding activity or antigen-neutralizing activity (Non-patent Document 7). This technology enables enhancement of antigen-binding activity through introduction of amino acid mutations into the CDR region of a variable region or such. The enhancement of antigen-binding activity enables to improve *in vitro* biological activity or reduce dosage, and further to improve *in vivo* efficacy (Non-patent Document 8). Currently, clinical trials are being conducted to assess Motavizumab (produced by affinity maturation), which is expected to have a superior effect than Palivizumab (a first generation pharmaceutical of anti-RSV antibody) (Non-patent Document 9). Alternatively, a more superior effect can be produced by binding to a different epitope. For example, it has been reported that Oftamumab which recognizes an epitope different from that recognized by Rituximab (an anti-CD20 antibody) shows a more

superior effect *in vivo* than Rituximab. Currently, clinical trials are being conducted to assess Oftamumab (Non-patent Document 10). There is no previous report describing a human, humanized, or chimeric anti-IL-6 receptor antibody having an affinity greater than 1 nM.

A problem encountered with current antibody pharmaceuticals is high production cost associated with the administration of extremely large quantities of protein. For example, the dosage of TOCILIZUMAB, a humanized anti-IL-6 receptor IgG1 antibody, has been estimated to be about 8 mg/kg/month by intravenous injection (Non-patent Document 4). Its preferred form of administration is thought to be subcutaneous formulation in chronic autoimmune diseases. In general, it is necessary that subcutaneous formulations are high concentration formulations. From the perspective of stability or such, the concentration limit for IgG-type antibody formulations is in general thought to be about 100 mg/ml (Non-patent Document 11). Low-cost, convenient second-generation antibody pharmaceuticals that can be administered subcutaneously in longer intervals can be provided by increasing the half-life of an antibody in the plasma to prolong its therapeutic effect and thereby reduce the amount of protein administered, and by conferring the antibody with high stability so that high concentration formulations can be prepared.

A possible method for improving antibody half-life in the plasma has been reported, and it artificially substitutes amino acids in the constant region (Non-patent Documents 12 and 13). However, introduction of non-natural sequences into the constant region is not preferred from the perspective of immunogenicity risk. Although it is preferable to introduce amino acid substitutions into the variable region rather than to the constant region from the perspective of immunogenicity, there is no report on improvement of antibody half-life in the plasma by substituting amino acids in the variable region, or improvement of the half-life of a human, humanized, or chimeric IL-6 receptor antibody in the plasma.

Methods for improving stability have been reported, and these include amino acid substitution or shuffling in the framework of the variable region to improve physicochemical stability (intermediate temperature of thermal denaturation) (Non-patent Documents 14 and 15). However, there is no previous report suggesting that such amino acid substitution improves the stability (suppresses the aggregation) in formulations that have a concentration higher than 100 mg/ml. There is also no previous report on stable human or humanized IL-6 receptor antibody molecules in formulations of higher-than-100 mg/ml concentrations.

Another important problem encountered in developing biopharmaceuticals is immunogenicity. In general, the immunogenicity of mouse antibodies is reduced by antibody humanization. It is assumed that immunogenicity risk can be further reduced by using a germline framework sequence as a template in antibody humanization (Non-patent Document 16). However, even Adalimumab, a fully human anti-TNF antibody, showed high-frequency

(13% to 17%) immunogenicity, and the therapeutic effect was found to be reduced in patients who showed immunogenicity (Non-patent Documents 17 and 18). T-cell epitopes may be present even in the CDR regions of human antibodies, and these T-cell epitopes in CDR are a possible cause of immunogenicity. *In silico* and *in vivo* methods for predicting T-cell epitopes have been reported (Non-patent Documents 19 and 20). It is assumed that immunogenicity risk can be reduced by removing T-cell epitopes predicted using such methods (Non-patent Document 21). TOCILIZUMAB is a humanized anti-IL-6 receptor IgG1 antibody obtained by humanizing a mouse PM-1 antibody. The antibody is yielded by CDR grafting using human NEW and REI framework sequences as template for H and L chains, respectively; however, a five-amino acid mouse sequence is retained in the antibody as framework and it is essential for retaining the antibody activity (Non-patent Document 22). There is no previous report on the substitution of a human sequence for the mouse sequence that remains in the framework of the humanized antibody TOCILIZUMAB, without loss of the activity. Furthermore, the CDR sequence of TOCILIZUMAB is a mouse sequence. Thus, there is a possibility that, like Adalimumab, TOCILIZUMAB contains T-cell epitopes in its CDR region, and may have a potential immunogenicity risk. Furthermore, TOCILIZUMAB belongs to the IgG1 subclass, and therefore can bind to Fcγ receptor. Since Fcγ receptor is expressed in antigen-presenting cells, molecules that bind to Fcγ receptor tend to be presented as antigens. It has been reported that immunogenicity is and can be enhanced by linking a protein or peptide to the Fc domain of IgG1 (Non-patent Document 23 and Patent Document 4). In clinical trials of TOCILIZUMAB, antibodies against TOCILIZUMAB were not detected when TOCILIZUMAB was used at an effective dose (8 mg/kg). However, immunogenicity was observed at the doses of 2 and 4 mg/kg (Patent Document 5). This suggests that the immunogenicity of the humanized anti-IL-6 receptor IgG1 antibody TOCILIZUMAB can be reduced. However, there has been no report on reducing the immunogenicity risk of TOCILIZUMAB (a humanized PM-1 antibody) by amino acid substitution.

In recent years, the safety of antibody pharmaceuticals has become of great importance. Interaction between the antibody Fc domain and Fcγ receptor is assumed to be a cause of serious adverse effect encountered in phase-I clinical trials of TGN1412 (Non-patent Document 24). In antibody pharmaceuticals aimed at neutralizing the biological activity of an antigen, binding of the Fc domain to Fcγ receptor, which is important for the effector function such as ADCC, is unnecessary. In addition, as described above, the binding to Fcγ receptor may be unfavorable from the perspective of immunogenicity and adverse effects.

A method for impairing the binding to Fcγ receptor is to alter the isotype of the IgG antibody from IgG1 to IgG2 or IgG4; however, this method cannot completely inhibit the binding (Non-patent Document 25). One of the methods reported for completely inhibiting the

binding to Fcγ receptor is to artificially alter the Fc domain. For example, the effector functions of anti-CD3 antibodies and anti-CD4 antibodies cause adverse effects. Thus, amino acids that are not present in the wild type sequence were introduced into the Fcγ receptor-binding domain of Fc (Non-patent Documents 26 and 27), and clinical trials are currently being conducted to assess anti-CD3 antibodies that do not bind to Fcγ receptor and anti-CD4 antibodies that have a mutated Fc domain (Non-patent Documents 24 and 28). Alternatively, Fcγ receptor-nonbinding antibodies can be prepared by altering the FcγR-binding domain of IgG1 (at positions 233, 234, 235, 236, 327, 330, and 331 in the EU numbering system) to an IgG2 or IgG4 sequence (Non-patent Document 29 and Patent Document 6). However, these molecules contain novel non-natural peptide sequences of nine to twelve amino acids, which may constitute a T-cell epitope peptide and thus pose immunogenicity risk. There is no previous report on Fcγ receptor-nonbinding antibodies that have overcome these problems.

Meanwhile, physicochemical properties of antibody proteins, in particular, homogeneity and stability, are very crucial in the development of antibody pharmaceuticals. For the IgG2 isotype, significant heterogeneity derived from disulfide bonds in the hinge region has been reported (Non-patent Document 30). It is not easy to manufacture them as a pharmaceutical in large-scale while maintaining the objective substances/related substances related heterogeneity derived from disulfide bonds between productions. Thus, single substances are desirable as much as possible for antibody molecules developed as pharmaceuticals.

IgG2 and IgG4 are unstable under acidic conditions. IgG type antibodies are in general exposed to acidic conditions in the purification process using Protein A and the virus inactivation process. Thus, there is a possibility that IgG2 and IgG4 undergo denaturation and aggregation during these processes. It is thus preferred that antibody molecules developed as pharmaceuticals are also stable under acidic conditions. Natural IgG2 and IgG4, and Fcγ receptor-nonbinding antibodies derived from IgG2 or IgG4 (Non-patent Documents 25 and 26 and Patent Document 6) have such problems. It is desirable to solve these problems when developing antibodies into pharmaceuticals.

IgG1-type antibodies are relatively stable under acidic conditions, and the degree of heterogeneity originated from disulfide bonds in the hinge region is also lower in this type of antibodies. However, IgG1-type antibodies are reported to undergo non-enzymatic peptide bond cleavage in the hinge region in solutions when they are stored as formulations, and Fab fragments are generated as impurities as a result (Non-patent Document 31). It is desirable to overcome the generation of impurity when developing antibodies into pharmaceuticals.

Furthermore, for heterogeneity of the C-terminal sequences of an antibody, deletion of C-terminal amino acid lysine residue, and amidation of the C-terminal amino group due to deletion of both of the two C-terminal amino acids, glycine and lysine, have been reported

(Non-patent Document 32). It is preferable to eliminate such heterogeneity when developing antibodies into pharmaceuticals.

The constant region of an antibody pharmaceutical aimed for neutralizing an antigen preferably has a sequence that overcomes all the problems described above. However, a constant region that meets all the requirements has not been reported.

There is no previous report on the development of second-generation molecules that exhibit an improved antigen-neutralizing activity and produce a prolonged therapeutic effect even when the frequency of administration is reduced, and which have reduced immunogenicity and improved safety and physicochemical properties, as compared to first-generation molecules. There is also no report on second-generation TOCILIZUMAB, which has more superiority in terms of the requirements described above by altering amino acid sequences of the variable and constant regions of the humanized anti-IL-6 receptor IgG1 antibody TOCILIZUMAB.

Documents of related prior arts for the present invention are described below.

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[Patent Document 4] US 20050261229A1
[Patent Document 5] WO 2004096273 (A1)
[Patent Document 6] WO 99/58572

Disclosure of the Invention

[Problems to be Solved by the Invention]

The present invention was achieved in view of the above circumstances. An objective of the present invention is to provide pharmaceutical compositions that comprise second-generation molecules, which are more superior than the humanized anti-IL-6 receptor IgG1 antibody TOCILIZUMAB, and methods for producing such pharmaceutical compositions. The second-generation molecules have been improved to exhibit enhanced antigen-neutralizing activity and pharmacokinetics (retention in plasma), and thus produce a prolonged therapeutic effect even when the frequency of administration is reduced; and they have also been improved to have reduced immunogenicity and improved safety and physicochemical properties, by altering amino acid sequences of the variable and constant regions of TOCILIZUMAB.

[Summary]

In a first aspect, there is provided an anti-IL-6 receptor antibody comprising a heavy chain variable region of SEQ ID NO: 17 and a light chain variable region of SEQ ID NO: 18, in which

- Ser at position 1 in the CDR1 of the heavy chain has been substituted with Asp,
- Thr at position 9 and Ser at position 16 in the CDR2 of the heavy chain have been substituted with Asn and Gly, respectively,
- Arg at position 13, Gln at position 16, Thr at position 23 and Thr at position 30 in the FR1 of the heavy chain have been substituted with Lys, Glu, Ala and Ser, respectively,
- Arg at position 8 in the FR2 of the heavy chain has been substituted with Glu,
- Met at position 4, Leu at position 5, Arg at position 16 and Val at position 27 in the FR3 of the heavy chain have been substituted with Ile, Ser, Lys and Ala, respectively,
- Gln at position 3 and Ser at position 5 in the FR4 of the heavy chain have been substituted with Glu and Thr, respectively,
- Arg at position 1 in the CDR1 of the light chain has been substituted with Gln,
- Thr at position 2 and Arg at position 4 in the CDR2 of the light chain have been substituted with Gly and Glu, respectively,
- Thr at position 5 in the CDR3 of the light chain has been substituted with Ser,
- Arg at position 18 in the FR1 of the light chain has been substituted with Ser,
- Lys at position 11 in the FR2 of the light chain has been substituted with Glu,
- Gln at position 23, Pro at position 24 and Ile at position 27 in the FR3 of the light chain have been substituted with Glu, Ala and Ala, respectively,
- Lys at position 10 in the FR4 of the light chain has been substituted with Glu.

In a second aspect, there is provided an anti-IL-6 receptor antibody comprising a heavy chain variable region of SEQ ID NO: 17 and a light chain variable region of SEQ ID NO: 18, in which

- Ser at position 1 in the CDR1 of the heavy chain has been substituted with Asp,
- Thr at position 9 and Ser at position 16 in the CDR2 of the heavy chain have been substituted with Asn and Gly, respectively,
- Ser at position 1 and Thr at position 5 in the CDR3 of the heavy chain have been substituted with Val and Ile, respectively,
- Arg at position 13, Gln at position 16, Thr at position 23 and Thr at position 30 in the FR1 of the heavy chain have been substituted with Lys, Glu, Ala and Ser, respectively,
- Arg at position 8 in the FR2 of the heavy chain has been substituted with Glu,
- Met at position 4, Leu at position 5, Arg at position 16 and Val at position 27 in the

FR3 of the heavy chain have been substituted with Ile, Ser, Lys and Ala, respectively,
- Gln at position 3 and Ser at position 5 in the FR4 of the heavy chain have been substituted with Glu and Thr, respectively,
- Arg at position 1 in the CDR1 of the light chain has been substituted with Gln,
- Thr at position 2 and Arg at position 4 in the CDR2 of the light chain have been substituted with Gly and Glu, respectively,
- Gln at position 1 and Thr at position 5 in the CDR3 of the light chain have been substituted with Gly and Arg, respectively,
- Arg at position 18 in the FR1 of the light chain has been substituted with Ser,
- Lys at position 11 in the FR2 of the light chain has been substituted with Glu,
- Gln at position 23, Pro at position 24 and Ile at position 27 in the FR3 of the light chain have been substituted with Glu, Ala and Ala, respectively,
- Lys at position 10 in the FR4 of the light chain has been substituted with Glu.

In a third aspect, there is provided an anti-IL-6 receptor antibody comprising a heavy chain variable region of SEQ ID NO: 17 and a light chain variable region of SEQ ID NO: 18, in which
- Ser at position 1 in the CDR1 of the heavy chain has been substituted with Asp,
- Thr at position 9 and Ser at position 16 in the CDR2 of the heavy chain have been substituted with Asn and Gly, respectively,
- Ser at position 1 and Thr at position 5 in the CDR3 of the heavy chain have been substituted with Val and Ile, respectively,
- Arg at position 13, Gln at position 16, Thr at position 23 and Thr at position 30 in the FR1 of the heavy chain have been substituted with Lys, Glu, Ala and Ser, respectively,
- Arg at position 8 in the FR2 of the heavy chain has been substituted with Glu,
- the amino acid sequence of the FR3 of the heavy chain has been substituted with that of SEQ ID NO: 184,
- Gln at position 3 and Ser at position 5 in the FR4 of the heavy chain have been substituted with Glu and Thr, respectively,
- Arg at position 1 in the CDR1 of the light chain has been substituted with Gln,
- Thr at position 2 and Arg at position 4 in the CDR2 of the light chain have been substituted with Gly and Glu, respectively,
- Gln at position 1 and Thr at position 5 in the CDR3 of the light chain have been substituted with Gly and Arg, respectively,
- Arg at position 18 in the FR1 of the light chain has been substituted with Ser,
- Lys at position 11 in the FR2 of the light chain has been substituted with Glu,
- Gln at position 23, Pro at position 24 and Ile at position 27 in the FR3 of the light

chain have been substituted with Glu, Ala and Ala, respectively,

- Lys at position 10 in the FR4 of the light chain has been substituted with Glu.

In a fourth aspect, there is provided an an anti-IL-6 receptor antibody comprising a heavy chain variable region of SEQ ID NO: 17 and a light chain variable region of SEQ ID NO: 18, in which

- Ser at position 1 in the CDR1 of the heavy chain has been substituted with Asp,
- the amino acid sequence of the CDR2 of the heavy chain has been substituted with that of SEQ ID NO: 122,
- Ser at position 1 and Thr at position 5 in the CDR3 of the heavy chain have been substituted with Leu and Ala, respectively,
- Arg at position 13, Gln at position 16, Thr at position 23 and Thr at position 30 in the FR1 of the heavy chain have been substituted with Lys, Glu, Ala and Ser, respectively,
- Arg at position 8 in the FR2 of the heavy chain has been substituted with Glu,
- the amino acid sequence of the FR3 of the heavy chain has been substituted with that of SEQ ID NO: 184,
- Ser at position 5 in the FR4 of the heavy chain has been substituted with Ile,
- Arg at position 1 and Gln at position 4 in the CDR1 of the light chain have been substituted with Gln and Glu respectively,
- the amino acid sequence of the CDR2 of the light chain has been substituted with that of SEQ ID NO: 126,
- Gln at position 1 and Thr at position 5 in the CDR3 of the light chain have been substituted with Gly and Arg, respectively,
- Arg at position 18 in the FR1 of the light chain has been substituted with Ser,
- Lys at position 11 in the FR2 of the light chain has been substituted with Glu,
- Gln at position 23, Pro at position 24 and Ile at position 27 in the FR3 of the light chain have been substituted with Glu, Ala and Ala, respectively,
- Lys at position 10 in the FR4 of the light chain has been substituted with Glu.

In a fifth aspect, there is provided an anti-IL-6 receptor antibody comprising a heavy chain variable region of SEQ ID NO: 17 and a light chain variable region of SEQ ID NO: 18, in which

- the amino acid sequence of the CDR1 of the heavy chain has been substituted with that of SEQ ID NO: 165,
- the amino acid sequence of the CDR2 of the heavy chain has been substituted with that of SEQ ID NO: 166,
- the amino acid sequence of the CDR3 of the heavy chain has been substituted with that of SEQ ID NO: 167,

- Arg at position 13, Gln at position 16, Thr at position 23 and Thr at position 30 in the FR1 of the heavy chain have been substituted with Lys, Glu, Ala and Ser, respectively,
- Arg at position 8 in the FR2 of the heavy chain has been substituted with Glu,
- the amino acid sequence of the FR3 of the heavy chain has been substituted with that of SEQ ID NO: 184,
- Gln at position 3 and Ser at position 5 in the FR4 of the heavy chain have been substituted with Glu and Thr, respectively,
- the amino acid sequence of the CDR1 of the light chain has been substituted with that of SEQ ID NO: 101,
- the amino acid sequence of the CDR2 of the light chain has been substituted with that of SEQ ID NO: 168,
- Gln at position 1 and Thr at position 5 in the CDR3 of the light chain have been substituted with Gly and Arg, respectively,
- Arg at position 18 in the FR1 of the light chain has been substituted with Ser,
- Lys at position 11 in the FR2 of the light chain has been substituted with Glu,
- Gln at position 23, Pro at position 24 and Ile at position 27 in the FR3 of the light chain have been substituted with Glu, Ala and Ala, respectively,
- Lys at position 10 in the FR4 of the light chain has been substituted with Glu.

In a sixth aspect, there is provided an anti-IL-6 receptor antibody comprising a heavy chain variable region of SEQ ID NO: 17 and a light chain variable region of SEQ ID NO: 18, in which

- the amino acid sequence of the CDR1 of the heavy chain has been substituted with that of SEQ ID NO: 169,
- the amino acid sequence of the CDR2 of the heavy chain has been substituted with that of SEQ ID NO: 174,
- the amino acid sequence of the FR1 of the heavy chain has been substituted with that of SEQ ID NO: 186,
- Arg at position 8 in the FR2 of the heavy chain has been substituted with Glu,
- the amino acid sequence of the FR3 of the heavy chain has been substituted with that of SEQ ID NO: 184,
- Gln at position 3 and Ser at position 5 in the FR4 of the heavy chain have been substituted with Glu and Thr, respectively,
- the amino acid sequence of the CDR1 of the light chain has been substituted with that of SEQ ID NO: 175,
- the amino acid sequence of the CDR2 of the light chain has been substituted with that of SEQ ID NO: 173,

- Gln at position 1 and Thr at position 5 in the CDR3 of the light chain have been substituted with Gly and Arg, respectively,
- Arg at position 18 in the FR1 of the light chain has been substituted with Ser,
- Lys at position 11 in the FR2 of the light chain has been substituted with Glu,
- Gln at position 23, Pro at position 24 and Ile at position 27 in the FR3 of the light chain have been substituted with Glu, Ala and Ala, respectively,
- Lys at position 10 in the FR4 of the light chain has been substituted with Glu.

[Means for Solving the Problems]

The present inventors conducted dedicated studies to generate second-generation molecules that are more superior than the first-generation humanized anti-IL-6 receptor IgG1 antibody TOCILIZUMAB, and have been improved to exhibit enhanced drug efficacy and pharmacokinetics, and thus produce a prolonged therapeutic effect even when the frequency of administration is reduced. They have also been improved to have reduced immunogenicity and improved safety and physicochemical properties (stability and homogeneity), by altering amino acid sequences of the variable and constant regions of TOCILIZUMAB. As a result, the present inventors discovered multiple CDR mutations in the variable regions of TOCILIZUMAB that enable to improve the antigen-binding activity (affinity). The present inventors thus successfully improved the affinity significantly using a combination of such mutations. The present inventors also successfully improved pharmacokinetics by altering the variable region sequence to lower the isoelectric point of an antibody. Furthermore, the present inventors successfully reduced immunogenicity risk by removing some of the *in silico*-predicted T-cell epitope peptides in the variable regions and the mouse sequences that remain in the framework of TOCILIZUMAB. In addition, the present inventors successfully increased the stability at

higher concentrations. Furthermore, the present inventors also successfully discovered novel constant region sequences that do not bind to Fcγ receptor and that improve the stability under acidic conditions, heterogeneity originated from disulfide bonds in the hinge region, heterogeneity originated from the H-chain C terminus, and stability in high concentration formulations, while minimizing the generation of new T-cell epitope peptides in the constant region of TOCILIZUMAB. The present inventors successfully discovered second-generation molecules that are more superior to TOCILIZUMAB by combining amino acid sequence alterations in the CDR, variable, and constant regions.

The present invention relates to pharmaceutical compositions comprising a humanized anti-IL-6 receptor IgG1 antibody that has been improved to exhibit more superior antigen (IL-6 receptor)-binding activity, more prolonged retention in plasma, more excellent safety and physicochemical properties (stability and homogeneity), and further reduced immunogenicity risk, by altering the amino acid sequences of variable and constant regions of the humanized anti-IL-6 receptor IgG1 antibody TOCILIZUMAB; and methods for producing such pharmaceutical compositions. More specifically, the present invention provides:

[1] an anti-IL-6 receptor antibody of any one of:

(a) an antibody that comprises a heavy chain variable region comprising CDR1 in which Ser at position 1 in the amino acid sequence of SEQ ID NO: 1 has been substituted with another amino acid;

(b) an antibody that comprises a heavy chain variable region comprising CDR1 in which Trp at position 5 in the amino acid sequence of SEQ ID NO: 1 has been substituted with another amino acid;

(c) an antibody that comprises a heavy chain variable region comprising CDR2 in which Tyr at position 1 in the amino acid sequence of SEQ ID NO: 2 has been substituted with another amino acid;

(d) an antibody that comprises a heavy chain variable region comprising CDR2 in which Thr at position 8 in the amino acid sequence of SEQ ID NO: 2 has been substituted with another amino acid;

(e) an antibody that comprises a heavy chain variable region comprising CDR2 in which Thr at position 9 in the amino acid sequence of SEQ ID NO: 2 has been substituted with another amino acid;

(f) an antibody that comprises a heavy chain variable region comprising CDR3 in which Ser at position 1 in the amino acid sequence of SEQ ID NO: 3 has been substituted with another amino acid;

(g) an antibody that comprises a heavy chain variable region comprising CDR3 in which Leu at position 2 in the amino acid sequence of SEQ ID NO: 3 has been substituted with another amino

acid;

(h) an antibody that comprises a heavy chain variable region comprising CDR3 in which Thr at position 5 in the amino acid sequence of SEQ ID NO: 3 has been substituted with another amino acid;

(i) an antibody that comprises a heavy chain variable region comprising CDR3 in which Ala at position 7 in the amino acid sequence of SEQ ID NO: 3 has been substituted with another amino acid;

(j) an antibody that comprises a heavy chain variable region comprising CDR3 in which Met at position 8 in the amino acid sequence of SEQ ID NO: 3 has been substituted with another amino acid;

(k) an antibody that comprises a heavy chain variable region comprising CDR3 in which Ser at position 1 and Thr at position 5 in the amino acid sequence of SEQ ID NO: 3 have been substituted with other amino acids;

(l) an antibody that comprises a heavy chain variable region comprising CDR3 in which Leu at position 2, Ala at position 7, and Met at position 8 in the amino acid sequence of SEQ ID NO: 3 have been substituted with other amino acids;

(m) an antibody that comprises a light chain variable region comprising CDR1 in which Arg at position 1 in the amino acid sequence of SEQ ID NO: 4 has been substituted with another amino acid;

(n) an antibody that comprises a light chain variable region comprising CDR1 in which Gln at position 4 in the amino acid sequence of SEQ ID NO: 4 has been substituted with another amino acid;

(o) an antibody that comprises a light chain variable region comprising CDR1 in which Tyr at position 9 in the amino acid sequence of SEQ ID NO: 4 has been substituted with another amino acid;

(p) an antibody that comprises a light chain variable region comprising CDR1 in which Asn at position 11 in the amino acid sequence of SEQ ID NO: 4 has been substituted with another amino acid;

(q) an antibody that comprises a light chain variable region comprising CDR2 in which Thr at position 2 in the amino acid sequence of SEQ ID NO: 5 has been substituted with another amino acid;

(r) an antibody that comprises a light chain variable region comprising CDR3 in which Gln at position 1 in the amino acid sequence of SEQ ID NO: 6 has been substituted with another amino acid;

(s) an antibody that comprises a light chain variable region comprising CDR3 in which Gly at position 3 in the amino acid sequence of SEQ ID NO: 6 has been substituted with another amino

acid;

(t) an antibody that comprises a light chain variable region comprising CDR1 in which Tyr at position 9 in the amino acid sequence of SEQ ID NO: 4 has been substituted with another amino acid, and CDR3 in which Gly at position 3 in the amino acid sequence of SEQ ID NO: 6 has been substituted with another amino acid;

(u) an antibody that comprises a light chain variable region comprising CDR3 in which Thr at position 5 in the amino acid sequence of SEQ ID NO: 6 has been substituted with another amino acid;

(v) an antibody that comprises a light chain variable region comprising CDR3 in which Gln at position 1 and Thr at position 5 in the amino acid sequence of SEQ ID NO: 6 have been substituted with other amino acids;

(w) an antibody that comprises a heavy chain variable region comprising CDR2 in which Thr at position 9 in the amino acid sequence of SEQ ID NO: 2 has been substituted with another amino acid, and CDR3 in which Ser at position 1 and Thr at position 5 in the amino acid sequence of SEQ ID NO: 3 have been substituted with other amino acids;

(x) an antibody that comprises the heavy chain variable region of (k) and the light chain variable region of (v); and

(y) the antibody of (x) that further comprises the CDR2 of (e);

[2] an anti-IL-6 receptor antibody that comprises a light chain variable region comprising CDR2 in which Thr at position 2 in the amino acid sequence of SEQ ID NO: 5 has been substituted with another amino acid;

[3] an anti-IL-6 receptor antibody of any one of:

(a) an antibody that comprises a heavy chain variable region comprising FR1 in which Arg at position 13 in the amino acid sequence of SEQ ID NO: 7 has been substituted with another amino acid;

(b) an antibody that comprises a heavy chain variable region comprising FR1 in which Gln at position 16 in the amino acid sequence of SEQ ID NO: 7 has been substituted with another amino acid;

(c) an antibody that comprises a heavy chain variable region comprising FR1 in which Thr at position 23 in the amino acid sequence of SEQ ID NO: 7 has been substituted with another amino acid;

(d) an antibody that comprises a heavy chain variable region comprising FR1 in which Thr at position 30 in the amino acid sequence of SEQ ID NO: 7 has been substituted with another amino acid;

(e) an antibody that comprises a heavy chain variable region comprising FR1 in which Arg at position 13, Gln at position 16, Thr at position 23, and Thr at position 30 in the amino acid

sequence of SEQ ID NO: 7 have been substituted with other amino acids;

(f) an antibody that comprises a heavy chain variable region comprising FR2 in which Arg at position 8 in the amino acid sequence of SEQ ID NO: 8 has been substituted with another amino acid;

(g) an antibody that comprises a heavy chain variable region comprising FR3 in which Met at position 4 in the amino acid sequence of SEQ ID NO: 9 has been substituted with another amino acid;

(h) an antibody that comprises a heavy chain variable region comprising FR3 in which Leu at position 5 in the amino acid sequence of SEQ ID NO: 9 has been substituted with another amino acid;

(i) an antibody that comprises a heavy chain variable region comprising FR3 in which Arg at position 16 in the amino acid sequence of SEQ ID NO: 9 has been substituted with another amino acid;

(j) an antibody that comprises a heavy chain variable region comprising FR3 in which Val at position 27 in the amino acid sequence of SEQ ID NO: 9 has been substituted with another amino acid;

(k) an antibody that comprises a heavy chain variable region comprising FR3 in which Met at position 4, Leu at position 5, Arg at position 16, and Val at position 27 in the amino acid sequence of SEQ ID NO: 9 have been substituted with other amino acids;

(l) an antibody that comprises a heavy chain variable region comprising FR4 in which Gln at position 3 in the amino acid sequence of SEQ ID NO: 10 has been substituted with another amino acid;

(m) an antibody that comprises a light chain variable region comprising FR1 in which Arg at position 18 in the amino acid sequence of SEQ ID NO: 11 has been substituted with another amino acid;

(n) an antibody that comprises a light chain variable region comprising FR2 in which Lys at position 11 in the amino acid sequence of SEQ ID NO: 12 has been substituted with another amino acid;

(o) an antibody that comprises a light chain variable region comprising FR3 in which Gln at position 23 in the amino acid sequence of SEQ ID NO: 13 has been substituted with another amino acid;

(p) an antibody that comprises a light chain variable region comprising FR3 in which Pro at position 24 in the amino acid sequence of SEQ ID NO: 13 has been substituted with another amino acid;

(q) an antibody that comprises a light chain variable region comprising FR3 in which Ile at position 27 in the amino acid sequence of SEQ ID NO: 13 has been substituted with another

amino acid;

(r) an antibody that comprises a light chain variable region comprising FR3 in which Gln at position 23, Pro at position 24, and Ile at position 27 in the amino acid sequence of SEQ ID NO: 13 have been substituted with other amino acids;

(s) an antibody that comprises a light chain variable region comprising FR4 in which Lys at position 10 in the amino acid sequence of SEQ ID NO: 14 has been substituted with another amino acid;

(t) an antibody that comprises a heavy chain variable region comprising FR4 in which Ser at position 5 in the amino acid sequence of SEQ ID NO: 10 has been substituted with another amino acid;

(u) an antibody that comprises a heavy chain variable region comprising FR4 in which Gln at position 3 and Ser at position 5 in the amino acid sequence of SEQ ID NO: 10 have been substituted with other amino acids;

(v) an antibody that comprises a heavy chain variable region comprising FR3 comprising the amino acid sequence of SEQ ID NO: 184;

(w) an antibody that comprises a heavy chain variable region comprising the FR1 of (e), FR2 of (f), FR3 of (k), and FR4 of (l) or (u);

(x) an antibody that comprises a light chain variable region comprising the FR1 of (m), FR2 of (n), FR3 of (r), and FR4 of (s); and

(y) an antibody that comprises the heavy chain variable region of (w) and the light chain variable region of (x);

[4] an anti-IL-6 receptor antibody of any one of:

(a) an antibody that comprises a heavy chain variable region comprising CDR1 in which Ser at position 1 in the amino acid sequence of SEQ ID NO: 1 has been substituted with another amino acid;

(b) an antibody that comprises a heavy chain variable region comprising CDR2 in which Thr at position 9 in the amino acid sequence of SEQ ID NO: 2 has been substituted with another amino acid;

(c) an antibody that comprises a heavy chain variable region comprising CDR2 in which Ser at position 16 in the amino acid sequence of SEQ ID NO: 2 has been substituted with another amino acid;

(d) an antibody that comprises a heavy chain variable region comprising CDR2 in which Thr at position 9 and Ser at position 16 in the amino acid sequence of SEQ ID NO: 2 have been substituted with other amino acids;

(e) an antibody that comprises a light chain variable region comprising CDR1 in which Arg at position 1 in the amino acid sequence of SEQ ID NO: 4 has been substituted with another amino

acid;

(f) an antibody that comprises a light chain variable region comprising CDR2 in which Thr at position 2 in the amino acid sequence of SEQ ID NO: 5 has been substituted with another amino acid;

(g) an antibody that comprises a light chain variable region comprising CDR2 in which Arg at position 4 in the amino acid sequence of SEQ ID NO: 5 has been substituted with another amino acid;

(h) an antibody that comprises a light chain variable region comprising CDR2 in which Thr at position 2 and Arg at position 4 in the amino acid sequence of SEQ ID NO: 5 have been substituted with other amino acids;

(i) an antibody that comprises a light chain variable region comprising CDR3 in which Thr at position 5 in the amino acid sequence of SEQ ID NO: 6 has been substituted with another amino acid;

(j) an antibody that comprises a heavy chain variable region comprising the CDR1 of (a), CDR2 of (d), and CDR3 comprising the amino acid sequence of SEQ ID NO: 3;

(k) an antibody that comprises a light chain variable region comprising the CDR1 of (e), CDR2 of (h), and CDR3 of (i); and

(l) an antibody that comprises the heavy chain variable region of (j) and the light chain variable region of (k);

[5] an anti-IL-6 receptor antibody of any one of:

(a) an antibody that comprises a heavy chain variable region comprising CDR1 in which Ser at position 1 in the amino acid sequence of SEQ ID NO:1 has been substituted with another amino acid, CDR2 in which Thr at position 9 and Ser at position 16 in the amino acid sequence of SEQ ID NO: 2 have been substituted with other amino acids, and CDR3 in which Ser at position 1 and Thr at position 5 in the amino acid sequence of SEQ ID NO: 3 have been substituted with other amino acids;

(b) an antibody that comprises a light chain variable region comprising CDR1 in which Arg at position 1 in the amino acid sequence of SEQ ID NO: 4 has been substituted with another amino acid, CDR2 in which Thr at position 2 and Arg at position 4 in the amino acid sequence of SEQ ID NO:5 have been substituted with other amino acids, and CDR3 in which Gln at position 1 and Thr at position 5 in the amino acid sequence of SEQ ID NO:6 have been substituted with other amino acids;

(c) an antibody that comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 22;

(d) an antibody that comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO: 23;

(e) an antibody that comprises the heavy chain variable region of (a) and the light chain variable region of (b); and

(f) an antibody that comprises the heavy chain variable region of (c) and the light chain variable region of (d);

[6] a human antibody constant region of any one of:

(a) a human antibody constant region that comprises deletions of both Gly at position 329 (446 in the EU numbering system) and Lys at position 330 (447 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 19;

(b) a human antibody constant region that comprises deletions of both Gly at position 325 (446 in the EU numbering system) and Lys at position 326 (447 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20; and

(c) a human antibody constant region that comprises deletions of both Gly at position 326 (446 in the EU numbering system) and Lys at position 327 (447 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 21;

[7] an IgG2 constant region in which the amino acids at positions 209 (330 in the EU numbering system), 210 (331 in the EU numbering system), and 218 (339 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20 have been substituted with other amino acids;

[8] an IgG2 constant region in which the amino acid at position 276 (397 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20 has been substituted with another amino acid;

[9] an IgG2 constant region in which the amino acid at position 14 (131 in the EU numbering system), 102 (219 in the EU numbering system), and/or 16 (133 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20 has been substituted with another amino acid;

[10] the IgG2 constant region of [9], wherein the amino acids at positions 20 (137 in the EU numbering system) and 21 (138 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20 have been substituted with other amino acids;

[11] an IgG2 constant region in which His at position 147 (268 in the EU numbering system), Arg at position234 (355 in the EU numbering system), and/or Gln at position 298 (419 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20 has been substituted with another amino acid;

[12] an IgG2 constant region in which the amino acids at positions 209 (330 in the EU numbering system), 210 (331 in the EU numbering system), 218 (339 in the EU numbering system), 276 (397 in the EU numbering system), 14 (131 in the EU numbering system), 16 (133 in the EU numbering system), 102 (219 in the EU numbering system), 20 (137 in the EU numbering system), and 21 (138 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20 have been substituted with other amino acids;

[13] the IgG2 constant region of [12], which further comprises deletions of both Gly at position 325 (446 in the EU numbering system) and Lys at position 326 (447 in the EU numbering system);

[14] an IgG2 constant region in which the amino acids at positions 276 (397 in the EU numbering system), 14 (131 in the EU numbering system), 16 (133 in the EU numbering system), 102 (219 in the EU numbering system), 20 (137 in the EU numbering system), and 21 (138 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20 have been substituted with other amino acids;

[15] the IgG2 constant region of [14], which further comprises deletions of both Gly at position 325 (446 in the EU numbering system) and Lys at position 326 (447 in the EU numbering system);

[16] an IgG2 constant region in which the Cys at position 14 (131 in the EU numbering system), Arg at position 16 (133 in the EU numbering system), Cys at position 102 (219 in the EU numbering system), Glu at position 20 (137 in the EU numbering system), Ser at position 21 (138 in the EU numbering system), His at position 147 (268 in the EU numbering system), Arg at position 234 (355 in the EU numbering system), and Gln at position 298 (419 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20 have been substituted with other amino acids;

[17] the IgG2 constant region of [16], which further comprises deletions of both Gly at position 325 (446 in the EU numbering system) and Lys at position 326 (447 in the EU numbering system);

[18] an IgG2 constant region in which the Cys at position 14 (131 in the EU numbering system), Arg at position 16 (133 in the EU numbering system), Cys at position 102 (219 in the EU numbering system), Glu at position 20 (137 in the EU numbering system), Ser at position 21 (138 in the EU numbering system), His at position 147 (268 in the EU numbering system), Arg at position 234 (355 in the EU numbering system), Gln at position 298 (419 in the EU numbering system), and Asn at position 313 (434 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20 have been substituted with other amino acids;

[19] the IgG2 constant region of [18], which further comprises deletions of both Gly at position 325 (446 in the EU numbering system) and Lys at position 326 (447 in the EU numbering system);

[20] an IgG4 constant region in which the amino acid at position 289 (409 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 21 has been substituted with another amino acid;

[21] an IgG4 constant region in which the amino acids at position 289 (409 in the EU numbering system), positions 14, 16, 20, 21, 97, 100, 102, 103, 104, and 105 (131, 133, 137, 138, 214, 217,

219, 220, 221, and 222 in the EU numbering system, respectively), and positions 113, 114, and 115 (233, 234, and 235 in the EU numbering system, respectively), have been substituted with other amino acids, and the amino acid at position 116 (236 in the EU numbering system) has been deleted from the amino acid sequence of SEQ ID NO: 21;

[22] the IgG4 constant region of [21], which further comprises deletions of both Gly at position 326 (446 in the EU numbering system) and Lys at position 327 (447 in the EU numbering system);

[23] an IgG2 constant region in which Ala at position 209 (330 in the EU numbering system), Pro at position 210 (331 in the EU numbering system), Thr at position 218 (339 in the EU numbering system), Cys at position 14 (131 in the EU numbering system), Arg at position 16 (133 in the EU numbering system), Cys at position 102 (219 in the EU numbering system), Glu at position 20 (137 in the EU numbering system), and Ser at position 21 (138 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20 have been substituted with other amino acids;

[24] the IgG2 constant region of [23], which further comprises deletions of both Gly at position 325 (446 in the EU numbering system) and Lys at position 326 (447 in the EU numbering system);

[25] an IgG2 constant region in which Cys at position 14 (131 in the EU numbering system), Arg at position 16 (133 in the EU numbering system), Cys at position 102 (219 in the EU numbering system), Glu at position 20 (137 in the EU numbering system), and Ser at position 21 (138 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20 have been substituted with other amino acids;

[26] the IgG2 constant region of [25], which further comprises deletions of both Gly at position 325 (446 in the EU numbering system) and Lys at position 326 (447 in the EU numbering system);

[27] a constant region comprising the amino acid sequence of SEQ ID NO: 24;

[28] a constant region comprising the amino acid sequence of SEQ ID NO: 118;

[29] a constant region comprising the amino acid sequence of SEQ ID NO: 25;

[30] a constant region comprising the amino acid sequence of SEQ ID NO: 151;

[31] a constant region comprising the amino acid sequence of SEQ ID NO: 152;

[32] a constant region comprising the amino acid sequence of SEQ ID NO: 153;

[33] a constant region comprising the amino acid sequence of SEQ ID NO: 164;

[34] a human antibody constant region comprising the amino acid sequence of SEQ ID NO: 194 (M40ΔGK);

[35] a human antibody constant region comprising the amino acid sequence of SEQ ID NO: 192 (M86ΔGK);

[36] an antibody comprising the constant region of any one of [6] to [32];
[37] the antibody of [36], which binds to an IL-6 receptor;
[38] an anti-IL-6 receptor antibody whose binding activity to an IL-6 receptor is 1 nM or less;
[39] an anti-IL-6 receptor antibody, wherein the measured isoelectric point of the full-length antibody is 7.0 or lower or the theoretical isoelectric point of the variable region is 5.0 or lower;
[40] an anti-IL-6 receptor antibody, wherein the increase in the ratio of antibody aggregate after one month at 25°C in a buffer containing 20 mM Histidine-HCl and 150 mM NaCl at pH 6.5 to 7.0 is 0.3% or less when the concentration of the antibody is 100 mg/ml; and
[41] a pharmaceutical composition comprising the antibody of any one of [36] to [40].

Brief Description of the Drawings

Fig. 1 is a graph showing the BaF/gp130-neutralizing activities of WT and RD_6.

Fig. 2 is a graph showing a sensorgram for the interaction between rhIL-s6R (R&D systems) and WT.

Fig. 3 is a graph showing a sensorgram for the interaction between rhIL-s6R (R&D systems) and RD_6.

Fig. 4-1 is a diagram showing a list of CDR mutations that improve the affinity or neutralizing activity in comparison with WT.

Fig. 4-2 is the continuation of Fig. 4-1.

Fig. 5 is a diagram showing a list of CDR mutations that in combination improve the affinity or neutralizing activity.

Fig. 6 is a graph showing the BaF/gp130-neutralizing activities of WT and RDC23.

Fig. 7 is a graph showing a sensorgram for the interaction between rhIL-s6R (R&D systems) and RDC23.

Fig. 8 is a graph showing a sensorgram for the interaction between rhsIL-6R and WT.

Fig. 9 is a graph showing a sensorgram for the interaction between rhsIL-6R and RDC23.

Fig. 10 is a graph showing a sensorgram for the interaction between SR344 and WT.

Fig. 11 is a graph showing a sensorgram for the interaction between SR344 and RDC23.

Fig. 12 is a graph showing the BaF/gp130-neutralizing activities of WT and H53L28.

Fig. 13 is a graph showing a sensorgram for the interaction between SR344 and H53/L28.

Fig. 14 is a graph showing transitions in the plasma concentrations of WT and H53/L28 after intravenous administration to mice.

Fig. 15 is a graph showing transitions in the plasma concentrations of WT and H53/L28 after subcutaneous administration to mice.

Fig. 16 is a graph showing the BaF/gp130-neutralizing activities of WT and PF1.

Fig. 17 is a graph showing a sensorgram for the interaction between SR344 and PF1.

Fig. 18 is a graph showing the result of testing the stability of WT and PF1 at high concentrations.

Fig. 19 is a graph showing transitions in the plasma concentrations of WT and PF1 after intravenous administration to human IL-6 receptor transgenic mice.

Fig. 20 is a graph showing transitions in the plasma concentrations of free human soluble IL-6 receptor after intravenous administration of WT or PF1 to human IL-6 receptor transgenic mice.

Fig. 21 is a graph showing the result of using gel filtration chromatography to analyze the content of aggregates in WT-IgG1, WT-IgG2, WT-IgG4, IgG2-M397V, and IgG4-R409K purified by hydrochloric acid elution.

Fig. 22 is a diagram showing the result of cation exchange chromatography (IEC) analysis of WT-IgG1, WT-IgG2, and WT-IgG4.

Fig. 23 is a diagram showing predicted disulfide bonding in the hinge region of WT-IgG2.

Fig. 24 is a diagram showing predicted disulfide bonding in the hinge region of WT-IgG2-SKSC.

Fig. 25 is a diagram showing the result of cation exchange chromatography (IEC) analysis of WT-IgG2 and IgG2-SKSC.

Fig. 26 is a diagram showing the result of cation exchange chromatography (IEC) analysis of humanized PM-1 antibody, H chain C-terminal ΔK antibody, and H chain C-terminal ΔGK antibody.

Fig. 27 shows comparison of the amounts WT-IgG1, WT-IgG2, WT-IgG4, WT-M14ΔGK, WT-M17ΔGK, and WT-M11ΔGK bound to FcγRI.

Fig. 28 is a graph showing comparison of the amounts WT-IgG1, WT-IgG2, WT-IgG4, WT-M14ΔGK, WT-M17ΔGK, and WT-M11ΔGK bound to FcγRIIa.

Fig. 29 is a graph showing comparison of the amounts WT-IgG1, WT-IgG2, WT-IgG4, WT-M14ΔGK, WT-M17ΔGK, and WT-M11ΔGK bound to FcγRIIb.

Fig. 30 is a graph showing comparison of the amounts WT-IgG1, WT-IgG2, WT-IgG4, WT-M14ΔGK, WT-M17ΔGK, and WT-M11ΔGK bound to FcγRIIIa (Val).

Fig. 31 is a graph showing the increase of aggregation in a stability test for WT-IgG1, WT-M14ΔGK, WT-M17ΔGK, and WT-M11ΔGK at high concentrations.

Fig. 32 is a graph showing the increase of Fab fragments in a stability test for WT-IgG1, WT-M14ΔGK, WT-M17ΔGK, and WT-M11ΔGK at high concentrations.

Fig. 33 is a diagram showing the result of cation exchange chromatography (IEC)

analysis of WT-IgG2, WT-M14ΔGK, and WT-M31ΔGK.

Fig. 34 is a graph showing the BaF/gp130-neutralizing activities of WT and F2H/L39-IgG1.

Fig. 35 is a graph showing the plasma concentration time courses of antibodies after subcutaneous administration of WT, PF1, or F2H/L39-IgG1 at 1.0 mg/kg to cynomolgus monkeys.

Fig. 36 is a graph showing the time courses of CRP concentration in the groups of cynomolgus monkeys administered with WT or F2H/L39-IgG1.

Fig. 37 is a graph showing the time courses of free cynomolgus monkey IL-6 receptor concentration in the groups of cynomolgus monkeys administered with WT or F2H/L39-IgG1.

Fig. 38 is a graph showing the time courses of plasma concentrations of WT-IgG1 and WT-M14 after intravenous administration to human FcRn transgenic mice.

Fig. 39 is a graph showing the time courses of plasma concentrations of WT-IgG1, WT-M14, and WT-M58 after intravenous administration to human FcRn transgenic mice.

Fig. 40 is a graph showing the time courses of plasma concentrations of WT-IgG1, WT-M44, WT-M58, and WT-M73 after intravenous administration to human FcRn transgenic mice.

Fig. 41 is a diagram showing a cation exchange chromatography-based assessment of the effect on heterogeneity by the constant region of anti IL-6 receptor antibodies WT and F2H/L39, anti-IL-31 receptor antibody H0L0, and anti-RANKL antibody DNS.

Fig. 42 is a diagram showing a cation exchange chromatography-based assessment of the effect on heterogeneity by the CH1 domain cysteine of anti IL-6 receptor antibodies WT and F2H/L39.

Fig. 43 is a diagram showing a DSC-based assessment of the effect on denaturation peak by the CH1 domain cysteine of anti IL-6 receptor antibody WT.

Fig. 44 is a graph showing the activities of TOCILIZUMAB, the control, and Fv5-M83 to neutralize BaF/g130.

Fig. 45 is a graph showing the activities of TOCILIZUMAB, Fv3-M73, and Fv4-M73 to neutralize BaF/gp130.

Fig. 46 is a graph showing the plasma concentration time courses of TOCILIZUMAB, the control, Fv3-M73, Fv4-M73, and Fv5-M83 in cynomolgus monkeys after intravenous administration.

Fig. 47 is a graph showing the time courses of CRP concentration in cynomolgus monkeys after intravenous administration of TOCILIZUMAB, the control, Fv3-M73, Fv4-M73, or Fv5-M83.

Fig. 48 is a graph showing the time courses of percentage of soluble IL-6 receptor

neutralization in cynomolgus monkeys after intravenous administration of TOCILIZUMAB, the control, Fv3-M73, Fv4-M73, or Fv5-M83.

Mode for Carrying Out the Invention

The present invention provides pharmaceutical compositions comprising second-generation molecules that are more superior to the humanized anti-IL-6 receptor IgG1 antibody TOCILIZUMAB, and have been improved to exhibit enhanced drug efficacy and pharmacokinetics, and thus produce a prolonged therapeutic effect even when the frequency of administration is reduced. They have also been improved to have reduced immunogenicity and improved safety and physicochemical properties, by altering amino acid sequences of the variable and constant regions of TOCILIZUMAB; and methods for producing such pharmaceutical compositions. The present invention also provides antibody constant regions that are suitable for pharmaceuticals.

The present invention relates to anti-IL-6 receptor antibodies exhibiting superior antigen-binding activity, neutralizing activity, retention in plasma, stability, and/or homogeneity, and reduced immunogenicity risk.

Preferably, the anti-IL-6 receptor antibody is a humanized PM-1 antibody (TOCILIZUMAB). More specifically, the present invention provides humanized PM-1 antibodies with enhanced antigen-binding activity, humanized PM-1 antibodies with enhanced neutralizing activity, humanized PM-1 antibodies showing improved pharmacokinetics, humanized PM-1 antibodies with reduced immunogenicity risk, humanized PM-1 antibodies with improved stability, and humanized PM-1 antibodies with improved homogeneity, all of which have been achieved through amino acid substitution.

Humanized PM-1 antibodies bind to the human IL-6 receptor, and thus inhibit the binding between human IL-6 and the human IL-6 receptor. Herein, SEQ IDs in the Sequence Listing correspond to the amino acid sequences of humanized PM-1 antibodies shown below.

Heavy chain amino acid sequence: SEQ ID NO: 15

Light chain amino acid sequence: SEQ ID NO: 16

Heavy chain variable region amino acid sequence: SEQ ID NO: 17

Light chain variable region amino acid sequence: SEQ ID NO: 18

Heavy chain CDR1 (HCDR1) amino acid sequence: SEQ ID NO: 1

Heavy chain CDR2 (HCDR2) amino acid sequence: SEQ ID NO: 2

Heavy chain CDR3 (HCDR3) amino acid sequence: SEQ ID NO: 3

Heavy chain FR1 (HFR1) amino acid sequence: SEQ ID NO: 7

Heavy chain FR2 (HFR2) amino acid sequence: SEQ ID NO: 8

Heavy chain FR3 (HFR3) amino acid sequence: SEQ ID NO: 9

Heavy chain FR4 (HFR4) amino acid sequence: SEQ ID NO: 10
Light chain CDR1 (LCDR1) amino acid sequence: SEQ ID NO: 4
Light chain CDR2 (LCDR2) amino acid sequence: SEQ ID NO: 5
Light chain CDR3 (LCDR3) amino acid sequence: SEQ ID NO: 6
Light chain FR1 (LFR1) amino acid sequence: SEQ ID NO: 11
Light chain FR2 (LFR2) amino acid sequence: SEQ ID NO: 12
Light chain FR3 (LFR3) amino acid sequence: SEQ ID NO: 13
Light chain FR4 (LFR4) amino acid sequence: SEQ ID NO: 14

<Antibodies with enhanced affinity and neutralizing activity>

The present invention provides anti-human IL-6 receptor antibodies exhibiting strong human IL-6 receptor-binding and/or neutralizing activity. More specifically, the present invention provides the following antibodies of (a) to (y), and methods for producing the antibodies:

(a) An anti-human IL-6 receptor antibody comprising a heavy chain CDR1 in which Ser at position 1 in the amino acid sequence of SEQ ID NO: 1 (HCDR1) has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution to Trp (RD_68), Thr (RD_37), Asp (RD_8), Asn (RD_11), Arg (RD_31), Val (RD_32), Phe (RD_33), Ala (RD_34), Gln (RD_35), Tyr (RD_36), Leu (RD_38), His (RD_42), Glu (RD_45), or Cys (RD_46) is preferred.

A sequence resulting from the substitution of Trp for Ser at position 1 in the amino acid sequence of SEQ ID NO: 1 is shown in SEQ ID NO: 26.

A sequence resulting from the substitution of Thr for Ser at position 1 in the amino acid sequence of SEQ ID NO: 1 is shown in SEQ ID NO: 27.

A sequence resulting from the substitution of Asp for Ser at position 1 in the amino acid sequence of SEQ ID NO: 1 is shown in SEQ ID NO: 28.

A sequence resulting from the substitution of Asn for Ser at position 1 in the amino acid sequence of SEQ ID NO: 1 is shown in SEQ ID NO: 29.

A sequence resulting from the substitution of Arg for Ser at position 1 in the amino acid sequence of SEQ ID NO: 1 is shown in SEQ ID NO: 30.

A sequence resulting from the substitution of Val for Ser at position 1 in the amino acid sequence of SEQ ID NO: 1 is shown in SEQ ID NO: 31.

A sequence resulting from the substitution of Phe for Ser at position 1 in the amino acid sequence of SEQ ID NO: 1 is shown in SEQ ID NO: 32.

A sequence resulting from the substitution of Ala for Ser at position 1 in the amino acid sequence of SEQ ID NO: 1 is shown in SEQ ID NO: 33.

A sequence resulting from the substitution of Gln for Ser at position 1 in the amino acid sequence of SEQ ID NO: 1 is shown in SEQ ID NO: 34.

A sequence resulting from the substitution of Tyr for Ser at position 1 in the amino acid sequence of SEQ ID NO: 1 is shown in SEQ ID NO: 35.

A sequence resulting from the substitution of Leu for Ser at position 1 in the amino acid sequence of SEQ ID NO: 1 is shown in SEQ ID NO: 36.

A sequence resulting from the substitution of His for Ser at position 1 in the amino acid sequence of SEQ ID NO: 1 is shown in SEQ ID NO: 37.

A sequence resulting from the substitution of Glu for Ser at position 1 in the amino acid sequence of SEQ ID NO: 1 is shown in SEQ ID NO: 38.

A sequence resulting from the substitution of Cys for Ser at position 1 in the amino acid sequence of SEQ ID NO: 1 is shown in SEQ ID NO: 39.

(b) An anti-human IL-6 receptor antibody comprising a heavy chain CDR1 in which Trp at position 5 in the amino acid sequence of SEQ ID NO: 1 (HCDR1) has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution to Ile (RD_9) or Val (RD_30) is preferred.

A sequence resulting from the substitution of Ile for Trp at position 5 in the amino acid sequence of SEQ ID NO: 1 is shown in SEQ ID NO: 40.

A sequence resulting from the substitution of Val for Trp at position 5 in the amino acid sequence of SEQ ID NO: 1 is shown in SEQ ID NO: 41.

(c) An anti-human IL-6 receptor antibody comprising a heavy chain CDR2 in which Tyr at position 1 in the amino acid sequence of SEQ ID NO: 2 (HCDR2) has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution to Phe (RD_82) is preferred.

A sequence resulting from the substitution of Phe for Tyr at position 1 in the amino acid sequence of SEQ ID NO: 2 is shown in SEQ ID NO: 42.

(d) An anti-human IL-6 receptor antibody comprising a heavy chain CDR2 in which Thr at position 8 in the amino acid sequence of SEQ ID NO: 2 (HCDR2) has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution to Arg (RD_79) is preferred.

A sequence resulting from the substitution of Arg for Thr at position 8 in the amino acid sequence of SEQ ID NO: 2 is shown in SEQ ID NO: 43.

(e) An anti-human IL-6 receptor antibody comprising a heavy chain CDR2 in which Thr

at position 9 in the amino acid sequence of SEQ ID NO: 2 (HCDR2) has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution to Ser (RD_12) or Asn (RD_61) is preferred.

A sequence resulting from the substitution of Ser for Thr at position 9 in the amino acid sequence of SEQ ID NO: 2 is shown in SEQ ID NO: 44.

A sequence resulting from the substitution of Asn for Thr at position 9 in the amino acid sequence of SEQ ID NO: 2 is shown in SEQ ID NO: 45.

(f) An anti-human IL-6 receptor antibody comprising a heavy chain CDR3 in which Ser at position 1 in the amino acid sequence of SEQ ID NO: 3 (HCDR3) has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution to Ile (RD_2), Val (RD_4), Thr (RD_80), or Leu (RD_5) is preferred.

A sequence resulting from the substitution of Ile for Ser at position 1 in the amino acid sequence of SEQ ID NO: 3 is shown in SEQ ID NO: 46.

A sequence resulting from the substitution of Val for Ser at position 1 in the amino acid sequence of SEQ ID NO: 3 is shown in SEQ ID NO: 47.

A sequence resulting from the substitution of Thr for Ser at position 1 in the amino acid sequence of SEQ ID NO: 3 is shown in SEQ ID NO: 48.

A sequence resulting from the substitution of Leu for Ser at position 1 in the amino acid sequence of SEQ ID NO: 3 is shown in SEQ ID NO: 49.

(g) An anti-human IL-6 receptor antibody comprising a heavy chain CDR3 in which Leu at position 2 in the amino acid sequence of SEQ ID NO: 3 (HCDR3) has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution to Thr (RD_84) is preferred.

A sequence resulting from the substitution of Thr for Leu at position 2 in the amino acid sequence of SEQ ID NO: 3 is shown in SEQ ID NO: 50.

(h) An anti-human IL-6 receptor antibody comprising a heavy chain CDR3 in which Thr at position 5 in the amino acid sequence of SEQ ID NO: 3 (HCDR3) has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution to Ala (RD_3) or Ile (RD_83) is preferred. In addition, the substitution of Ser (RDC_14H) for Thr at position 5 is also preferred.

A sequence resulting from the substitution of Ala for Thr at position 5 in the amino acid sequence of SEQ ID NO: 3 is shown in SEQ ID NO: 51.

A sequence resulting from the substitution of Ile for Thr at position 5 in the amino acid sequence of SEQ ID NO: 3 is shown in SEQ ID NO: 52.

A sequence resulting from the substitution of Ser for Thr at position 5 in the amino acid sequence of SEQ ID NO: 3 is shown in SEQ ID NO: 53.

(i) An anti-human IL-6 receptor antibody comprising a heavy chain CDR3 in which Ala at position 7 in the amino acid sequence of SEQ ID NO: 3 (HCDR3) has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution to Ser (RD_81) or Val (PF_3H) is preferred.

A sequence resulting from the substitution of Ser for Ala at position 7 in the amino acid sequence of SEQ ID NO: 3 is shown in SEQ ID NO: 54.

A sequence resulting from the substitution of Val for Ala at position 7 in the amino acid sequence of SEQ ID NO: 3 is shown in SEQ ID NO: 55.

(j) An anti-human IL-6 receptor antibody comprising a heavy chain CDR3 in which Met at position 8 in the amino acid sequence of SEQ ID NO: 3 (HCDR3) has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution to Leu (PF_4H) is preferred.

A sequence resulting from the substitution of Leu for Met at position 8 in the amino acid sequence of SEQ ID NO: 3 is shown in SEQ ID NO: 56.

(k) An anti-human IL-6 receptor antibody comprising a heavy chain CDR3 in which Ser at position 1 and Thr at position 5 in the amino acid sequence of SEQ ID NO: 3 (HCDR3) have been substituted with other amino acids.

The type of amino acid after substitution is not particularly limited; however, substitutions of Leu for Ser at position 1 and Ala for Thr at position 5 (RD_6) are preferred. Other preferred substitutions include: substitutions of Val for Ser at position 1 and Ala for Thr at position 5 (RDC_2H); substitutions of Ile for Ser at position 1 and Ala for Thr at position 5 (RDC_3H); substitutions of Thr for Ser at position 1 and Ala for Thr at position 5 (RDC_4H); substitutions of Val for Ser at position 1 and Ile for Thr at position 5 (RDC_5H); substitutions of Ile for Ser at position 1 and Ile for Thr at position 5 (RDC_6H); substitutions of Thr for Ser at position 1 and Ile for Thr at position 5 (RDC_7H); and substitutions of Leu for Ser at position 1 and Ile for Thr at position 5 (RDC_8H).

A sequence resulting from the substitutions of Leu for Ser at position 1 and Ala for Thr at position 5 in the amino acid sequence of SEQ ID NO: 3 is shown in SEQ ID NO: 57.

A sequence resulting from the substitutions of Val for Ser at position 1 and Ala for Thr at position 5 in the amino acid sequence of SEQ ID NO: 3 is shown in SEQ ID NO: 58.

A sequence resulting from the substitutions of Ile for Ser at position 1 and Ala for Thr at position 5 in the amino acid sequence of SEQ ID NO: 3 is shown in SEQ ID NO: 59.

A sequence resulting from the substitutions of Thr for Ser at position 1 and Ala for Thr at position 5 in the amino acid sequence of SEQ ID NO: 3 is shown in SEQ ID NO: 60.

A sequence resulting from the substitutions of Val for Ser at position 1 and Ile for Thr at position 5 in the amino acid sequence of SEQ ID NO: 3 is shown in SEQ ID NO: 61.

A sequence resulting from the substitutions of Ile for Ser at position 1 and Ile for Thr at position 5 in the amino acid sequence of SEQ ID NO: 3 is shown in SEQ ID NO: 62.

A sequence resulting from the substitutions of Thr for Ser at position 1 and Ile for Thr at position 5 in the amino acid sequence of SEQ ID NO: 3 is shown in SEQ ID NO: 63.

A sequence resulting from the substitutions of Leu for Ser at position 1 and Ile for Thr at position 5 in the amino acid sequence of SEQ ID NO: 3 is shown in SEQ ID NO: 64.

(l) An anti-human IL-6 receptor antibody comprising a heavy chain CDR3 in which Leu at position 2, Ala at position 7, and Met at position 8 in the amino acid sequence of SEQ ID NO: 3 (HCDR3) have been substituted with other amino acids.

The type of amino acid after substitution is not particularly limited; however, substitutions of Thr for Leu at position 2, Val for Ala at position 7, and Leu for Met at position 8 (RD_78) are preferred.

A sequence resulting from the substitutions of Thr for Leu at position 2, Val for Ala at position 7, and Leu for Met at position 8 in the amino acid sequence of SEQ ID NO: 3 is shown in SEQ ID NO: 65.

(m) An anti-human IL-6 receptor antibody comprising a light chain CDR1 in which Arg at position 1 in the amino acid sequence of SEQ ID NO: 4 (LCDR1) has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution to Phe (RD_18) is preferred.

A sequence resulting from the substitution of Phe for Arg at position 1 in the amino acid sequence of SEQ ID NO: 4 is shown in SEQ ID NO: 66.

(n) An anti-human IL-6 receptor antibody comprising a light chain CDR1 in which Gln at position 4 in the amino acid sequence of SEQ ID NO: 4 (LCDR1) has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution to Arg (RD_26) or Thr (RD_20) is preferred.

A sequence resulting from the substitution of Arg for Gln at position 4 in the amino acid sequence of SEQ ID NO: 4 is shown in SEQ ID NO: 67.

A sequence resulting from the substitution of Thr for Gln at position 4 in the amino acid

sequence of SEQ ID NO: 4 is shown in SEQ ID NO: 68.

(o) An anti-human IL-6 receptor antibody comprising a light chain CDR1 in which Tyr at position 9 in the amino acid sequence of SEQ ID NO: 4 (LCDR1) has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution to Phe (RD_73) is preferred.

A sequence resulting from the substitution of Phe for Tyr at position 9 in the amino acid sequence of SEQ ID NO: 4 is shown in SEQ ID NO: 69.

(p) An anti-human IL-6 receptor antibody comprising a light chain CDR1 in which Asn at position 11 in the amino acid sequence of SEQ ID NO: 4 (LCDR1) has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution to Ser (RD_27) is preferred.

A sequence resulting from the substitution of Ser for Asn at position 11 in the amino acid sequence of SEQ ID NO: 4 is shown in SEQ ID NO: 70.

(q) An anti-human IL-6 receptor antibody comprising a light chain CDR2 in which Thr at position 2 in the amino acid sequence of SEQ ID NO: 5 (LCDR2) has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution to Gly is preferred.

A sequence resulting from the substitution of Gly for Thr at position 2 in the amino acid sequence of SEQ ID NO: 5 is shown in SEQ ID NO: 71.

(r) An anti-human IL-6 receptor antibody comprising a light chain CDR3 in which Gln at position 1 in the amino acid sequence of SEQ ID NO: 6 (LCDR3) has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however substitution to Gly (RD_28), Asn (RD_29), or Ser (RDC_15L) is preferred.

A sequence resulting from the substitution of Gly for Gln at position 1 in the amino acid sequence of SEQ ID NO: 6 is shown in SEQ ID NO: 72.

A sequence resulting from the substitution of Asn for Gln at position 1 in the amino acid sequence of SEQ ID NO: 6 is shown in SEQ ID NO: 73.

A sequence resulting from the substitution of Ser for Gln at position 1 in the amino acid sequence of SEQ ID NO: 6 is shown in SEQ ID NO: 74.

(s) An anti-human IL-6 receptor antibody comprising a light chain CDR3 in which Gly at position 3 in the amino acid sequence of SEQ ID NO: 6 has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution to Ser is preferred.

A sequence resulting from the substitution of Ser for Gly at position 3 in the amino acid sequence of SEQ ID NO: 6 is shown in SEQ ID NO: 75.

(t) An anti-human IL-6 receptor antibody comprising a light chain CDR1 in which Tyr at position 9 in the amino acid sequence of SEQ ID NO: 4 (LCDR1) has been substituted with another amino acid, and a light chain CDR3 in which Gly at position 3 in the amino acid sequence of SEQ ID NO: 6 (LCDR3) has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, Tyr at position 9 in the amino acid sequence of SEQ ID NO: 4 (LCDR1) is preferably substituted with Phe, while Gly at position 3 in the amino acid sequence of SEQ ID NO: 6 (LCDR3) is preferably substituted with Ser (RD_72).

(u) An anti-human IL-6 receptor antibody comprising a light chain CDR3 in which Thr at position 5 in the amino acid sequence of SEQ ID NO: 6 (LCDR3) has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution to Arg (RD_23) or Ser is preferred.

A sequence resulting from the substitution of Arg for Thr at position 5 in the amino acid sequence of SEQ ID NO: 6 is shown in SEQ ID NO: 76.

A sequence resulting from the substitution of Ser for Thr at position 5 in the amino acid sequence of SEQ ID NO: 6 is shown in SEQ ID NO: 77.

(v) An anti-human IL-6 receptor antibody comprising a light chain CDR3 in which Gln at position 1 and Thr at position 5 in the amino acid sequence of SEQ ID NO: 6 (LCDR3) have been substituted with other amino acids.

The type of amino acid after substitution is not particularly limited; however, substitutions of Gly for Gln at position 1 and Ser for Thr at position 5 (RD_22) are preferred. Other preferred substitutions include substitutions of Gly for Gln at position 1 and Arg for Thr at position 5 (RDC_11L).

A sequence resulting from the substitutions of Gly for Gln at position 1 and Ser for Thr at position 5 in the amino acid sequence of SEQ ID NO: 6 is shown in SEQ ID NO: 78.

A sequence resulting from the substitutions of Gly for Gln at position 1 and Arg for Thr at position 5 in the amino acid sequence of SEQ ID NO: 6 is shown in SEQ ID NO: 79.

(w) An anti-human IL-6 receptor antibody comprising a heavy chain CDR2 in which Thr at position 9 in the amino acid sequence of SEQ ID NO: 2 (HCDR2) has been substituted with another amino acid, and a heavy chain CDR3 in which Ser at position 1 and Thr at position 5 in the amino acid sequence of SEQ ID NO: 3 (HCDR3) have been substituted with other amino

acids.

Thr at position 9 in the amino acid sequence of SEQ ID NO: 2 (HCDR2) is preferably replaced with Asn. Furthermore, preferred combinations of amino acids for substitutions of Ser at position 1 and Thr at position 5 in the amino acid sequence of SEQ ID NO: 3 (HCDR3) include: Leu and Ala (RDC_27H); Val and Ala (RDC_28H); Ile and Ala (RDC_30H); Thr and Ala (RDC_4H); Val and Ile (RDC_29H); Ile and Ile (RDC_32H); Thr and Ile (RDC_7H); and Leu and Ile (RDC_8H).

(x) An antibody that comprises a variable region comprising the heavy chain CDR3 of (k) and a variable region comprising the light chain CDR3 of (v).

(y) The antibody of (x), which further comprises the heavy chain CDR2 of (e).

The present invention provides antibodies comprising at least the amino acid substitution of any one of (a) to (y) described above and methods for producing the antibodies. Thus, the antibodies of the present invention can also comprise other amino acid substitutions in addition to the amino acid substitution of any one of (a) to (y) described above. Furthermore, the antibodies of the present invention also include antibodies comprising a combination of any amino acid substitutions of (a) to (y) described above. The amino acid substitutions of (a) to (y) described above include substitutions of the CDR amino acid sequences described above to other amino acids. Amino acid substitutions other than those of (a) to (y) described above include, for example, amino acid sequence substitutions, deletions, additions, and/or insertions in other CDR regions. Such substitutions also include amino acid sequence substitutions, deletions, additions, and/or insertions in the FR regions. Such substitutions further include substitutions, deletions, additions, and/or insertions in the constant regions.

Furthermore, the antibodies of the present invention also include antibodies in which a high affinity CDR discovered in the present invention is grafted into any framework other than a humanized PM-1 antibody. The antibodies of the present invention also include antibodies in which the loss of affinity as a result of grafting a high affinity CDR discovered in the present invention into any framework other than a humanized PM-1 antibody has been compensated by mutations introduced into the framework region to restore the original affinity (see, for example, Curr. Opin. Biotechnol. 1994 Aug;5(4):428-33), and antibodies in which the loss has been compensated by mutations introduced into the CDR region to restore the original affinity (see, for example, US 2006/0122377).

In the present invention, the amino acid substitution of any one of (a) to (y) described above is preferably introduced into a humanized PM-1 antibody. Humanized PM-1 antibodies introduced with the amino acid substitution of any one of (a) to (y) described above have strong IL-6 receptor-neutralizing activity. Humanized PM-1 antibodies introduced with the amino acid substitution of any one of (a) to (y) described above are effective as therapeutic agents for

IL-6-associated inflammatory diseases such as rheumatoid arthritis.

Antibodies comprising the amino acid substitution of any one of (a) to (y) described above can also be referred to as, for example, (1) or (2) described below. An example of antibody comprising the substitution of (a) is described here; other antibodies comprising the substitution of any one of (b) to (y) can also be referred to in the same way.

(1) An antibody that comprises a heavy chain variable region comprising CDR1 comprising an amino acid sequence in which Ser at position 1 in the amino acid sequence of SEQ ID NO: 1 has been substituted with another amino acid

(2) An antibody that comprises an H chain comprising CDR1 comprising an amino acid sequence in which Ser at position 1 in the amino acid sequence of SEQ ID NO: 1 has been substituted with another amino acid

<Antibodies with enhanced binding activity>

The present invention further provides anti-IL-6 receptor antibodies with strong IL-6 receptor-binding activity. Herein, "anti-IL-6 receptor antibodies with strong IL-6 receptor-binding activity" typically refers to antibodies whose affinity is measured to be 1 nM or less at 37°C under physiological conditions, preferably 0.1 nM or less, and more preferably 0.04 nM or less. Such anti-IL-6 receptor antibodies with strong IL-6 receptor binding activity are assumed to have an enhanced activity of neutralizing the biological activity of the antigen.

There is no limitation on the type of amino acid substitutions introduced to the present invention's anti-IL-6 receptor antibodies with strong IL-6 receptor binding activity. Such amino acid substitutions include, for example, the above-described amino acid substitutions.

The type of IL-6 receptor is not particularly limited; however, human IL-6 receptor is preferred.

The binding activity can be determined by methods known to those skilled in the art, for example, using Biacore (BIACORE) or such, based on surface plasmon resonance (SPR).

<Antibodies having a CDR sequence with reduced immunogenicity risk>

The present invention also provides anti-IL-6 receptor antibodies with reduced immunogenicity, in particular, humanized PM-1 antibodies. The immunogenicity is assumed to be enhanced when the sequence of an antibody contains a T-cell epitope that binds to HLA. Thus, the immunogenicity risk for an antibody can be reduced by removing the T-cell epitope from the antibody sequence through sequence substitution.

The present invention provides light chain variable regions of humanized anti-human IL-6 receptor antibodies with reduced immunogenicity, in particular, those of humanized PM-1 antibodies, from which T-cell epitopes have been removed through substituting other amino acids in the antibody amino acid sequences, in particular, CDR sequences. The present invention also provides antibodies comprising such light chain variable regions.

More specifically, the present invention provides light chain CDR2 in which Thr at position 2 in the amino acid sequence of SEQ ID NO: 5 (LCDR2) has been substituted with another amino acid. The present invention also provides light chain variable regions comprising such light chain CDR2. The present invention also provides anti-IL-6 receptor antibodies comprising such light chain variable region. The amino acid sequence after substitution is not particularly limited; however, substitution to Gly is preferred. A sequence comprising the substitution of Gly for Thr at position 2 in the amino acid sequence of SEQ ID NO: 5 is shown in SEQ ID NO: 71. The amino acid substitution is preferably introduced into a light chain variable region of a humanized PM-1 antibody.

<FR and CDR of H53/L28>

The present invention also provides anti-human IL-6 receptor antibodies with improved pharmacokinetic, increased stability, and/or reduced immunogenicity. The half-lives of IgGs sharing the same Fc domain in plasma have been found to be correlated to isoelectric points with a high correlation coefficient. Then, the present inventors tried altering the isoelectric points of the variable regions of two antibodies against different antigens, and successfully controlled their half-lives in plasma without altering their Fc domains irrespective of the antigen type. The rate of non-specific antibody uptake by endothelial cells is assumed to depend on the physicochemical Coulomb interaction between IgG and negatively charged cell surface. Lowering the isoelectric point of IgG impairs the Coulomb interaction, which reduces the non-specific uptake by endothelial cells, and as a result, the metabolism in endothelial cells is reduced. This can improve pharmacokinetics.

Specifically, the present invention provides anti-human IL-6 receptor antibodies with reduced isoelectric point and improved pharmacokinetics, by substituting amino acids in the amino acid sequence of an anti-IL-6 receptor antibody, in particular, a humanized PM-1 antibody. Specifically, the humanized PM-1 antibody is altered to reduce its isoelectric point by substituting other amino acids at H13 (amino acid at position 13 in SEQ ID NO: 7), H16 (amino acid at position 16 in SEQ ID NO: 7), H43 (amino acid at position 8 in SEQ ID NO: 8), H81 (amino acid at position 16 in SEQ ID NO: 9), H105 (amino acid at position 3 in SEQ ID NO: 10), L18 (amino acid at position 18 in SEQ ID NO: 11), L45 (amino acid at position 11 in SEQ ID NO: 12), L79 (amino acid at position 23 in SEQ ID NO: 13), L107 (amino acid at position 10 in SEQ ID NO: 14), H31 (amino acid at position 1 in SEQ ID NO: 1), L24 (amino acid at position 1 in SEQ ID NO: 4), and/or L53 (amino acid at position 4 in SEQ ID NO: 5), where positions are numbered according to Kabat's numbering system (Kabat EA *et al.*, 1991 Sequences of Proteins of Immunological Interest. NIH). These substitutions can lower the isoelectric point of a humanized PM-1 antibody without affecting its binding activity and stability. Some amino acid residues originated from the mouse sequence remain unsubstituted in the humanized PM-1

antibody to maintain its binding activity even after humanization of the mouse sequence. More specifically, amino acids at H27 (amino acid at position 27 in SEQ ID NO: 7), H28 (amino acid at position 28 in SEQ ID NO: 7), H29 (amino acid at position 29 in SEQ ID NO: 7), H30 (amino acid at position 30 in SEQ ID NO: 7), and H71 in the humanized PM-1 antibody (positions are numbered according to Kabat's numbering system described above) are of the mouse sequence. HFR1 can be converted into a human sequence by substituting H13, H16, H23, and H30, which enables to produce an antibody whose immunogenicity risk is lower than that of the humanized PM-1 antibody. Furthermore, since the humanized PM-1 antibody is an antibody humanized by CDR grafting, its stability may be further improved. Antibodies can be stabilized, for example, by substituting hydrophilic amino acids for amino acid residues exposed on the surface of the antibody variable region. Alternatively, antibodies can also be stabilized by altering the CDR sequence to a consensus sequence. The humanized PM-1 antibody can be stabilized by a substitution of Ile for Met at H69 (amino acid position 4 in SEQ ID NO: 9) (stabilization of the hydrophobic core), Ser for Leu at H70 (amino acid at position 5 in SEQ ID NO: 9) (conversion of the surface-exposed residue to a hydrophilic residue), Asn for Thr at H58 (amino acid at position 9 in SEQ ID NO: 2) (alternation of the heavy chain CDR2 to a consensus sequence), Gly for Ser at H65 (amino acid at position 16 in SEQ ID NO: 2) (substitution of Gly in the β turn region and alternation of the heavy chain CDR2 to a consensus sequence), or Ser for Thr at L93 (amino acid at position 5 in SEQ ID NO: 6) (conversion of the surface-exposed residue to a hydrophilic residue) (positions are numbered according to Kabat's numbering system described above). Alternatively, *in silico*-predicted T-cell epitopes can be removed by substituting Gly for Thr at L51 at position 2 in LCDR2 (SEQ ID NO: 5) described above, and this can reduce the immunogenicity risk without affecting the binding activity and stability. Anti-IL-6 receptor antibodies with improved stability and antibody pharmacokinetics, as well as reduced immunogenicity can be obtained by using these amino acid substitutions in combination.

Such antibodies include, for example, the antibodies of (1) to (37) below.

(1) An antibody that comprises a heavy chain variable region comprising FR1 in which Arg at position 13 in the amino acid sequence of SEQ ID NO: 7 has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution to Lys is preferred.

A sequence resulting from the substitution of Lys for Arg at position 13 in the amino acid sequence of SEQ ID NO: 7 is shown in SEQ ID NO: 80.

(2) An antibody that comprises a heavy chain variable region comprising FR1 in which Gln at position 16 in the amino acid sequence of SEQ ID NO: 7 has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution of Glu is preferred.

A sequence resulting from the substitution of Glu for Gln at position 16 in the amino acid sequence of SEQ ID NO: 7 is shown in SEQ ID NO: 81.

(3) An antibody that comprises a heavy chain variable region comprising FR1 in which Thr at position 23 in the amino acid sequence of SEQ ID NO: 7 has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution to Ala is preferred.

A sequence resulting from the substitution of Ala for Thr at position 23 in the amino acid sequence of SEQ ID NO: 7 is shown in SEQ ID NO: 82.

(4) An antibody that comprises a heavy chain variable region comprising FR1 in which Thr at position 30 in the amino acid sequence of SEQ ID NO: 7 has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution to Ser is preferred.

A sequence resulting from the substitution of Ser for Thr at position 30 in the amino acid sequence of SEQ ID NO: 7 is shown in SEQ ID NO: 83.

(5) An antibody that comprises a heavy chain variable region comprising FR1 in which Arg at position 13, Gln at position 16, Thr at position 23, and Thr at position 30 in the amino acid sequence of SEQ ID NO: 7 have been substituted with other amino acids.

The type of amino acid after substitution is not particularly limited; however, substitutions of Lys for Arg at position 13, Glu for Gln at position 16, Ala for Thr at position 23, and Ser for Thr at position 30 are preferred.

A sequence resulting from the substitutions of Lys for Arg at position 13, Glu for Gln at position 16, Ala for Thr at position 23, and Ser for Thr at position 30 in the amino acid sequence of SEQ ID NO: 7 is shown in SEQ ID NO: 84.

(6) An antibody that comprises a heavy chain variable region comprising FR2 in which Arg at position 8 in the amino acid sequence of SEQ ID NO: 8 has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution to Glu is preferred.

A sequence resulting from the substitution of Glu for Arg at position 8 in the amino acid sequence of SEQ ID NO: 8 is shown in SEQ ID NO: 85.

(7) An antibody that comprises a heavy chain variable region comprising FR3 in which Met at position 4 in the amino acid sequence of SEQ ID NO: 9 has been substituted with another

amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution to Ile is preferred.

A sequence resulting from the substitution of Ile for Met at position 4 in the amino acid sequence of SEQ ID NO: 9 is shown in SEQ ID NO: 86.

(8) An antibody that comprises a heavy chain variable region comprising FR3 in which Leu at position 5 in the amino acid sequence of SEQ ID NO: 9 has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution to Ser is preferred.

A sequence resulting from the substitution of Ser for Leu at position 5 in the amino acid sequence of SEQ ID NO: 9 is shown in SEQ ID NO: 87.

(9) An antibody that comprises a heavy chain variable region comprising FR3 in which Arg at position 16 in the amino acid sequence of SEQ ID NO: 9 has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution to Lys is preferred.

A sequence resulting from the substitution of Lys for Arg at position 16 in the amino acid sequence of SEQ ID NO: 9 is shown in SEQ ID NO: 88.

(10) An antibody that comprises a heavy chain variable region comprising FR3 in which Val at position 27 in the amino acid sequence of SEQ ID NO: 9 has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution to Ala is preferred.

A sequence resulting from the substitution of Ala for Val at position 27 in the amino acid sequence of SEQ ID NO: 9 is shown in SEQ ID NO: 89.

(11) An antibody that comprises a heavy chain variable region comprising FR3 in which Met at position 4, Leu at position 5, Arg at position 16, and Val at position 27 in the amino acid sequence of SEQ ID NO: 9 (HFR3) have been substituted with other amino acids.

The type of amino acid after substitution is not particularly limited; however, substitutions of Ile for Met at position 4, Ser for Leu at position 5, Lys for Arg at position 16, and Ala for Val at position 27 are preferred.

A sequence resulting from the substitutions of Ile for Met at position 4, Ser for Leu at position 5, Lys for Arg at position 16, and Ala for Val at position 27 in the amino acid sequence of SEQ ID NO: 9 is shown in SEQ ID NO: 90.

(12) An antibody that comprises a heavy chain variable region comprising FR4 in which

Gln at position 3 in the amino acid sequence of SEQ ID NO: 10 (HFR4) has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution to Glu is preferred.

A sequence resulting from the substitution of Glu for Gln at position 3 in the amino acid sequence of SEQ ID NO: 10 is shown in SEQ ID NO: 91.

(13) An antibody that comprises a light chain variable region comprising FR1 in which Arg at position 18 in the amino acid sequence of SEQ ID NO: 11 (LFR1) has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution to Ser is preferred.

A sequence resulting from the substitution of Ser for Arg at position 18 in the amino acid sequence of SEQ ID NO: 11 is shown in SEQ ID NO: 92.

(14) An antibody that comprises a light chain variable region comprising FR2 in which Lys at position 11 in the amino acid sequence of SEQ ID NO: 12 (LFR2) has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution to Glu is preferred.

A sequence resulting from the substitution of Glu for Lys at position 11 in the amino acid sequence of SEQ ID NO: 12 is shown in SEQ ID NO: 93.

(15) An antibody that comprises a light chain variable region comprising FR3 in which Gln at position 23 in the amino acid sequence of SEQ ID NO: 13 has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution to Glu is preferred.

A sequence resulting from the substitution of Glu for Gln at position 23 in the amino acid sequence of SEQ ID NO: 13 is shown in SEQ ID NO: 94.

(16) An antibody that comprises a light chain variable region comprising FR3 in which Pro at position 24 in the amino acid sequence of SEQ ID NO: 13 has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution to Ala is preferred.

A sequence resulting from the substitution of Ala for Pro at position 24 in the amino acid sequence of SEQ ID NO: 13 is shown in SEQ ID NO: 95.

(17) An antibody that comprises a light chain variable region comprising FR3 in which Ile at position 27 in the amino acid sequence of SEQ ID NO: 13 has been substituted with

another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution to Ala is preferred.

A sequence resulting from the substitution of Ala for Ile at position 27 in the amino acid sequence of SEQ ID NO: 13 is shown in SEQ ID NO: 96.

(18) An antibody that comprises a light chain variable region comprising FR3 in which Gln at position 23, Pro at position 24, and Ile at position 27 in the amino acid sequence of SEQ ID NO: 13 (LFR3) have been substituted with other amino acids.

The type of amino acid after substitution is not particularly limited; however, substitutions of Glu for Gln at position 23, Ala for Pro at position 24, and Ala for Ile at position 27 are preferred.

A sequence resulting from the substitutions of Glu for Gln at position 23, Ala for Pro at position 24, and Ala for Ile at position 27 in the amino acid sequence of SEQ ID NO: 13 is shown in SEQ ID NO: 97.

(19) An antibody that comprises a light chain variable region comprising FR4 in which Lys at position 10 in the amino acid sequence of SEQ ID NO: 14 (LFR4) has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution to Glu is preferred.

A sequence resulting from the substitution of Glu for Lys at position 10 in the amino acid sequence of SEQ ID NO: 14 is shown in SEQ ID NO: 98.

(20) An antibody that comprises a heavy chain variable region comprising FR4 in which Ser at position 5 in the amino acid sequence of SEQ ID NO: 10 (HFR4) has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution to Thr is preferred.

A sequence resulting from the substitution of Thr for Ser at position 5 in the amino acid sequence of SEQ ID NO: 10 is shown in SEQ ID NO: 132.

(21) An antibody that comprises a heavy chain variable region comprising FR4 in which Gln at position 3 and Ser at position 5 in the amino acid sequence of SEQ ID NO: 10 (HFR4) have been substituted with other amino acids.

The type of amino acid after substitution is not particularly limited; however, substitutions of Glu for Gln at position 3 and Thr for Ser at position 5 are preferred.

A sequence resulting from the substitutions of Glu for Gln at position 3 and Thr for Ser at position 5 in the amino acid sequence of SEQ ID NO: 10 is shown in SEQ ID NO: 133.

(22) An antibody that comprises a heavy chain variable region of a humanized PM-1

antibody comprising the amino acid substitutions of (5), (6), (11), and (21).

(23) An antibody that comprises a light chain variable region of a humanized PM-1 antibody comprising the amino acid substitutions of (13), (14), (18), and (19).

(24) An antibody that comprises the heavy chain variable region of (22) and the light chain variable region of (23).

(25) An antibody that comprises a heavy chain variable region comprising CDR1 in which Ser at position 1 in the amino acid sequence of SEQ ID NO: 1 (HCDR1) has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution of Asp is preferred.

A sequence resulting from the substitution of Asp for Ser at position 1 in the amino acid sequence of SEQ ID NO: 1 is shown in SEQ ID NO: 28.

(26) An antibody that comprises a heavy chain variable region comprising CDR2 in which Ser at position 16 in the amino acid sequence of SEQ ID NO: 2 has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution of Gly is preferred.

A sequence resulting from the substitution of Gly for Ser at position 16 in the amino acid sequence of SEQ ID NO: 2 is shown in SEQ ID NO: 99.

(27) An antibody that comprises a heavy chain variable region comprising CDR2 in which Thr at position 9 and Ser at position 16 in the amino acid sequence of SEQ ID NO: 2 (HCDR2) have been substituted with other amino acids.

The type of amino acid after substitution is not particularly limited; however, substitutions of Asn for Thr at position 9 and Gly for Ser at position 16 are preferred.

A sequence resulting from the substitutions of Asn for Thr at position 9 and Gly for Ser at position 16 in the amino acid sequence of SEQ ID NO: 2 is shown in SEQ ID NO: 100.

(28) An antibody that comprises a light chain variable region comprising CDR1 in which Arg at position 1 in the amino acid sequence of SEQ ID NO: 4 (LCDR1) has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution of Gln is preferred.

A sequence resulting from the substitution of Gln for Arg at position 1 in the amino acid sequence of SEQ ID NO: 4 is shown in SEQ ID NO: 101.

(29) An antibody that comprises a light chain variable region comprising CDR2 in which Arg at position 4 in the amino acid sequence of SEQ ID NO: 5 has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution to Glu is preferred.

A sequence resulting from the substitution of Glu for Arg at position 4 in the amino acid sequence of SEQ ID NO: 5 is shown in SEQ ID NO: 102.

(30) An antibody that comprises a light chain variable region comprising CDR2 in which Thr at position 2 and Arg at position 4 in the amino acid sequence of SEQ ID NO: 5 (LCDR2) have been substituted with other amino acids.

The type of amino acid after substitution is not particularly limited; however, substitutions of Gly for Thr at position 2 and Glu for Arg at position 4 are preferred.

A sequence resulting from the substitutions of Gly for Thr at position 2 and Glu for Arg at position 4 in the amino acid sequence of SEQ ID NO: 5 is shown in SEQ ID NO: 103.

(31) An antibody that comprises a light chain variable region comprising CDR3 in which Thr at position 5 in the amino acid sequence of SEQ ID NO: 6 (LCDR3) has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution to Ser is preferred.

A sequence resulting from the substitution of Ser for Thr at position 5 in the amino acid sequence of SEQ ID NO: 6 is shown in SEQ ID NO: 77.

(32) An antibody that comprises a heavy chain variable region comprising the amino acid substitutions of (25) and (27).

(33) An antibody that comprises a light chain variable region comprising the amino acid substitutions of (28), (30), and (31).

(34) An antibody that comprises the heavy chain variable region of (32) and the light chain variable region of (33).

(35) An antibody that comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 104 (VH of H53/L28).

(36) An antibody that comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO: 105 (VL of H53/L28).

(37) An antibody that comprises the heavy chain variable region of (35) and the light chain variable region of (36).

Any amino acid substitutions of (1) to (37) described above are preferably introduced into a humanized PM-1 antibody. The present invention provides antibodies comprising at least the amino acid substitution of any one of (1) to (37) described above and methods for producing those antibodies. Thus, the antibodies of the present invention also include antibodies comprising other amino acid substitutions in addition to the amino acid substitution of any one of (1) to (37) described above. The antibodies of the present invention also include

antibodies comprising combinations of multiple amino acid substitutions of (1) to (37) described above. The amino acid substitutions of (1) to (37) described above include, for example, substitutions in the amino acid sequences of FR and CDR described above. Amino acid substitutions other than those of (1) to (37) described above include other substitutions, deletions, additions, and/or insertions in FR and CDR sequences than those described above. The amino acid substitutions also include substitutions, deletions, additions, and/or insertions in the amino acid sequences of constant regions.

Furthermore, in addition to those described above, amino acid alternations that result in a lower isoelectric point without loss of the activity of anti-IL-6 receptor antibody, include, for example, substitutions of Lys at position 15 and/or Ser at position 16 in the amino acid sequence of SEQ ID NO: 2 with other amino acids. The type of amino acid after substitution is not particularly limited; however, substitutions of Gln for Lys at position 15 and Asp for Ser at position 16 are preferred. A sequence comprising the substitutions of Gln for Lys at position 15 and Asp for Ser at position 16 in the amino acid sequence of SEQ ID NO: 2 is shown in SEQ ID NO: 121. Alternatively, such amino acid substitutions may also be introduced into the amino acid sequence of SEQ ID NO: 100. A sequence comprising the substitutions of Gln for Lys at position 15 and Asp for Gly at position 16 in the amino acid sequence of SEQ ID NO: 100 is shown in SEQ ID NO: 122. Thus, the present invention provides antibodies that comprise a heavy chain variable region comprising CDR2 in which Lys at position 15 and/or Ser at position 16 in the amino acid sequence of SEQ ID NO: 2 or 100 have been substituted with other amino acids.

Other alterations that result in a lower isoelectric point include substitution of Gln at position 4 in the amino acid sequence of SEQ ID NO: 4 has been substituted with another amino acid. The type of amino acid after substitution is not particularly limited; however, substitution to Glu is preferred. An amino acid sequence comprising the substitution of Glu for Gln at position 4 in the amino acid sequence of SEQ ID NO: 4 is shown in SEQ ID NO: 123. Alternatively, this amino acid substitution may also be introduced into the amino acid sequence of SEQ ID NO: 101. An amino acid sequence comprising the substitution of Glu for Gln at position 4 in the amino acid sequence of SEQ ID NO: 101 is shown in SEQ ID NO: 124. Thus, the present invention provides antibodies that comprise a light chain variable region comprising CDR1 in which Gln at position 4 in the amino acid sequence of SEQ ID NO: 4 or 101 has been substituted with another amino acid.

Other alterations that result in a lower isoelectric point include substitution of His at position 6 in the amino acid sequence of SEQ ID NO: 5 with another amino acid. The type of amino acid after substitution is not particularly limited; however, substitution to Glu is preferred. An amino acid sequence comprising the substitution of Glu for His at position 6 in the amino

acid sequence of SEQ ID NO: 5 is shown in SEQ ID NO: 125. Alternatively, this amino acid substitution may also be introduced into the amino acid sequence of SEQ ID NO: 103. An amino acid sequence comprising the substitution of Glu for His at position 6 in the amino acid sequence of SEQ ID NO: 103 is shown in SEQ ID NO: 126. Thus, the present invention provides antibodies that comprise a light chain variable region comprising CDR2 in which His at position 6 in the amino acid sequence of SEQ ID NO: 5 or 103 has been substituted with another amino acid.

Furthermore, alternations that result in reduced immunogenicity risk include substitution of Val for Ala at position 27 (H89 in Kabat's numbering system) in the amino acid sequence of heavy chain FR3 of SEQ ID NO: 90. An amino acid sequence comprising the substitution of Val for Ala at position 27 in the amino acid sequence of SEQ ID NO: 90 is shown in SEQ ID NO: 127. Thus, the present invention provides antibodies that comprise a heavy chain variable region comprising FR3 in which Val has been substituted for Ala at position 27 in the amino acid sequence of SEQ ID NO: 90.

The only mouse sequence that remains in the amino acid sequences of heavy chain FR3 of SEQ ID NO: 9 and 90 is Arg at position 6 (H71 in Kabat's numbering system). Anti-human IL-6 receptor antibodies having a framework consisting entirely of human sequences can be produced by using as a FR3 sequence, the human sequence of human VH1 subclass (SEQ ID NO: 128) or human VH3 subclass (SEQ ID NO: 129) where Arg is conserved at H71. Thus, the present invention provides antibodies that comprise a heavy chain variable region comprising the FR3 of SEQ ID NO: 128 or 129.

Furthermore, alternations that improve stability include substitution of Ile for Ser at position 5 (H107 in Kabat's numbering system) in the amino acid sequence of heavy chain FR4 of SEQ ID NO: 10. An amino acid sequence comprising the substitution of Ile for Ser at position 5 in the amino acid sequence of SEQ ID NO: 10 is shown in SEQ ID NO: 130. Alternatively, this amino acid sequence may also be introduced into the amino acid sequence of SEQ ID NO: 91. An amino acid sequence comprising the substitution of Ile for Ser at position 5 in the amino acid sequence of SEQ ID NO: 91 is shown in SEQ ID NO: 131. Thus, the present invention provides antibodies that comprise a heavy chain variable region comprising FR4 in which Ile has been substituted for Ser at position 5 in the amino acid sequence of SEQ ID NO: 10 or 91.

Such amino acid substitutions are preferably introduced into the humanized PM-1 antibody, H53/L28 (an antibody comprising the heavy chain variable region of SEQ ID NO: 104 and the light chain variable region of SEQ ID NO: 105), or PF1 antibody (an antibody comprising the heavy chain variable region of SEQ ID NO: 22 and the light chain variable region of SEQ ID NO: 23). The present invention provides antibodies comprising at least such

amino acid substitutions and methods for producing the antibodies. Thus, the antibodies of the present invention include antibodies comprising, in addition to such amino acid substitutions, the amino acid substitution of any one of (1) to (37) described above and/or other amino acid substitutions than those of (1) to (37) described above. Amino acid substitutions other than those of (1) to (37) described above include other substitutions, deletions, additions, and/or insertions in FR and CDR sequences than those described above. The amino acid substitutions also include substitutions, deletions, additions, and/or insertions in the amino acid sequences of constant regions.

<Anti-human IL-6 receptor antibodies with low isoelectric point>

The present invention also provides anti-IL-6 receptor antibodies with a low isoelectric point. The antibodies of the present invention with low isoelectric point include antibodies in which the measured isoelectric point of the whole antibody is low and antibodies in which the theoretical isoelectric point of the variable region (VH/VL) is low.

Herein, "anti-IL-6 receptor antibodies in which the measured isoelectric point of the whole antibody is low" typically refers to antibodies in which the measured isoelectric point is 7.5 or less, preferably 7.0 or less, and more preferably 6.0 or less. The measured isoelectric point can be determined by methods known to those skilled in the art, for example, non-denaturation gel isoelectric focusing or capillary isoelectric focusing.

Herein, "anti-IL-6 receptor antibodies in which the theoretical isoelectric point of the variable region is low" typically refers to antibodies in which the theoretical isoelectric point is 5.5 or less, preferably 5.0 or less, and more preferably 4.0 or less. The theoretical isoelectric point can be determined by methods known to those skilled in the art. For example, the theoretical isoelectric points of VH and VL of a variable region can be computed by using software such as GENETYX (GENETYX CORPORATION).

There is no limitation on the type of amino acid substitution to be introduced to obtain anti-IL-6 receptor antibodies of the present invention with low isoelectric point. Such amino acid substitutions include, for example, the amino acid substitutions described above. Such anti-IL-6 receptor antibodies with low isoelectric point are assumed to show enhanced pharmacokinetics.

The type of IL-6 receptor is not particularly limited; however, human IL-6 receptor is preferred.

<Anti-human IL-6 receptor antibodies that are stable at high concentrations>

Furthermore, the present invention provides anti-IL-6 receptor antibodies that are stable at high concentrations.

Herein, "stable at high concentrations" means that the increase in the proportion of aggregates of anti-IL-6 receptor antibody ([peak area for aggregate in gel filtration

chromatogram]/[total peak area in gel filtration chromatogram] x 100) generated in a high-concentration antibody solution (100 mg/ml) at 25°C in one month is 0.3% or less, preferably 0.2% or less, and more preferably 0.1% or less when the antibody is in a buffer of pH 6.5 to 7.0 properly selected for subcutaneous administration, for example, 20 mM histidine-HCl, 150 mM NaCl. The concentration of anti-IL-6 receptor antibody may be 100 mg/ml or higher, for example, 200 or 300 mg/ml.

There is no limitation on the anti-IL-6 receptor antibodies of the present invention that are stable at high concentrations. The antibodies can be prepared, for example, with the above-described amino acid substitutions or such.

The type of IL-6 receptor is not particularly limited; however, human IL-6 receptor is preferred.

The present invention also provides humanized PM-1 antibodies comprising any one of the amino acid substitutions of (1) to (37) described above and further comprising any of the amino acid substitutions of (a) to (y) described above to improve their binding activity and/or neutralizing activity. In an embodiment, such antibodies include those comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 22 (PF1_H) and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 23 (PF1_L) (PF1), but are not limited thereto.

Furthermore, the present invention provides anti-IL-6 receptor antibodies of any of the following:

(A) a heavy chain variable region that comprises CDR1 comprising the amino acid sequence of SEQ ID NO: 165 (CDR1 of VH5-M83), CDR2 comprising the amino acid sequence of SEQ ID NO: 166 (CDR2 of VH5-M83), and CDR3 comprising the amino acid sequence of SEQ ID NO: 167 (CDR3 of VH5-M83);

(B) a light chain variable region that comprises CDR1 comprising the amino acid sequence of SEQ ID NO: 101 (CDR1 of VL5), CDR2 comprising the amino acid sequence of SEQ ID NO: 168 (CDR2 of VL5), and CDR3 comprising the amino acid sequence of SEQ ID NO: 79 (CDR3 of VL5);

(C) an antibody that comprises the heavy chain variable region of (A) and the light chain variable region of (B);

(D) a heavy chain variable region that comprises CDR1 comprising the amino acid sequence of SEQ ID NO: 169 (CDR1 of VH3-M73), CDR2 comprising the amino acid sequence of SEQ ID NO: 170 (CDR2 of VH3-M73), and CDR3 comprising the amino acid sequence of SEQ ID NO: 171 (CDR3 of VH3-M73);

(E) a light chain variable region that comprises CDR1 comprising the amino acid sequence of SEQ ID NO: 172 (CDR1 of VL3), CDR2 comprising the amino acid sequence of SEQ ID NO:

173 (CDR2 of VL3), and CDR3 comprising the amino acid sequence of SEQ ID NO: 79 (CDR3 of VL3);

(F) an antibody that comprises the heavy chain variable region of (D) and the light chain variable region of (E);

(G) a heavy chain variable region that comprises CDR1 comprising the amino acid sequence of SEQ ID NO: 169 (CDR1 of VH4-M73), CDR2 comprising the amino acid sequence of SEQ ID NO: 174 (CDR2 of VH4-M73), and CDR3 comprising the amino acid sequence of SEQ ID NO: 171 (CDR3 of VH4-M73);

(H) a light chain variable region that comprises CDR1 comprising the amino acid sequence of SEQ ID NO: 175 (CDR1 of VL1), CDR2 comprising the amino acid sequence of SEQ ID NO: 173 (CDR2 of VL1), and CDR3 comprising the amino acid sequence of SEQ ID NO: 79 (CDR3 of VL1); and

(I) an antibody that comprises the heavy chain variable region of (G) and the light chain variable region of (H).

Furthermore, the present invention provides anti-IL-6 receptor antibodies of any of the following:

(a) an antibody that comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 159 (H96-IgG1 variable region);

(b) an antibody that comprises a heavy chain variable region in which at least one of amino acids of Trp at position 35, Tyr at position 51, Ser at position 63, Lys at position 65, Gly at position 66, Val at position 99, Ile at position 103, Tyr at position 108, Glu at position 111, and Thr at position 113 in the amino acid sequence of SEQ ID NO: 159 (H96-IgG1 variable region) has been substituted with another amino acid;

(c) an antibody that comprises a heavy chain variable region comprising an amino acid sequence in which Lys at position 65, Gly at position 66, Val at position 99, Ile at position 103, Glu at position 111, and Thr at position 113 in the amino acid sequence of SEQ ID NO: 159 (H96-IgG1 variable region) have been substituted with other amino acids;

(d) an antibody that comprises a heavy chain variable region comprising an amino acid sequence in which Trp at position 35, Tyr at position 51, Ser at position 63, Lys at position 65, Gly at position 66, Val at position 99, Ile at position 103, and Tyr at position 108 in the amino acid sequence of SEQ ID NO: 159 (H96-IgG1 variable region) have been substituted with other amino acids;

(e) an antibody that comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 160 (F2H-IgG1 variable region);

(f) an antibody that comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 161 (VH5-M83 variable region);

(g) an antibody that comprises a light chain variable region comprising an amino acid sequence in which Gln at position 27 and/or His at position 55 in the amino acid sequence of SEQ ID NO: 23 (PF1L) have been substituted with other amino acids;

(h) an antibody that comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO: 162 (L39 variable region);

(i) an antibody that comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO: 163 (VL5-kappa variable region);

(j) an antibody that comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 176 (VH3-M73 variable region);

(k) an antibody that comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 178 (VH4-M73 variable region);

(l) an antibody that comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO: 177 (VL3-kappa variable region);

(m) an antibody that comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO: 179 (VL1-kappa variable region);

(n) an antibody that comprises the heavy chain variable region of (e) and the light chain variable region of (h);

(o) an antibody that comprises the heavy chain variable region of (f) and the light chain variable region of (i) (combination of FV5-M83 variable regions);

(p) an antibody that comprises the heavy chain variable region of (j) and the light chain variable region of (l) (combination of FV4-M73 variable regions); and

(q) an antibody that comprises the heavy chain variable region of (k) and the light chain variable region of (m) (combination of FV3-M73 variable regions).

The type of amino acid after substitution is not particularly limited in the amino acid substitution of the heavy chain variable regions of (a) to (d) above; however, substitutions of Val for Trp at position 35, Phe for Tyr at position 51, Thr for Ser at position 63, Gln for Lys at position 65, Asp for Gly at position 66, Leu for Val at position 99, Ala for Ile at position 103, Val for Tyr at position 108, Gln for Glu at position 111, Ile for Thr at position 113 are preferred. Alternatively, the type of amino acid after substitution is not particularly limited in the amino acid substitution of the light chain variable region of (g) above; however, substitutions of Glu for Gln at position 27 and Glu for His at position 55 are preferred. Amino acid substitutions, deletions, insertions, and/or additions other than the amino acid substitution described above may be included.

The antibody constant regions of the present invention are not particularly limited, and any constant regions may be used. For example, constant regions comprising a natural sequence such as IgG1, IgG2, and IgG4 and altered constant regions prepared by introducing

amino acid substitutions, deletions, additions, and/or insertions into a constant region comprising a natural sequence can be used. The examples of such altered constant regions include the constant regions described below.

The examples of antibodies using the variable regions of the present invention mentioned above include:

(1) an antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 134 (H96-IgG1);

(2) an antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 135 (F2H-IgG1);

(3) an antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 137 (VH5-IgG1);

(4) an antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 139 (VH5-M83);

(5) an antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 136 (L39);

(6) an antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 138 (VL5-kappa);

(7) an antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 180 (VH3-M73);

(8) an antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 182 (VH4-M73);

(9) an antibody that comprises a light chain comprising the amino acid sequence of SEQ ID NO: 181 (VL3-kappa);

(10) an antibody that comprises a light chain comprising the amino acid sequence of SEQ ID NO: 183 (VL1-kappa);

(11) an antibody that comprises the heavy chain of (2) and the light chain of (5);

(12) an antibody that comprises the heavy chain of (3) and the light chain of (6);

(13) an antibody that comprises the heavy chain of (4) and the light chain of (6) (FV5-M83);

(14) an antibody that comprises the heavy chain of (7) and the light chain of (9) (FV4-M73);

(15) an antibody that comprises the heavy chain of (8) and the light chain of (10) (FV3-M73); and

(16) an antibody having an activity equivalent to that of any of the antibodies of (1) to (15).

Herein, "having equivalent activity" means that the antigen binding activity and/or neutralizing activity are equivalent. "Equivalent activity" in the present invention does not necessarily mean completely identical activity, but may be, for example, 50% or more of the activity, preferably 70% or more, and more preferably 90% or more.

Furthermore, the present invention provides CDR and FR of any of the following:

(i) a heavy chain FR1 that comprises the amino acid sequence of SEQ ID NO: 84 (heavy chain FR1 of VH5);

(ii) a heavy chain FR1 that comprises the amino acid sequence of SEQ ID NO: 186 (heavy chain FR1 of VH3 and VH4);

(iii) a heavy chain FR2 that comprises the amino acid sequence of SEQ ID NO: 85 (heavy chain FR2 of VH3, VH4, and VH5);

(iv) a heavy chain FR3 that comprises the amino acid sequence of SEQ ID NO: 184 (heavy chain FR3 of VH3, VH4, and VH5);

(v) a heavy chain FR4 that comprises the amino acid sequence of SEQ ID NO: 133 (heavy chain FR4 of VH3, VH4, and VH5);

(vi) a light chain FR1 that comprises the amino acid sequence of SEQ ID NO: 92 (light chain FR1 of VL1, VL3, and VL5);

(vii) a light chain FR2 that comprises the amino acid sequence of SEQ ID NO: 93 (light chain FR2 of VL1, VL3, and VL5);

(viii) a light chain FR3 that comprises the amino acid sequence of SEQ ID NO: 97 (light chain FR3 of VL1, VL3, and VL5);

(ix) a light chain FR4 that comprises the amino acid sequence of SEQ ID NO: 98 (light chain FR4 of VL1, VL3, and VL5);

(x) a heavy chain CDR1 that comprises the amino acid sequence of SEQ ID NO: 169 (heavy chain CDR1 of VH3 and VH4);

(xi) a heavy chain CDR1 that comprises the amino acid sequence of SEQ ID NO: 165 (heavy chain CDR1 of VH5);

(xii) a heavy chain CDR2 that comprises the amino acid sequence of SEQ ID NO: 170 (heavy chain CDR2 of VH3);

(xiii) a heavy chain CDR2 that comprises the amino acid sequence of SEQ ID NO: 174 (heavy chain CDR2 of VH4);

(xiv) a heavy chain CDR2 that comprises the amino acid sequence of SEQ ID NO: 166 (heavy chain CDR2 of VH5);

(xv) a heavy chain CDR3 that comprises the amino acid sequence of SEQ ID NO: 171 (heavy chain CDR3 of VH3 and VH4);

(xvi) a heavy chain CDR3 that comprises the amino acid sequence of SEQ ID NO: 167 (heavy chain CDR3 of VH5);

(xvii) a light chain CDR1 that comprises the amino acid sequence of SEQ ID NO: 175 (light chain CDR1 of VL1;

(xviii) a light chain CDR1 that comprises the amino acid sequence of SEQ ID NO: 172 (light

chain CDR1 of VL3);
(xix) a light chain CDR1 that comprises the amino acid sequence of SEQ ID NO: 101 (light chain CDR1 of VL5);
(xx) a light chain CDR2 that comprises the amino acid sequence of SEQ ID NO: 173 (light chain CDR2 of VL1 and VL3);
(xxi) a light chain CDR2 that comprises the amino acid sequence of SEQ ID NO: 168 (light chain CDR2 of VL5); and
(xxii) a light chain CDR3 that comprises the amino acid sequence of SEQ ID NO: 79 (light chain CDR3 of VL1, VL3, and VL5).

The antibodies of the present invention also include fragments and processed products of antibodies comprising any of the amino acid substitutions described above. Such antibody fragments include, for example, Fab, F(ab')2, Fv, single chain Fv (scFv) in which H and L chains are linked together via an appropriate linker, single domain H chain and single domain L chain (for example, Nat. Biotechnol. 2005 Sep;23(9):1126-36), Unibody (WO 2007059782 A1), and SMIP (WO 2007014278 A2). The origin of antibodies is not particularly limited. The antibodies include human, mouse, rat, and rabbit antibodies. The antibodies of the present invention may be chimeric, humanized, fully humanized antibodies, or such.

Specifically, such antibody fragments are obtained by treating antibodies with an enzyme, for example, papain or pepsin, or by constructing genes to encode such antibody fragments, inserting them into expression vectors, and then expressing them in appropriate host cells (see, for example, Co, M. S. *et al.*, J. Immunol. (1994) 152, 2968-2976; Better, M. and Horwitz, A. H. Methods in Enzymology (1989) 178, 476-496; Plückthun, A.; Skerra, A., Methods in Enzymology (1989) 178, 497-515; Lamoyi, E., Methods in Enzymology (1989) 121, 652-663; Rousseaux, J. *et al.*, Methods in Enzymology (1989) 121, 663-66; Bird, R. E. *et al.*, TIBTECH (1991) 9, 132-137).

scFv is obtained by linking V regions of antibody H and L chains. In such scFv, the H chain V region is linked to the L chain V region via a linker, preferably a peptide linker (Huston, J. S. *et al.*, Proc. Natl. Acad. Sci. USA (1988) 85, 5879-5883). The H chain and L chain V regions in an scFv may be derived from any of the antibodies described above. The peptide linker to link the V regions includes, for example, arbitrary single chain peptides of 12 to 19 amino acid residues.

<Antibody constant regions>

The present invention also provides the antibody constant regions of (i) to (xxi) described below, which have been improved through amino acid substitution. The constant region refers to IgG1, IgG2, or IgG4 type constant region. The amino acid sequences of human IgG1, IgG2, and IgG4 constant regions are known (human IgG1 constant region, SEQ ID NO:

19; human IgG2 constant region, SEQ ID NO: 20; and human IgG4 constant region, SEQ ID NO: 21). The sequence of human IgG4 constant region has been altered to improve the stability of the hinge region (Mol. Immunol. 1993 Jan;30(1):105-8). The present invention also provides antibodies that comprise such an amino acid substitution-containing antibody constant region. The antibody constant regions are preferably human antibody constant regions.

The amino acid substitution-containing antibody constant regions of the present invention may comprise other amino acid substitutions or modifications as long as they comprise the amino acid substitution of any one of (i) to (xxi) described below. Therefore, IgG2 constant regions comprising the amino acid substitutions of the present invention in the IgG2 constant region comprising the amino acid sequence of SEQ ID NO: 20 include IgG2 constant regions that comprise one or more amino acid substitutions and/or modifications in the amino acid sequence of SEQ ID NO: 20 and further comprise the amino acid substitutions of the present invention, as well as IgG2 constant regions that comprise the amino acid substitutions of the present invention and further comprise one or more amino acid substitutions and/or modifications. The same applies to IgG1 constant regions comprising the amino acid sequence of SEQ ID NO: 19 and IgG4 constant regions comprising the amino acid sequence of SEQ ID NO: 21.

Furthermore, the sugar chain at position 297 in the EU numbering system (see sequences of proteins of immunological interest, NIH Publication No.91-3242) may be of any sugar-chain structure, or there may not be any sugar chain linked at this site (for example, constant regions produced in host cells where glycosylation does not occur, such as *E. coli*).

(i) Improvement of the stability of IgG2 constant region at acidic conditions

In an embodiment, the IgG2 constant region of the present invention comprising amino acid substitutions includes IgG2 constant regions in which Met at position 276 (position 397 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20 has been substituted with another amino acid. The type of amino acid after substitution is not particularly limited; however, substitution to Val is preferred. The antibody stability under acidic conditions can be improved by substituting Met at position 276 (position 397 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20 with another amino acid.

(ii) Improvement of the heterogeneity of IgG2 constant region

In an embodiment, the IgG2 constant region of the present invention comprising amino acid substitutions includes IgG2 constant regions in which Cys at position 14 (position 131 in the EU numbering system), Arg at position 16 (position 133 in the EU numbering system), and Cys at position 102 (position 219 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20 have been substituted with other amino acids. The type of amino acid after substitution is not particularly limited; however, substitutions of Ser for Cys at position 14

(position 131 in the EU numbering system), Lys for Arg at position 16 (position 133 in the EU numbering system), and Ser for Cys at position 102 (position 219 in the EU numbering system) (IgG2-SKSC) are preferred.

These substitutions can reduce the heterogeneity originated from the hinge region of IgG2. The IgG2 constant regions of the present invention comprising amino acid substitutions include IgG2 constant regions comprising at least one of the three types of amino acid substitutions described above; however, the IgG2 constant regions preferably comprise substitutions of Cys at position 14 and Cys at position 102 with other amino acids or all three types of the amino acid substitutions described above.

(iii) Impairment of the binding of IgG2 constant region to FcγR

In an embodiment, the present invention also provides IgG2 constant regions comprising an amino acid sequence in which Ala at position 209 (EU330), Pro at position 210 (EU331), and/or Thr at position 218 (EU339) of the amino acid sequence of SEQ ID NO: 20 have been substituted with Ser, Ser, and Ala, respectively. The substitutions for Ala at position 209 (EU330) and for Pro at position 210 (EU331) have already been reported to enable the impairment of the Fcγ receptor binding (Eur. J. Immunol. 1999 Aug;29(8):2613-24). From the perspective of immunogenicity risk, however, these alterations are not preferred because they result in generation of non-human derived peptides that can become T-cell epitopes. However, the Fcγ receptor binding of IgG2 can be reduced by substituting Ala for Thr at position 218 (EU339) at the same time, and the 9-12 amino acid peptides which can become T-cell epitopes are derived from human only.

The IgG2 constant regions of the present invention comprising amino acid substitutions comprise at least one of the three types of amino acid substitutions described above; however, the IgG2 constant regions preferably comprise all three types of the amino acid substitutions described above. In a preferred embodiment, the IgG2 constant regions of the present invention comprising amino acid substitutions include IgG2 constant regions comprising an amino acid sequence in which Ala at position 209 (EU330), Pro at position 210 (EU331), and Thr at position 218 (EU339) in the amino acid sequence of SEQ ID NO: 20 have been substituted with Ser, Ser, and Ala, respectively.

(iv) Improvement of the C-terminal heterogeneity of IgG2 constant region

The present invention provides IgG2 constant regions comprising an amino acid sequence in which Gly at position 325 (position 446 in the EU numbering system) and Lys at position 326 (position 447 in the EU numbering system) have been deleted in the amino acid sequence of SEQ ID NO: 20. The heterogeneity originated from the C terminus of antibody H chain can be reduced only when both of the amino acids are deleted.

(v) Improvement of the pharmacokinetics by altering IgG2 constant region

An embodiment of the IgG2 constant regions with amino acid substitutions of the present invention includes IgG2 constant regions in which His at position 147 (position 268 in the EU numbering system), Arg at position 234 (position 355 in the EU numbering system), and Gln at position 298 (position 419 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20 have been substituted with other amino acids. These amino acid substitutions enable to improve antibody pharmacokinetics. The type of amino acid after substitution is not particularly limited; however, substitutions of Gln for His at position 147 (position 268 in the EU numbering system), Gln for Arg at position 234 (position 355 in the EU numbering system), and Glu for Gln at position 298 (position 419 in the EU numbering system) are preferred. The IgG2 constant regions with amino acid substitutions of the present invention include IgG2 constant regions comprising at least one of the three types of the amino acid substitutions described above; however, the IgG2 constant regions preferably comprise all three types of the amino acid substitutions described above.

(vi) Improvement of the stability of IgG4 constant region at acidic conditions

The present invention provides IgG4 constant regions comprising an amino acid sequence in which Arg at position 289 (position 409 in the EU numbering system) of the amino acid sequence of SEQ ID NO: 21 has been substituted with another amino acid. The type of amino acid after substitution is not particularly limited; however, substitution to Lys is preferred. The antibody stability under acidic conditions can be improved by substituting Arg at position 289 (position 409 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 21 with another amino acid.

(vii) Improvement of the C-terminal heterogeneity of IgG4 constant region

The present invention provides IgG4 constant regions comprising an amino acid sequence in which Gly at position 326 (position 446 in the EU numbering system) and Lys at position 327 (position 447 in the EU numbering system) have been deleted in the amino acid sequence of SEQ ID NO: 21. The heterogeneity originated from the C terminus of antibody H chain can be reduced only when both of the amino acids are deleted.

(viii) Improvement of the C-terminal heterogeneity of IgG1 constant region

The present invention provides IgG1 constant regions comprising an amino acid sequence in which Gly at position 329 (position 446 in the EU numbering system) and Lys at position 330 (position 447 in the EU numbering system) have been deleted in the amino acid sequence of SEQ ID NO: 19. The heterogeneity originated from the C terminus of antibody H chain can be reduced only when both of the amino acids are deleted.

(ix)

The present invention provides IgG1 constant regions comprising an amino acid sequence in which Asn at position 317 (position 434 in the EU numbering system) in the amino

acid sequence of SEQ ID NO: 19 has been substituted with another amino acid.

The type of amino acid after substitution is not particularly limited; however, substitution to Ala is preferred.

(x)

The present invention provides IgG2 constant regions comprising an amino acid sequence in which Ala at position 209 (position 330 in the EU numbering system), Pro at position 210 (position 331 in the EU numbering system), Thr at position 218 (position 339 in the EU numbering system), Met at position 276 (position 397 in the EU numbering system), Cys at position 14 (position 131 in the EU numbering system), Arg at position 16 (position 133 in the EU numbering system), Cys at position 102 (position 219 in the EU numbering system), Glu at position 20 (position 137 in the EU numbering system), and Ser at position 21 (position 138 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20 have been substituted with other amino acids.

The type of amino acid after substitution is not particularly limited; however, substitutions of Ser for Ala at position 209, Ser for Pro at position 210, Ala for Thr at position 218, Val for Met at position 276, Ser for Cys at position 14, Lys for Arg at position 16, Ser for Cys at position 102, Gly for Glu at position 20, and Gly for Ser at position 21 are preferred.

(xi)

The present invention provides IgG2 constant regions comprising an amino acid sequence in which Ala at position 209 (position 330 in the EU numbering system), Pro at position 210 (position 331 in the EU numbering system), Thr at position 218 (position 339 in the EU numbering system), Met at position 276 (position 397 in the EU numbering system), Cys at position 14 (position 131 in the EU numbering system), Arg at position 16 (position 133 in the EU numbering system), Cys at position 102 (position 219 in the EU numbering system), Glu at position 20 (position 137 in the EU numbering system), and Ser at position 21 (position 138 in the EU numbering system) have been substituted with other amino acids, and simultaneously Gly at position 325 (position 446 in the EU numbering system) and Lys at position 326 (position 447 in the EU numbering system) have been deleted in the amino acid sequence of SEQ ID NO: 20.

The type of amino acid after substitution is not particularly limited; however, substitutions of Ser for Ala at position 209, Ser for Pro at position 210, Ala for Thr at position 218, Val for Met at position 276, Ser for Cys at position 14, Lys for Arg at position 16, Ser for Cys at position 102, Gly for Glu at position 20, and Gly for Ser at position 21 are preferred.

(xii)

The present invention provides IgG2 constant regions comprising an amino acid sequence in which Met at position 276 (position 397 in the EU numbering system), Cys at

position 14 (position 131 in the EU numbering system), Arg at position 16 (position 133 in the EU numbering system), Cys at position 102 (position 219 in the EU numbering system), Glu at position 20 (position 137 in the EU numbering system), and Ser at position 21 (position 138 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20 have been substituted with other amino acids.

The type of amino acid after substitution is not particularly limited; however, substitutions of Val for Met at position 276, Ser for Cys at position 14, Lys for Arg at position 16, Ser for Cys at position 102, Gly for Glu at position 20, and Gly for Ser at position 21 are preferred.

(xiii)

The present invention provides IgG2 constant regions comprising an amino acid sequence in which Met at position 276 (position 397 in the EU numbering system), Cys at position 14 (position 131 in the EU numbering system), Arg at position 16 (position 133 in the EU numbering system), Cys at position 102 (position 219 in the EU numbering system), Glu at position 20 (position 137 in the EU numbering system), and Ser at position 21 (position 138 in the EU numbering system) have been substituted with other amino acids, and simultaneously Gly at position 325 (position 446 in the EU numbering system) and Lys at position 326 (position 447 in the EU numbering system) have been deleted in the amino acid sequence of SEQ ID NO: 20.

The type of amino acid after substitution is not particularly limited; however, substitutions of Val for Met at position 276, Ser for Cys at position 14, Lys for Arg at position 16, Ser for Cys at position 102, Gly for Glu at position 20, and Gly for Ser at position 21 are preferred.

(xiv)

The present invention provides IgG2 constant regions comprising an amino acid sequence in which Cys at position 14 (position 131 in the EU numbering system), Arg at position 16 (position 133 in the EU numbering system), Cys at position 102 (position 219 in the EU numbering system), Glu at position 20 (position 137 in the EU numbering system), Ser at position 21 (position 138 in the EU numbering system), His at position 147 (position 268 in the EU numbering system), Arg at position 234 (position 355 in the EU numbering system), and Gln at position 298 (position 419 in the EU numbering system) have been substituted with other amino acids, and simultaneously Gly at position 325 (position 446 in the EU numbering system) and Lys at position 326 (position 447 in the EU numbering system) have been deleted in the amino acid sequence of SEQ ID NO: 20.

The type of amino acid after substitution is not particularly limited; however, substitutions of Ser for Cys at position 14, Lys for Arg at position 16, Ser for Cys at position 102,

Gly for Glu at position 20, Gly for Ser at position 21, Gln for His at position 147, Gln for Arg at position 234, and Glu for Gln at position 298 are preferred.

(xv)

The present invention provides IgG2 constant regions comprising an amino acid sequence in which Cys at position 14 (position 131 in the EU numbering system), Arg at position 16 (position 133 in the EU numbering system), Cys at position 102 (position 219 in the EU numbering system), Glu at position 20 (position 137 in the EU numbering system), Ser at position 21 (position 138 in the EU numbering system), His at position 147 (position 268 in the EU numbering system), Arg at position 234 (position 355 in the EU numbering system), Gln at position 298 (position 419 in the EU numbering system), and Asn at position 313 (position 434 in the EU numbering system) have been substituted with other amino acids, and simultaneously Gly at position 325 (position 446 in the EU numbering system) and Lys at position 326 (position 447 in the EU numbering system) have been deleted in the amino acid sequence of SEQ ID NO: 20.

The type of amino acid after substitution is not particularly limited; however, substitutions of Ser for Cys at position 14, Lys for Arg at position 16, Ser for Cys at position 102, Gly for Glu at position 20, Gly for Ser at position 21, Gln for His at position 147, Gln for Arg at position 234, Glu for Gln at position 298, and Ala for Asn at position 313 are preferred.

(xvi)

The present invention provides IgG4 constant regions comprising an amino acid sequence in which Arg at position 289 (position 409 in the EU numbering system), Cys at position 14, Arg at position 16, Glu at position 20, Ser at position 21, Arg at position 97, Ser at position 100, Tyr at position 102, Gly at position 103, Pro at position 104, and Pro at position 105 (positions 131, 133, 137, 138, 214, 217, 219, 220, 221, and 222 in the EU numbering system, respectively), Glu at position 113, Phe at position 114, and Leu at position 115 (positions 233, 234, and 235 in the EU numbering system, respectively) have been substituted with other amino acids, and simultaneously Gly at position 116 (position 236 in the EU numbering system) has been deleted in the amino acid sequence of SEQ ID NO: 21.

The type of amino acid after substitution is not particularly limited; however, substitutions of Ser for Cys at position 14 (position 131 in the EU numbering system), Lys for Arg at position 16 (position 133 in the EU numbering system), Gly for Glu at position 20 (position 137 in the EU numbering system), Gly for Ser at position 21 (position 138 in the EU numbering system), Thr for Arg at position 97 (position 214 in the EU numbering system), Arg for Ser at position 100 (position 217 in the EU numbering system), Ser for Tyr at position 102 (position 219 in the EU numbering system), Cys for Gly at position 103 (position 220 in the EU numbering system), Val for Pro at position 104 (position 221 in the EU numbering system), Glu

for Pro at position 105 (position 222 in the EU numbering system), Pro for Glu at position 113 (position 233 in the EU numbering system), Val for Phe at position 114 (position 234 in the EU numbering system), Ala for Leu at position 115 (position 235 in the EU numbering system), and Lys for Arg at position 289 (position 409 in the EU numbering system) are preferred.

(xvii)

The present invention provides IgG4 constant regions comprising an amino acid sequence in which Arg at position 289 (position 409 in the EU numbering system), Cys at position 14, Arg at position 16, Glu at position 20, Ser at position 21, Arg at position 97, Ser at position 100, Tyr at position 102, Gly at position 103, Pro at position 104, and Pro at position 105 (positions 131, 133, 137, 138, 214, 217, 219, 220, 221, and 222 in the EU numbering system, respectively), Glu at position 113, Phe at position 114, and Leu at position 115 (positions 233, 234, and 235 in the EU numbering system, respectively) have been substituted with other amino acids, and simultaneously Gly at position 116 (position 236 in the EU numbering system), Gly at position 326 (position 446 in the EU numbering system), and Lys at position 327 (position 447 in the EU numbering system) have been deleted in the amino acid sequence of SEQ ID NO: 21.

The type of amino acid after substitution is not particularly limited; however, substitutions of Ser for Cys at position 14 (position 131 in the EU numbering system), Lys for Arg at position 16 (position 133 in the EU numbering system), Gly for Glu at position 20 (position 137 in the EU numbering system), Gly for Ser at position 21 (position 138 in the EU numbering system), Thr for Arg at position 97 (position 214 in the EU numbering system), Arg for Ser at position 100 (position 217 in the EU numbering system), Ser for Tyr at position 102 (position 219 in the EU numbering system), Cys for Gly at position 103 (position 220 in the EU numbering system), Val for Pro at position 104 (position 221 in the EU numbering system), Glu for Pro at position 105 (position 222 in the EU numbering system), Pro for Glu at position 113 (position 233 in the EU numbering system), Val for Phe at position 114 (position 234 in the EU numbering system), Ala for Leu at position 115 (position 235 in the EU numbering system), and Lys for Arg at position 289 (position 409 in the EU numbering system) are preferred.

(xviii)

The present invention provides IgG1 constant regions comprising an amino acid sequence in which Asn at position 317 (position 434 in the EU numbering system) has been substituted with another amino acid, and simultaneously Gly at position 329 (position 446 in the EU numbering system) and Lys at position 330 (position 447 in the EU numbering system) have been deleted in the amino acid sequence of SEQ ID NO: 19.

The type of amino acid after substitution of Asn at position 317 (position 434 in the EU numbering system) is not particularly limited; however, substitution to Ala is preferred.

(xix)

Below is a preferred embodiment of IgG2 of the present invention, which has reduced heterogeneity in the hinge region and/or reduced Fcγ receptor-binding activity.

Antibodies comprising an IgG2 constant region comprising an amino acid sequence in which Ala at position 209, Pro at position 210, Thr at position 218, Cys at position 14, Arg at position 16, Cys at position 102, Glu at position 20, and Ser at position 21 in the amino acid sequence of SEQ ID NO: 20 have been substituted with other amino acids.

The type of amino acid after substitution is not particularly limited; however, substitutions of Ser for Ala at position 209 (position 330 in the EU numbering system), Ser for Pro at position 210 (position 331 in the EU numbering system), Ala for Thr at position 218 (position 339 in the EU numbering system), Ser for Cys at position 14 (position 131 in the EU numbering system), Lys for Arg at position 16 (position 133 in the EU numbering system), Ser for Cys at position 102 (position 219 in the EU numbering system), Gly for Glu at position 20 (position 137 in the EU numbering system), and Gly for Ser at position 21 (position 138 in the EU numbering system) are preferred.

Such IgG2 constant regions include, for example, IgG2 constant regions comprising the amino acid sequence of SEQ ID NO: 191 (M86).

In another preferred embodiment, IgG2 constant regions of the present invention include IgG2 constant regions resulting from the deletion of Gly at position 325 and Lys at position 326 in the above-described IgG2 constant regions to reduce C-terminal heterogeneity. Such antibodies include, for example, IgG2 that comprises a constant region comprising the amino acid sequence of SEQ ID NO: 192 (M86ΔGK).

(xx)

Below is another preferred embodiment of the IgG2 constant regions of the present invention, which have reduced heterogeneity in the hinge region.

IgG2 constant regions comprising an amino acid sequence in which Cys at position 14, Arg at position 16, Cys at position 102, Glu at position 20, and Ser at position 21 in the amino acid sequence of SEQ ID NO: 20 have been substituted with other amino acids.

The type of amino acid after substitution is not particularly limited; however, substitutions of Ser for Cys at position 14 (position 131 in the EU numbering system), Lys for Arg at position 16 (position 133 in the EU numbering system), Ser for Cys at position 102 (position 219 in the EU numbering system), Gly for Glu at position 20 (position 137 in the EU numbering system), and Gly for Ser at position 21 (position 138 in the EU numbering system) are preferred.

Such IgG2 constant regions include, for example, IgG2 constant regions comprising the amino acid sequence of SEQ ID NO: 193 (M40).

In another preferred embodiment, the IgG2 constant regions of the present invention

include IgG2 constant regions further comprising the deletion of Gly at position 325 and Lys at position 326 in the above-described IgG2 constant regions. Such antibodies include, for example, IgG2 constant regions comprising the amino acid sequence of SEQ ID NO: 194 (M40ΔGK).

(xxi) M14ΔGK, M17ΔGK, M11ΔGK, M31ΔGK, M58, M73, M83, M86ΔGK, and M40ΔGK

The present invention also provides an antibody constant region comprising the amino acid sequence of SEQ ID NO: 24 (M14ΔGK). The present invention also provides an antibody constant region comprising the amino acid sequence of SEQ ID NO: 116 (M17ΔGK). The present invention also provides an antibody constant region comprising the amino acid sequence of SEQ ID NO: 25 (M11ΔGK). The present invention further provides an antibody constant region comprising the amino acid sequence of SEQ ID NO: 118 (M31ΔGK). The present invention further provides an antibody constant region comprising the amino acid sequence of SEQ ID NO: 151 (M58). The present invention further provides an antibody constant region comprising the amino acid sequence of SEQ ID NO: 153 (M73). The present invention also provides an antibody constant region comprising the amino acid sequence of SEQ ID NO: 164 (M83). The present invention further provides an antibody constant region comprising the amino acid sequence of SEQ ID NO: 192 (M86ΔGK). The present invention further provides an antibody constant region comprising the amino acid sequence of SEQ ID NO: 194 (M40ΔGK). These antibody constant regions have been optimized to have reduced Fcγ receptor binding activity, reduced immunogenicity risk, improved stability under acidic conditions, reduced heterogeneity, improved pharmacokinetics, and/or higher stability in preparations in comparison with the IgG1 constant region.

The present invention provides antibodies comprising the antibody constant region of any one of (i) to (xxi) described above. There is no limitation on the type of antigen and origin of antibody, as long as the antibodies comprise an antibody constant region described above. The preferred antibodies include, for example, antibodies that bind to IL-6 receptor. Alternatively, the preferred antibodies include, for example, humanized antibodies. Such antibodies include, for example, antibodies comprising the variable region of humanized PM-1 antibody. Such a variable region of humanized PM-1 antibody may comprise any of the above-described amino acid substitutions, or other amino acid substitutions, deletions, additions, and/or insertions. Specifically, the substitutions include, for example, alterations that improve the affinity of (a) to (y) described above; alterations that lower the isoelectric point of (i) to (viii) described above, alternations that improve the stability of (α) to (ζ) described below; and alternations that reduce immunogenicity, but are not limited thereto.

In one embodiment, such antibodies include antibodies that comprise a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 113 (PF_1+M14ΔGK) and

a light chain variable region comprising the amino acid sequence of SEQ ID NO: 23 (PF1_L) (the light chain constant region may be kappa or lambda, or an altered form thereof) (PF1), but are not limited thereto.

Alternatively, the antibody constant regions described above and/or antibody molecules comprising an antibody constant region described above can be linked as a form of Fc fusion molecule to antibody-like binding molecule (scaffold molecules), bioactive peptides, binding peptides, or such.

The antibodies of the present invention can also be obtained by, for example, the following methods in addition to those described in the Examples. In one embodiment to obtain antibodies of the present invention, one or more amino acid residues are first substituted with amino acids of interest in at least one region selected from the group consisting of CDR, FR, and constant regions of an anti-IL-6 receptor antibody known to those skilled in the art. Methods for obtaining anti-IL-6 receptor antibodies known to those skilled in the art are not limited. Methods for substituting one or more amino acid residues with amino acids of interest in at least one region selected from the group consisting of the CDR, FR, and constant regions include, for example, site-directed mutagenesis (Hashimoto-Gotoh, T., Mizuno, T., Ogasahara, Y., and Nakagawa, M. An oligodeoxyribonucleotide-directed dual amber method for site-directed mutagenesis. Gene (1995) 152, 271-275; Zoller, M. J., and Smith, M. Oligonucleotide-directed mutagenesis of DNA fragments cloned into M13 vectors. Methods Enzymol. (1983) 100, 468-500; Kramer, W., Drutsa, V., Jansen, H. W., Kramer, B., Pflugfelder, M., and Fritz, H. J. The gapped duplex DNA approach to oligonucleotide-directed mutation construction. Nucleic Acids Res. (1984) 12, 9441-9456; Kramer W., and Fritz H. J. Oligonucleotide-directed construction of mutations via gapped duplex DNA Methods. Enzymol. (1987) 154, 350-367; Kunkel, T. A. Rapid and efficient site-specific mutagenesis without phenotypic selection. Proc. Natl. Acad. Sci. USA (1985) 82, 488-492). These methods can be used to substitute target amino acids in antibodies with amino acids of interest. Methods for substituting amino acids include library technologies such as framework shuffling (Mol. Immunol. 2007 Apr;44(11): 3049-60) and CDR repair (US2006/0122377). Using these methods, amino acids can be substituted into appropriate frameworks and CDRs.

In another embodiment to obtain antibodies, an antibody that binds to IL-6 receptor is first prepared by methods known to those skilled in the art. When the prepared antibody is derived from a nonhuman animal, it can be humanized. Then, the prepared antibody is tested to assess whether it has neutralizing activity by using methods known to those skilled in the art. The binding activity and neutralizing activity of antibodies can be determined, for example, by the methods described in the Examples; however, such methods are not limited thereto. Next, one or more amino acid residues in at least one selected from the group consisting of CDR, FR,

and constant regions of antibody are substituted with amino acids of interest.

More specifically, the present invention relates to methods for producing antibodies with improved neutralizing activity, binding activity, or stability, or reduced immunogenicity, which comprise the steps of:

(a) expressing a DNA encoding an H chain in which one or more amino acid residues in at least one region selected from the group consisting of CDR, FR, and constant regions are substituted with amino acids of interest, and a DNA encoding an L chain in which one or more amino acid residues in at least one region selected from the group consisting of CDR and FR regions are substituted with amino acids of interest; and

(b) collecting the expression products of step (a).

The first step of the production methods of the present invention is expressing a DNA encoding a mutant anti-IL-6 receptor antibody H chain in which one or more amino acid residues in at least one region selected from the group consisting of CDR, FR, and constant regions are substituted with amino acids of interest, and a DNA encoding an anti-IL-6 receptor antibody L chain in which one or more amino acid residues in at least one region selected from the group consisting of CDR and FR regions are substituted with amino acids of interest. A DNA encoding an H chain in which one or more amino acid residues in at least one region selected from the group consisting of CDR, FR, and constant regions are substituted with amino acids of interest can be prepared, for example, by obtaining a DNA encoding the CDR, FR, or constant region of a wild type H chain, and introducing an appropriate substitution so that a codon encoding a particular amino acid in at least one selected from the group consisting of the CDR, FR, and constant regions encodes an amino acid of interest. Furthermore, a DNA encoding an L chain in which one or more amino acid residues in at least one selected from the group consisting of CDR and FR regions are substituted with amino acids of interest can be prepared, for example, by obtaining a DNA encoding the CDR and/or FR regions of a wild type L chain and introducing an appropriate substitution so that a codon encoding a particular amino acid in the CDR and/or FR regions encodes an amino acid of interest.

Alternatively, a DNA encoding an H chain in which one or more amino acid residues in at least one selected from the group consisting of CDR, FR, and constant regions are substituted with amino acids of interest can also be prepared by designing and then chemically synthesizing a DNA encoding a protein in which one or more amino acid residues in at least one selected from the group consisting of CDR, FR, and constant regions of the wild type H chain are substituted with amino acids of interest. Furthermore, a DNA encoding an L chain in which one or more amino acid residues in the CDR and/or FR regions are substituted with amino acids of interest can also be prepared by designing and then chemically synthesizing a DNA encoding a protein in which one or more amino acid residues in the CDR and/or FR regions of a wild type

L chain are substituted with amino acids of interest.

The type of amino acid substitution includes the substitutions described herein, but is not limited thereto.

Alternatively, a DNA encoding an H chain in which one or more amino acid residues in at least one region selected from the group consisting of CDR, FR, and constant regions are substituted with amino acids of interest can also be prepared as a combination of partial DNAs. Such combinations of partial DNAs include, for example, the combination of a DNA encoding a variable region and a DNA encoding a constant region, and the combination of a DNA encoding an Fab region and a DNA encoding an Fc region, but are not limited thereto. A DNA encoding an L chain can also be prepared as a combination of partial DNAs.

Methods for expressing the above-described DNAs include the methods described below. For example, an H chain expression vector is constructed by inserting a DNA encoding an H chain variable region into an expression vector along with a DNA encoding an H chain constant region. Likewise, an L chain expression vector is constructed by inserting a DNA encoding an L chain variable region into an expression vector along with a DNA encoding an L chain constant region. Alternatively, these H and L chain genes may be inserted into a single vector. Expression vectors include, for example, SV40 virus-based vectors, EB virus-based vectors, and BPV (papilloma virus)-based vectors, but are not limited thereto.

Host cells are co-transformed with an antibody expression vector constructed by the methods described above. Such host cells include the above-described cells such as CHO (Chinese hamster ovary) cells as well as microorganisms such as *E. coli*, yeast, and *Bacillus subtilis*, and plants and animals (Nature Biotechnology (2007) 25, 563-565; Nature Biotechnology (1998) 16, 773-777; Biochemical and Biophysical Research Communications (1999) 255, 444-450; Nature Biotechnology (2005) 23, 1159-1169; Journal of Virology (2001) 75, 2803-2809; Biochemical and Biophysical Research Communications (2003) 308, 94-100). The transformation can be preferably achieved by using electroporation, the lipofectin method (R. W. Malone *et al.*, Proc. Natl. Acad. Sci. USA (1989) 86, 6077; P. L. Felgner *et al.*, Proc. Natl. Acad. Sci. USA (1987) 84, 7413), calcium phosphate method (F. L. Graham & A. J. van der Eb, Virology (1973) 52, 456-467), DEAE-Dextran method, and the like.

In the next step of antibody production, the expression products obtained in step (a) are collected. The expression products can be collected, for example, by culturing the transformants and then separating the products from the transformed cells or culture media. Separation and purification of antibodies can be achieved by an appropriate combination of methods such as centrifugation, ammonium sulfate fractionation, salting out, ultrafiltration, columns of 1q, FcRn, Protein A, and Protein G, affinity chromatography, ion exchange chromatography, and gel filtration chromatography.

Those skilled in the art can appropriately prepare the constant regions of the present invention according to the methods for preparing antibodies.

The present invention further relates to methods for enhancing the activity of an anti-IL-6 receptor antibody to bind or neutralize an IL-6 receptor, which comprise at least one step selected from the group consisting of:

(A) substituting Ser at position 1 in the amino acid sequence of SEQ ID NO: 1 (HCDR1) with another amino acid;

(B) substituting Trp at position 5 in the amino acid sequence of SEQ ID NO: 1 (HCDR1) with another amino acid;

(C) substituting Tyr at position 1 in the amino acid sequence of SEQ ID NO: 2 (HCDR2) with another amino acid;

(D) substituting Thr at position 8 in the amino acid sequence of SEQ ID NO: 2 (HCDR2) with another amino acid;

(E) substituting Thr at position 9 in the amino acid sequence of SEQ ID NO: 2 (HCDR2) with another amino acid;

(F) substituting Ser at position 1 in the amino acid sequence of SEQ ID NO: 3 (HCDR3) with another amino acid;

(G) substituting Leu at position 2 in the amino acid sequence of SEQ ID NO: 3 (HCDR3) with another amino acid;

(H) substituting Thr at position 5 in the amino acid sequence of SEQ ID NO: 3 (HCDR3) with another amino acid;

(I) substituting Ala at position 7 in the amino acid sequence of SEQ ID NO: 3 (HCDR3) with another amino acid;

(J) substituting Met at position 8 in the amino acid sequence of SEQ ID NO: 3 (HCDR3) with another amino acid;

(K) substituting Ser at position 1 and Thr at position 5 in the amino acid sequence of SEQ ID NO: 3 (HCDR3) with other amino acids;

(L) substituting Leu at position 2, Ala at position 7, and Met at position 8 in the amino acid sequence of SEQ ID NO: 3 (HCDR3) with other amino acids;

(M) substituting Arg at position 1 in the amino acid sequence of SEQ ID NO: 4 (LCDR1) with another amino acid;

(N) substituting Gln at position 4 in the amino acid sequence of SEQ ID NO: 4 (LCDR1) with another amino acid;

(O) substituting Tyr at position 9 in the amino acid sequence of SEQ ID NO: 4 (LCDR1) with another amino acid;

(P) substituting Asn at position 11 in the amino acid sequence of SEQ ID NO: 4 (LCDR1) with

another amino acid;

(Q) substituting Thr at position 2 in the amino acid sequence of SEQ ID NO: 5 (LCDR2) with another amino acid;

(R) substituting Gln at position 1 in the amino acid sequence of SEQ ID NO: 6 (LCDR3) with another amino acid;

(S) substituting Gly at position 3 in the amino acid sequence of SEQ ID NO: 6 (LCDR3) with another amino acid;

(T) substituting Tyr at position 9 in the amino acid sequence of SEQ ID NO: 4 (LCDR1) and Gly at position 3 in the amino acid sequence of SEQ ID NO: 6 (LCDR3) with other amino acids;

(U) substituting Thr at position 5 in the amino acid sequence of SEQ ID NO: 6 (LCDR3) with another amino acid;

(V) substituting Gln at position 1 and Thr at position 5 in the amino acid sequence of SEQ ID NO: 6 (LCDR3) with other amino acids; and

(W) substituting Thr at position 9 in the amino acid sequence of SEQ ID NO: 2 (HCDR2), and Ser at position 1 and Thr at position 5 in the amino acid sequence of SEQ ID NO: 3 (HCDR3) with other amino acids; or

(X) a step comprising (V) and (W).

In (A) described above, the type of amino acid after substitution is not particularly limited as long as the affinity is improved; however, substitution to Trp, Thr, Asp, Asn, Arg, Val, Phe, Ala, Gln, Tyr, Leu, His, Glu, or Cys is preferred.

In (B) described above, the type of amino acid after substitution is not particularly limited as long as the affinity is improved; however, substitution to Ile or Val is preferred.

In (C) described above, the type of amino acid after substitution is not particularly limited as long as the affinity is improved; however, substitution to Phe is preferred.

In (D) described above, the type of amino acid after substitution is not particularly limited as long as the affinity is improved; however, substitution to Arg is preferred.

In (E) described above, the type of amino acid after substitution is not particularly limited as long as the affinity is improved; however, substitution to Ser or Asn is preferred.

In (F) described above, the type of amino acid after substitution is not particularly limited as long as the affinity is improved; however, substitution to Ile, Val, Thr, or Leu is preferred.

In (G) described above, the type of amino acid after substitution is not particularly limited as long as the affinity is improved; however, substitution to Thr is preferred.

In (H) described above, the type of amino acid after substitution is not particularly limited as long as the affinity is improved; however, substitution to Ala, Ile, or Ser is preferred. Other preferred substitutions include substitution of Ser for Thr at position 5.

In (I) described above, the type of amino acid after substitution is not particularly limited as long as the affinity is improved; however, substitution to Ser or Val is preferred.

In (J) described above, the type of amino acid after substitution is not particularly limited as long as the affinity is improved; however, substitution to Leu is preferred.

In (K) described above, the type of amino acid after substitution is not particularly limited as long as the affinity is improved; however, substitutions of Leu for Ser at position 1 and Ala for Thr at position 5 are preferred. Other preferred substitutions include those of Val for Ser at position 1 and Ala for Thr at position 5; Ile for Ser at position 1 and Ala for Thr at position 5; Thr for Ser at position 1 and Ala for Thr at position 5; Val for Ser at position 1 and Ile for Thr at position 5; Ile for Ser at position 1 and Ile for Thr at position 5; Thr for Ser at position 1 and Ile for Thr at position 5; and Leu for Ser at position 1 and Ile for Thr at position 5.

In (L) described above, the type of amino acid after substitution is not particularly limited as long as the affinity is improved; however, substitution of Thr for Leu at position 2, Val for Ala at position 7, and Leu for Met at position 8 are preferred.

In (M) described above, the type of amino acid after substitution is not particularly limited as long as the affinity is improved; however, substitution to Phe is preferred.

In (N) described above, the type of amino acid after substitution is not particularly limited as long as the affinity is improved; however, substitution to Arg or Thr is preferred.

In (O) described above, the type of amino acid after substitution is not particularly limited as long as the affinity is improved; however, substitution to Phe is preferred.

In (P) described above, the type of amino acid after substitution is not particularly limited as long as the affinity is improved; however, substitution to Ser is preferred.

In (Q) described above, the type of amino acid after substitution is not particularly limited as long as the affinity is improved; however, substitution to Gly is preferred.

In (R) described above, the type of amino acid after substitution is not particularly limited as long as the affinity is improved; however, substitution to Gly, Asn, or Ser is preferred.

In (S) described above, the type of amino acid after substitution is not particularly limited as long as the affinity is improved; however, substitution to Ser is preferred.

In (T) described above, the type of amino acid after substitution is not particularly limited as long as the affinity is improved; however, substitutions of Phe for Tyr in the amino acid sequence of SEQ ID NO: 4 (LCDR1) and Ser for Gly in the amino acid sequence of SEQ ID NO: 6 (LCDR3) are preferred.

In (U) described above, the type of amino acid after substitution is not particularly limited as long as the affinity is improved; however, substitution to Arg or Ser is preferred.

In (V) described above, the type of amino acid after substitution is not particularly limited as long as the affinity is improved; however, substitutions of Gly for Gln at position 1

and Ser for Thr at position 5 are preferred. Other preferred substitutions include those of Gly for Gln at position 1 and Arg for Thr at position 5.

In (W) described above, substitution of Asn for Thr at position 9 in the amino acid sequence of SEQ ID NO: 2 (HCDR2) is preferred. The preferred combinations of amino acids after substitution for Ser at position 1 and Thr at position 5 in the amino acid sequence of SEQ ID NO: 3 (HCDR3) include Leu and Ala, Val and Ala, Ile and Ala, Thr and Ala, Val and Ile, Ile and Ile, Thr and Ile, and Leu and Ile.

In the steps of (A) to (X) above, the method for amino acid substitution is not particularly limited. The substitution can be achieved, for example, by site-directed mutagenesis described above or a method described in the Examples. When an amino acid is substituted in a heavy chain variable region, the original amino acid sequence of the heavy chain variable region before substitution is preferably an amino acid sequence of the heavy chain variable region of a humanized PM-1 antibody. Alternatively, when an amino acid is substituted in a light chain variable region, the original amino acid sequence of the light chain variable region before substitution is preferably an amino acid sequence of the light chain variable region of a humanized PM-1 antibody. Furthermore, it is preferable to introduce the amino acid substitutions of steps (A) to (X) described above into the humanized PM-1 antibody.

The methods of the present invention for enhancing the binding or neutralizing activity of an anti-IL-6 receptor antibody comprise at least any one of the steps of (A) to (X) described above. Specifically, the methods of the present invention may comprise two or more of the steps of (A) to (X) described above. Furthermore, the methods of the present invention may comprise other steps (for example, amino acid substitutions, deletions, additions and/or insertions other than those of (A) to (X) described above) as long as they comprise any one of the steps of (A) to (X) described above. Furthermore, for example, FR may comprise amino acid substitutions, deletions, additions and/or insertions, and the constant region may comprise amino acid substitutions, deletions, additions and/or insertions. It is preferable to introduce the amino acid substitutions described above into the humanized PM-1 antibody.

<Methods for reducing the immunogenicity risk of an anti-IL-6 receptor antibody>

The present invention also relates to methods for reducing the immunogenicity of an anti-IL-6 receptor antibody, which comprise the step of substituting Gly for Thr at position 2 in the amino acid sequence of SEQ ID NO: 5 (LCDR2). The methods of the present invention for reducing the immunogenicity of an anti-IL-6 receptor antibody may comprise other steps of amino acid substitution, as long as they comprise the step of substituting Gly for Thr at position 2 in the amino acid sequence of SEQ ID NO: 5 (LCDR2). The method for amino acid substitution is not particularly limited. The substitution can be achieved, for example, by site-directed mutagenesis described above or a method described in the Examples.

It is preferable to introduce the amino acid substitutions described above into the humanized PM-1 antibody or a variant thereof comprising substitutions, deletions, and/or insertions.

<Methods for lowering the isoelectric point of an anti-IL-6 receptor antibody>

The present invention also relates to methods for lowering the isoelectric point of an anti-IL-6 receptor antibody, which comprise at least one step selected from the group consisting of:

(i) substituting Gln at position 16 in the amino acid sequence of SEQ ID NO: 7 (HFR1) with another amino acid;

(ii) substituting Arg at position 8 in the amino acid sequence of SEQ ID NO: 8 (HFR2) with another amino acid;

(iii) substituting Arg at position 16 in the amino acid sequence of SEQ ID NO: 9 (HFR3) with another amino acid;

(iv) substituting Gln at position 3 in the amino acid sequence of SEQ ID NO: 10 (HFR4) with another amino acid;

(v) substituting Arg at position 18 in the amino acid sequence of SEQ ID NO: 11 (LFR1) with another amino acid;

(vi) substituting Lys at position 11 in the amino acid sequence of SEQ ID NO: 12 (LFR2) with another amino acid;

(vii) substituting Gln at position 23 in the amino acid sequence of SEQ ID NO: 13 (LFR3) with another amino acid;

(viii) substituting Lys at position 10 in the amino acid sequence of SEQ ID NO: 14 (LFR4) with another amino acid;

(ix) substituting Ser at position 1 in the amino acid sequence of SEQ ID NO: 1 (HCDR1) with another amino acid;

(x) substituting Arg at position 1 in the amino acid sequence of SEQ ID NO: 4 (LCDR1) with another amino acid;

(xi) substituting Arg at position 4 in the amino acid sequence of SEQ ID NO: 5 (LCDR2) with another amino acid;

(xii) substituting Arg at position 13 in the amino acid sequence of SEQ ID NO: 7 (HFR1) with another amino acid;

(xiii) substituting Lys at position 15 and/or Ser at position 16 in the amino acid sequence of SEQ ID NO: 2 (HFR1) or 100 with other amino acids;

(xiv) substituting Gln at position 4 in the amino acid sequence of SEQ ID NO: 4 (LCDR1) or 101 with another amino acid; and

(xv) substituting His at position 6 in the amino acid sequence of SEQ ID NO: 5 (LCDR2) or 103

with another amino acid.

In (i) described above, the type of amino acid after substitution is not particularly limited as long as the isoelectric point is lowered; however, substitution to Glu is preferred.

In (ii) described above, the type of amino acid after substitution is not particularly limited as long as the isoelectric point is lowered; however, substitution to Glu is preferred.

In (iii) described above, the type of amino acid after substitution is not particularly limited as long as the isoelectric point is lowered; however, substitution to Lys is preferred.

In (iv) described above, the type of amino acid after substitution is not particularly limited as long as the isoelectric point is lowered; however, substitution to Glu is preferred.

In (v) described above, the type of amino acid after substitution is not particularly limited as long as the isoelectric point is lowered; however, substitution to Ser is preferred.

In (vi) described above, the type of amino acid after substitution is not particularly limited as long as the isoelectric point is lowered; however, substitution to Glu is preferred.

In (vii) described above, the type of amino acid after substitution is not particularly limited as long as the isoelectric point is lowered; however, substitution to Glu is preferred.

In (viii) described above, the type of amino acid after substitution is not particularly limited as long as the isoelectric point is lowered; however, substitution to Glu is preferred.

In (ix) described above, the type of amino acid after substitution is not particularly limited as long as the isoelectric point is lowered; however, substitution to Asp is preferred.

In (x) described above, the type of amino acid after substitution is not particularly limited as long as the isoelectric point is lowered; however, substitution to Gln is preferred.

In (xi) described above, the type of amino acid after substitution is not particularly limited as long as the isoelectric point is lowered; however, substitution to Glu is preferred.

In (xii) described above, the type of amino acid after substitution is not particularly limited as long as the isoelectric point is lowered; however, substitution to Lys is preferred.

In (xiii) described above, the type of amino acid after substitution is not particularly limited as long as the isoelectric point is lowered; however, substitutions to Gln for Lys at position 15 and Asp for Ser at position 16 are preferred.

In (xiv) described above, the type of amino acid after substitution is not particularly limited as long as the isoelectric point is lowered; however, substitution to Glu is preferred.

In (xv) described above, the type of amino acid after substitution is not particularly limited as long as the isoelectric point is lowered; however, substitution to Glu is preferred.

In the steps of (i) to (xv) described above, the method for amino acid substitution is not particularly limited. The substitution can be achieved, for example, by site-directed mutagenesis described above or a method described in the Examples. When an amino acid is substituted in a heavy chain variable region, the original amino acid sequence of the heavy chain

variable region before substitution is preferably an amino acid sequence of the heavy chain variable region of a humanized PM-1 antibody. Alternatively, when an amino acid is substituted in a light chain variable region, the original amino acid sequence of the light chain variable region before substitution is preferably an amino acid sequence of the light chain variable region of a humanized PM-1 antibody. Furthermore, it is preferable to introduce the amino acid substitutions of the steps of (i) to (xv) described above into the humanized PM-1 antibody.

The methods of the present invention for lowering the isoelectric point of an anti-IL-6 receptor antibody comprise at least any one of the steps of (i) to (xv) described above. Specifically, the methods of the present invention may comprise two or more of the steps of (i) to (xv) described above. Furthermore, the methods of the present invention may comprise other steps (for example, amino acid substitutions, deletions, additions and/or insertions other than those of (i) to (xv) described above) as long as they comprise any one of the steps of (i) to (xv) described above. Furthermore, for example, the constant region may comprise amino acid substitutions, deletions, additions and/or insertions.

<Methods for improving the stability of an anti-IL-6 receptor antibody>

The present invention also relates to methods for increasing the stability of an anti-IL-6 receptor antibody, which comprise at least one step selected from the group consisting of:

(α) substituting Met at position 4 in the amino acid sequence of SEQ ID NO: 9 (HFR3) with another amino acid;

(β) substituting Leu at position 5 in the amino acid sequence of SEQ ID NO: 9 (HFR3) with another amino acid;

(γ) substituting Thr at position 9 in the amino acid sequence of SEQ ID NO: 2 (HCDR2) with another amino acid;

(δ) substituting Thr at position 5 in the amino acid sequence of SEQ ID NO: 6 (LCDR3) with another amino acid;

(ε) substituting Ser at position 16 in the amino acid sequence of SEQ ID NO: 2 (HCDR2) with another amino acid; and

(ζ) substituting Ser at position 5 in the amino acid sequence of SEQ ID NO: 10 (FR4) with another amino acid.

In (α) described above, the type of amino acid after substitution is not particularly limited as long as the stability is improved; however, substitution to Ile is preferred.

In (β) described above, the type of amino acid after substitution is not particularly limited as long as the stability is improved; however, substitution to Ser is preferred.

In (γ) described above, the type of amino acid after substitution is not particularly limited as long as the stability is improved; however, substitution to Asn is preferred.

In (δ) described above, the type of amino acid after substitution is not particularly limited as long as the stability is improved; however, substitution to Ser is preferred.

In (ε) described above, the type of amino acid after substitution is not particularly limited as long as the stability is improved; however, substitution to Gly is preferred.

In (ζ) described above, the type of amino acid after substitution is not particularly limited as long as the stability is improved; however, substitution to Ile is preferred.

In the steps of (α) to (ζ) described above, the method for amino acid substitution is not particularly limited. The substitution can be achieved, for example, by site-directed mutagenesis described above or a method described in the Examples. When an amino acid is substituted in a heavy chain variable region, the original amino acid sequence of the heavy chain variable region before substitution is preferably an amino acid sequence of the heavy chain variable region of a humanized PM-1 antibody. Alternatively, when an amino acid is substituted in a light chain variable region, the original amino acid sequence of the light chain variable region before substitution is preferably an amino acid sequence of the light chain variable region of a humanized PM-1 antibody. Furthermore, it is preferable to introduce the amino acid substitutions of (α) to (ζ) described above into the humanized PM-1 antibody.

The methods of the present invention for improving the stability of an anti-IL-6 receptor antibody comprise at least any one of the steps of (α) to (ζ) described above. Specifically, the methods of the present invention may comprise two or more of the steps of (α) to (ζ) described above. Furthermore, the methods of the present invention may comprise other steps (for example, amino acid substitutions, deletions, additions and/or insertions other than those of (α) to (ζ) described above) as long as they comprise any one of the steps of (α) to (ζ) described above. Furthermore, for example, the constant region may comprise amino acid substitutions, deletions, additions and/or insertions.

<Methods for reducing the immunogenicity of an anti-IL-6 receptor antibody>

The present invention also relates to methods for reducing the immunogenicity of an anti-IL-6 receptor antibody, in particular, a humanized PM-1 antibody, which comprise the step of substituting Lys for Arg at position 13, Glu for Gln at position 16, Ala for Thr at position 23, and/or Ser for Thr at position 30 in the amino acid sequence of SEQ ID NO: 7 (HFR1). The methods of the present invention for reducing the immunogenicity of an anti-IL-6 receptor antibody may comprise other steps of amino acid substitution, as long as they comprise the step of substituting Ser for Thr at position 30 in the amino acid sequence of SEQ ID NO: 7 (HFR1).

The present invention further relates to methods for reducing the immunogenicity of an anti-IL-6 receptor antibody, in particular, a humanized PM-1 antibody, which comprise the step of substituting Val for Ala at position 27 in the amino acid sequence of SEQ ID NO: 90 (HFR3). The methods of the present invention for reducing the immunogenicity of an anti-IL-6 receptor

antibody may comprise other steps of amino acid substitution, as long as they comprise the step of substituting Val for Ala at position 27 in the amino acid sequence of SEQ ID NO: 90 (HFR3).

The method for amino acid substitution is not particularly limited. The substitution can be achieved, for example, by site-directed mutagenesis described above or a method described in the Examples.

The present invention further relates to methods for reducing antibody immunogenicity, which comprise converting the FR3 of an anti-IL-6 receptor antibody, in particular, a humanized PM-1 antibody, H53/L28, or PF1 antibody, into an FR3 comprising the amino acid sequence of SEQ ID NO: 128 or 129.

<Methods for improving antibody stability under acidic conditions>

The present invention also relates to methods for improving antibody stability under acidic conditions, which comprise the step of substituting Met at position 276 (position 397 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20 (IgG2) with another amino acid. The methods of the present invention for improving antibody stability under acidic conditions may comprise other steps of amino acid substitution, as long as they comprise the step of substituting Met at position 276 (position 397 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20 (IgG2) with another amino acid. The type of amino acid after substitution is not particularly limited; however, substitution to Val is preferred. The method for amino acid substitution is not particularly limited. The substitution can be achieved, for example, by site-directed mutagenesis described above or a method described in the Examples.

The type of target antibody is not particularly limited; however, the antibody is preferably an anti-human IL-6 receptor antibody, more preferably a humanized PM-1 antibody or a variant thereof comprising substitutions, deletions, and/or insertions.

<Methods for reducing the heterogeneity originated from the hinge region of IgG2 constant region>

The present invention also relates to methods for reducing antibody heterogeneity, which comprise the step of substituting Cys at position 14 (position 131 in the EU numbering system), Arg at position 16 (position 133 in the EU numbering system), and/or Cys at position 102 (position 219 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20 (IgG2) with other amino acids. The type of amino acid after substitution is not particularly limited; however, substitutions of Ser for Cys at position 14, Lys for Arg at position 16, and Ser for Cys at position 102 are preferred. The methods of the present invention for reducing antibody heterogeneity may comprise other steps of amino acid substitution, as long as they comprise the step of substituting Cys at position 14 (position 131 in the EU numbering system), Arg at position 16 (position 133 in the EU numbering system), and/or Cys at position 102 (position 219 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20

(IgG2). The method for amino acid substitution is not particularly limited. The substitutions can be achieved, for example, by site-directed mutagenesis described above or a method described in the Examples. In the amino acid substitution, all of the three amino acids described above may be substituted or one or two (for example, positions 14 and 102) of them may be substituted.

The type of target antibody is not particularly limited; however, the antibody is preferably an anti-human IL-6 receptor antibody, more preferably a humanized PM-1 antibody or a variant thereof comprising substitutions, deletions, and/or insertions.

<Methods for reducing the heterogeneity originated from deletion of C-terminal amino acids in an IgG2 constant region>

The present invention also relates to methods for reducing antibody heterogeneity, which comprise the step of deleting Gly at position 325 (position 446 in the EU numbering system) and Lys at position 326 (position 447 in the EU numbering system) in an IgG2 constant region comprising the amino acid sequence of SEQ ID NO: 20. The methods of the present invention for reducing antibody heterogeneity may comprise other steps of amino acid substitution, as long as they comprise the step of deleting Gly at position 325 (position 446 in the EU numbering system) and Lys at position 326 (position 447 in the EU numbering system) in an IgG2 constant region comprising the amino acid sequence of SEQ ID NO: 20. The method for amino acid substitution is not particularly limited. The substitution can be achieved, for example, by site-directed mutagenesis described above or a method described in the Examples.

The type of target antibody is not particularly limited; however, the antibody is preferably an anti-human IL-6 receptor antibody, more preferably a humanized PM-1 antibody or a variant thereof comprising substitutions, deletions, and/or insertions.

<Methods for reducing the FcγR binding while maintaining the human sequence in the IgG2 constant region>

The present invention also relates to methods for reducing the FcγR binding of an antibody, which comprise the step of substituting Ser for Ala at position 209 (EU330), Ser for Pro at position 210 (EU331), and Ala for Thr at position 218 (EU339) in an IgG2 constant region comprising the amino acid sequence of SEQ ID NO: 20. The methods of the present invention for reducing the FcγR binding of an antibody may comprise other steps of amino acid substitution, as long as they comprise the step of substituting Ser for Ala at position 209 (EU330), Ser for Pro at position 210 (EU331), and Ala for Thr at position 218 (EU339) in an IgG2 constant region comprising the amino acid sequence of SEQ ID NO: 20. The method for amino acid substitution is not particularly limited. The substitution can be achieved, for example, by site-directed mutagenesis described above or a method described in the Examples.

<Methods for improving the pharmacokinetics by substituting amino acids of IgG2 constant

region>

The present invention also relates to methods for improving the pharmacokinetics of an antibody, which comprise the step of substituting His at position 147 (EU268), Arg at position 234 (EU355), and/or Gln at position 298 (EU419) in an IgG2 constant region comprising the amino acid sequence of SEQ ID NO: 20. The methods of the present invention for improving the pharmacokinetics of an antibody may comprise other steps of amino acid substitution, as long as they comprise the above-described step. The type of amino acid after substitution is not particularly limited; however, substitutions of Gln for His at position 147 (EU268), Gln for Arg at position 234 (EU355), and Glu for Gln at position 298 (EU419) are preferred.

The present invention also relates to methods for improving the pharmacokinetics of an antibody, which comprise the step of substituting Asn at position 313 (EU434) in an IgG2 constant region comprising the amino acid sequence of SEQ ID NO: 20 or 151 (M58). The type of amino acid after substitution is not particularly limited; however, substitution to Ala is preferred. The methods of the present invention for improving the pharmacokinetics of an antibody may comprise other steps of amino acid substitution, as long as they comprise the above-described step.

The type of target antibody is not particularly limited; however, the antibody is preferably an anti-human IL-6 receptor antibody, more preferably a humanized PM-1 antibody or a variant thereof comprising substitutions, deletions, and/or insertions.

The present invention also relates to methods for reducing antibody heterogeneity originated from the hinge region of IgG2, methods for improving antibody stability under acidic conditions, methods for reducing antibody heterogeneity originated from C-terminus, and/or methods for reducing the FcγR binding of an antibody, all of which comprise in an IgG2 constant region comprising the amino acid sequence of SEQ ID NO: 20 (M14ΔGK), the steps of:

(a) substituting Ser for Ala at position 209 (position 330 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20;

(b) substituting Ser for Pro at position 210 (position 331 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20;

(c) substituting Ala for Thr at position 218 (position 339 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20;

(d) substituting Val for Met at position 276 (position 397 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20;

(e) substituting Ser for Cys at position 14 (position 131 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20;

(f) substituting Lys for Arg at position 16 (position 133 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20;

(g) substituting Ser for Cys at position 102 (position 219 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20;
(h) substituting Gly for Glu at position 20 (position 137 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20;
(i) substituting Gly for Ser at position 21 (position 138 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20; and
(j) deleting Gly at position 325 and Lys at position 326 (positions 446 and 447 in the EU numbering system, respectively) in the amino acid sequence of SEQ ID NO: 20.

The methods of the present invention may comprise other steps such as amino acid substitution and deletion, as long as they comprise the steps described above. The methods for amino acid substitution and deletion are not particularly limited. The substitution and deletion can be achieved, for example, by site-directed mutagenesis described above or a method described in the Examples.

The type of target antibody is not particularly limited; however, it is preferably an anti-human IL-6 receptor antibody, more preferably a humanized PM-1 antibody or a variant thereof comprising substitutions, deletions, and/or insertions.

The present invention also relates to methods for reducing the heterogeneity originated from the hinge region of IgG2, methods for improving antibody stability under acidic conditions, and/or methods for reducing antibody heterogeneity originated from C-terminus, all of which comprise in an IgG2 constant region comprising the amino acid sequence of SEQ ID NO: 20 (M31ΔGK), the steps of:
(a) substituting Val for Met at position 276 (position 397 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20;
(b) substituting Ser for Cys at position 14 (position 131 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20;
(c) substituting Lys for Arg at position 16 (position 133 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20;
(d) substituting Ser for Cys at position 102 (position 219 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20;
(e) substituting Gly for Glu at position 20 (position 137 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20;
(f) substituting Gly for Ser at position 21 (position 138 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20; and
(g) deleting Gly at position 325 and Lys at position 326 (positions 446 and 447 in the EU numbering system, respectively) in the amino acid sequence of SEQ ID NO: 20.

The present invention also relates to methods for reducing antibody heterogeneity

originated from the hinge region of IgG2, methods for improving antibody pharmacokinetics, and/or methods for reducing antibody heterogeneity originated from C-terminus, all of which comprise in an IgG2 constant region comprising the amino acid sequence of SEQ ID NO: 20 (M58), the steps of:

(a) substituting Ser for Cys at position 14 (position 131 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20;

(b) substituting Lys for Arg at position 16 (position 133 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20;

(c) substituting Ser for Cys at position 102 (position 219 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20;

(d) substituting Gly for Glu at position 20 (position 137 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20;

(e) substituting Gly for Ser at position 21 (position 138 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20;

(f) substituting Gln for His at position 147 (position 268 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20;

(g) substituting Gln for Arg at position 234 (position 355 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20;

(h) substituting Glu for Gln at position 298 (position 419 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20; and

(i) deleting Gly at position 325 and Lys at position 326 (positions 446 and 447 in the EU numbering system, respectively) in the amino acid sequence of SEQ ID NO: 20.

The present invention also relates to methods for reducing antibody heterogeneity originated from the hinge region of IgG2, methods for improving antibody pharmacokinetics, and/or methods for reducing antibody heterogeneity originated from C-terminus, all of which comprise in an IgG2 constant region comprising the amino acid sequence of SEQ ID NO: 20 (M73), the steps of:

(a) substituting Ser for Cys at position 14 (position 131 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20;

(b) substituting Lys for Arg at position 16 (position 133 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20;

(c) substituting Ser for Cys at position 102 (position 219 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20;

(d) substituting Gly for Glu at position 20 (position 137 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20;

(e) substituting Gly for Ser at position 21 (position 138 in the EU numbering system) in the

amino acid sequence of SEQ ID NO: 20;
(f) substituting Gln for His at position 147 (position 268 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20;
(g) substituting Gln for Arg at position 234 (position 355 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20;
(h) substituting Glu for Gln at position 298 (position 419 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20;
(i) substituting Ala for Asn at position 313 (position 434 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20; and
(j) deleting Gly at position 325 and Lys at position 326 (positions 446 and 447 in the EU numbering system, respectively) in the amino acid sequence of SEQ ID NO: 20.

The present invention also relates to methods for reducing the heterogeneity originated from the hinge region of IgG2, methods for reducing antibody heterogeneity originated from C-terminus, and/or methods for reducing the FcγR binding of an antibody, all of which comprise, in an IgG2 constant region comprising the amino acid sequence of SEQ ID NO: 20 (M86ΔGK), the steps of:
(a) substituting Ala at position 209 (position 330 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20 with another amino acid;
(b) substituting Pro at position 210 (position 331 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20 with another amino acid;
(c) substituting Thr at position 218 (position 339 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20 with another amino acid;
(d) substituting Cys at position 14 (position 131 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20 with another amino acid;
(e) substituting Arg at position 16 (position 133 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20 with another amino acid;
(f) substituting Cys at position 102 (position 219 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20 with another amino acid;
(g) substituting Glu at position 20 (position 137 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20 with another amino acid;
(h) substituting Ser at position 21 (position 138 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20 with another amino acid; and
(i) deleting Gly at position 325 and Lys at position 326 (positions 446 and 447 in the EU numbering system, respectively) in the amino acid sequence of SEQ ID NO: 20.

The type of amino acid after substitution is not particularly limited; however, substitutions of Ser for Ala at position 209 (position 330 in the EU numbering system), Ser for

Pro at position 210 (position 331 in the EU numbering system), Ala for Thr at position 218 (position 339 in the EU numbering system), Ser for Cys at position 14 (position 131 in the EU numbering system), Lys for Arg at position 16 (position 133 in the EU numbering system), Ser for Cys at position 102 (position 219 in the EU numbering system), Gly for Glu at position 20 (position 137 in the EU numbering system), and Gly for Ser at position 21 (position 138 in the EU numbering system) are preferred.

The present invention further relates to methods for reducing the heterogeneity originated from the hinge region of IgG2 and/or methods for reducing antibody heterogeneity originated from C-terminus, which comprise in an IgG2 constant region comprising the amino acid sequence of SEQ ID NO: 20 (M40ΔGK), the steps of:

(a) substituting Cys at position 14 (position 131 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20 with another amino acid;

(b) substituting Arg at position 16 (position 133 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20 with another amino acid;

(c) substituting Cys at position 102 (position 219 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20 with another amino acid;

(d) substituting Glu at position 20 (position 137 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20 with another amino acid;

(e) substituting Ser at position 21 (position 138 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 20 with another amino acid; and

(f) deleting Gly at position 325 and Lys at position 326 (positions 446 and 447 in the EU numbering system, respectively) in the amino acid sequence of SEQ ID NO: 20.

The type of amino acid after substitution is not particularly limited; however, substitutions of Ser for Cys at position 14 (position 131 in the EU numbering system), Lys for Arg at position 16 (position 133 in the EU numbering system), Ser for Cys at position 102 (position 219 in the EU numbering system), Gly for Glu at position 20 (position 137 in the EU numbering system), and Gly for Ser at position 21 (position 138 in the EU numbering system) are preferred.

The methods of the present invention may comprise other steps such as amino acid substitution and deletion, as long as they comprise the steps described above. The methods for amino acid substitution and deletion are not particularly limited. The substitution and deletion can be achieved, for example, by site-directed mutagenesis described above or a method described in the Examples.

The type of target antibody is not particularly limited; however, it is preferably an anti-human IL-6 receptor antibody, more preferably a humanized PM-1 antibody or a variant thereof comprising substitutions, deletions, and/or insertions.

<Methods for improving the stability of an IgG4 constant region under acidic conditions>

The present invention also relates to methods for improving antibody stability under acidic conditions, which comprise the step of substituting Arg at position 289 (position 409 in the EU numbering system) of an IgG4 constant region comprising the amino acid sequence of SEQ ID NO: 21 (Mol. Immunol. 1993 Jan;30(1):105-8) with another amino acid. The methods of the present invention for improving antibody stability under acidic conditions may comprise other steps of amino acid substitution, as long as they comprise the step of substituting Arg at position 289 (position 409 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 21 (human IgG4 constant region) with another amino acid. The type of amino acid after substitution is not particularly limited; however, substitution to Lys is preferred. The method for amino acid substitution is not particularly limited. The substitution can be achieved, for example, by site-directed mutagenesis described above or a method described in the Examples.

The type of target antibody is not particularly limited; however, the antibody is preferably an anti-human IL-6 receptor antibody, more preferably a humanized PM-1 antibody or a variant thereof comprising substitutions, deletions, and/or insertions.

<Methods for reducing the heterogeneity originated from deletion of C-terminal amino acids in an IgG4 constant region>

The present invention also relates to methods for reducing the heterogeneity of an antibody, which comprise the step of deleting Gly at position 326 (position 446 in the EU numbering system) and Lys at position 327 (position 447 in the EU numbering system) in an IgG4 constant region comprising the amino acid sequence of SEQ ID NO: 21 (Mol. Immunol. 1993 Jan;30(1):105-8). The methods of the present invention for reducing the heterogeneity may comprise other steps of amino acid substitution, as long as they comprise the step of deleting Lys at position 327 (position 447 in the EU numbering system) and/or Gly at position 326 (position 446 in the EU numbering system) in an IgG4 constant region comprising the amino acid sequence of SEQ ID NO: 21 (Mol. Immunol. 1993 Jan;30(1):105-8). The method for amino acid substitution is not particularly limited. The substitution can be achieved, for example, by site-directed mutagenesis described above or a method described in the Examples.

The type of target antibody is not particularly limited; however, the antibody is preferably an anti-human IL-6 receptor antibody, more preferably a humanized PM-1 antibody or a variant thereof comprising substitutions, deletions, and/or insertions.

The present invention also relates to methods for improving the stability under acidic conditions, methods for reducing the heterogeneity originated from C-terminus, and/or methods for reducing the FcγR binding of an antibody, all of which comprise in an IgG4 constant region comprising the amino acid sequence of SEQ ID NO: 21 (Mol. Immunol. 1993 Jan;30(1):105-8) (M11ΔGK), the steps of:

(a) substituting Ser for Cys at position 14 (position 131 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 21;
(b) substituting Lys for Arg at position 16 (position 133 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 21;
(c) substituting Gly for Glu at position 20 (position 137 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 21;
(d) substituting Gly for Ser at position 21 (position 138 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 21;
(e) substituting Thr for Arg at position 97 (position 214 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 21;
(f) substituting Arg for Ser at position 100 (position 217 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 21;
(g) substituting Ser for Tyr at position 102 (position 219 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 21;
(h) substituting Cys for Gly at position 103 (position 220 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 21;
(i) substituting Val for Pro at position 104 (position 221 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 21;
(j) substituting Glu for Pro at position 105 (position 222 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 21;
(k) substituting Pro for Glu at position 113 (position 233 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 21;
(l) substituting Val for Phe at position 114 (position 234 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 21;
(m) substituting Ala for Leu at position 115 (position 235 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 21;
(n) deleting Gly at position 116 (position 236 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 21;
(o) substituting Lys for Arg at position 289 (position 409 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 21; and
(p) deleting Gly at position 236 and Lys at position 237 (positions 446 and 447 in the EU numbering system, respectively) in the amino acid sequence of SEQ ID NO: 21.

The methods of the present invention may comprise other steps, such as amino acid substitution and deletion, as long as they comprise the steps described above. The method for amino acid substitution and deletion are not particularly limited. The substitution and deletion can be achieved, for example, by site-directed mutagenesis described above or a method

described in the Examples.

The type of target antibody is not particularly limited; however, the antibody is preferably an anti-human IL-6 receptor antibody, more preferably a humanized PM-1 antibody or a variant thereof comprising substitutions, deletions, and/or insertions.

<Methods for reducing the heterogeneity originated from deletion of C-terminal amino acids in an IgG1 constant region>

The present invention also relates to methods for reducing antibody heterogeneity, which comprise the step of deleting Gly at position 329 (position 446 in the EU numbering system) and Lys at position 330 (position 447 in the EU numbering system) in an IgG1 constant region comprising the amino acid sequence of SEQ ID NO: 19. The methods of the present invention for reducing antibody heterogeneity may comprise other steps of amino acid substitutions, as long as they comprise the step of deleting Lys at position 330 (position 447 in the EU numbering system) and Gly at position 329 (position 446 in the EU numbering system) in an IgG1 constant region comprising the amino acid sequence of SEQ ID NO: 19. The method for amino acid substitution is not particularly limited. The substitution can be achieved, for example, by site-directed mutagenesis described above or a method described in the Examples.

<Methods for improving the pharmacokinetics by substituting amino acids of IgG1 constant region>

The present invention relates to methods for improving the antibody pharmacokinetics, which comprise the step of substituting Asn at position 317 (EU434) in an IgG1 constant region comprising the amino acid sequence of SEQ ID NO: 19 with another amino acid. The type of amino acid after substitution is not particularly limited; however, substitution to Ala is preferred. The methods of the present invention for improving the pharmacokinetics may comprise other steps of amino acid substitution, as long as they comprise the above-described step.

The present invention also relates to methods for improving the pharmacokinetics and/or methods for reducing the heterogeneity originated from C-terminus, both of which comprise, in an IgG1 constant region comprising the amino acid sequence of SEQ ID NO: 19 (M83), the steps of:

(a) substituting Ala for Asn at position 317 (EU 434) in the amino acid sequence of SEQ ID NO: 19; and

(b) deleting Lys at position 330 (position 447 in the EU numbering system) and Gly at position 329 (position 446 in the EU numbering system) in the amino acid sequence of SEQ ID NO: 19.

The type of target antibody is not particularly limited; however, the antibody is preferably an anti-human IL-6 receptor antibody, more preferably a humanized PM-1 antibody or a variant thereof comprising substitutions, deletions, and/or insertions.

The constant regions of the present invention described above can be combined with any antibody variable regions, and preferably with variable regions derived from antibodies against human IL-6 receptor. Variable regions of antibodies against human IL-6 receptor include, for example, variable regions of a humanized PM-1 antibody. The variable regions of a humanized PM-1 antibody may not comprise any amino acid substitutions or may comprise substitutions such as those described above.

The present invention provides pharmaceutical compositions comprising an antibody of the present invention. The pharmaceutical compositions of the present invention are useful in treating diseases associated with IL-6, such as rheumatoid arthritis.

The pharmaceutical compositions of the present invention can be formulated, in addition to the antibodies, with pharmaceutically acceptable carriers by known methods. For example, the compositions can be used parenterally, when the antibodies are formulated in a sterile solution or suspension for injection using water or any other pharmaceutically acceptable liquid. For example, the compositions can be formulated by appropriately combining the antibodies with pharmaceutically acceptable carriers or media, specifically, sterile water or physiological saline, vegetable oils, emulsifiers, suspending agents, surfactants, stabilizers, flavoring agents, excipients, vehicles, preservatives, binding agents, and such, by mixing them at a unit dose and form required by generally accepted pharmaceutical implementations. The content of the active ingredient in such a formulation is adjusted so that an appropriate dose within the required range can be obtained.

Sterile compositions for injection can be formulated using vehicles such as distilled water for injection, according to standard protocols.

Aqueous solutions used for injection include, for example, physiological saline and isotonic solutions containing glucose or other adjuvants such as D-sorbitol, D-mannose, D-mannitol, and sodium chloride. These can be used in conjunction with suitable solubilizers such as alcohol, specifically ethanol, polyalcohols such as propylene glycol and polyethylene glycol, and non-ionic surfactants such as Polysorbate 80™ and HCO-50.

Oils include sesame oils and soybean oils, and can be combined with solubilizers such as benzyl benzoate or benzyl alcohol. These may also be formulated with buffers, for example, phosphate buffers or sodium acetate buffers; analgesics, for example, procaine hydrochloride; stabilizers, for example, benzyl alcohol or phenol; or antioxidants. The prepared injections are typically aliquoted into appropriate ampules.

The administration is preferably carried out parenterally, and specifically includes injection, intranasal administration, intrapulmonary administration, and percutaneous administration. For example, injections can be administered systemically or locally by intravenous injection, intramuscular injection, intraperitoneal injection, or subcutaneous

injection.

Furthermore, the method of administration can be appropriately selected according to the age and symptoms of the patient. A single dose of the pharmaceutical composition containing an antibody or a polynucleotide encoding an antibody can be selected, for example, from the range of 0.0001 to 1,000 mg per kg of body weight. Alternatively, the dose may be, for example, in the range of 0.001 to 100,000 mg/person. However, the dose is not limited to these values. The dose and method of administration vary depending on the patient's body weight, age, and symptoms, and can be appropriately selected by those skilled in the art.

As used herein, the three-letter and single-letter codes for respective amino acids are as follows:

Alanine: Ala (A)
Arginine: Arg (R)
Asparagine: Asn (N)
Aspartic acid: Asp (D)
Cysteine: Cys (C)
Glutamine: Gln (Q)
Glutamic acid: Glu (E)
Glycine: Gly (G)
Histidine: His (H)
Isoleucine: Ile (I)
Leucine: Leu (L)
Lysine: Lys (K)
Methionine: Met (M)
Phenylalanine: Phe (F)
Proline: Pro (P)
Serine: Ser (S)
Threonine: Thr (T)
Tryptophan: Trp (W)
Tyrosine: Tyr (Y)
Valine: Val (V)

All prior art documents cited herein are incorporated by reference in their entirety.

Examples

Hereinbelow, the present invention is specifically described with reference to the Examples, but it is not to be construed as being limited thereto.

[Example 1] Improvement of antigen-binding activity through CDR alteration using affinity maturation technology

Preparation of SR344

A CHO cell line constitutively expressing a sequence of N-terminal 1st to 344th amino acids of soluble human IL-6R (hereinafter SR344) reported in J. Biochem. (1990) 108, 673-676 (Yamasaki *et al.*, Science (1988) 241, 825-828 (GenBank #X12830)) was prepared.

SR344 was purified from the culture supernatant of SR344-expresssing CHO cells using three types of column chromatography: Blue Sepharose 6 FF column chromatography, affinity chromatography with an SR344-specific antibody-immobilized column, and gel filtration column chromatography.

The culture supernatant was directly loaded onto a Blue Sepharose 6 FF column (GE Healthcare Bio-Sciences) equilibrated with 20 mM Tris-HCl buffer (pH 8.0), and the non-adsorbed fraction was thoroughly washed off using the same buffer. Then, the column was washed with the same buffer containing 300 mM KCl. The adsorbed protein was then eluted using the same buffer in the presence of 300 mM KCl with a linear concentration gradient of 0 to 0.5 M KSCN. Fractions eluted with the KSCN concentration gradient were analyzed by Western blotting using an SR344-specific antibody, and fractions containing SR344 were collected.

The SR344-specific antibody-immobilized column was pre-equilibrated with Tris-buffered saline (TBS). The SR344 fraction obtained in the first step was concentrated by ultrafiltration using Amicon Ultra-15 (MILLIPORE; molecular weight cut-off of 10 kDa), and diluted two fold with TBS before it was loaded onto the column. After the column was washed with TBS, the adsorbed protein was eluted with 100 mM glycine-HCl buffer (pH 2.5). The eluted fractions were neutralized by adding 1 M Tris (pH 8.1). The obtained fractions were analyzed by SDS-PAGE to collect SR344-containing fractions.

The fraction obtained in the second step was concentrated using Amicon Ultra-15 (molecular weight cut-off of 10 kDa) and loaded onto a Superdex 200 column (GE Healthcare Bio-Sciences) equilibrated with PBS. The fraction eluted as the major peak was used as the final purified sample of SR344.

Establishment of a human gp130-expressing BaF3 cell line

A BaF3 cell line expressing human gp130 was established by the procedure described below, to obtain a cell line that proliferates in an IL-6-dependent manner.

A full-length human gp130 cDNA (Hibi *et al.*, Cell (1990) 63, 1149-1157 (GenBank #NM_002184)) was amplified by PCR and cloned into the expression vector pCOS2Zeo to

construct pCOS2Zeo/gp130. pCOS2Zeo is an expression vector constructed by removing the DHFR gene expression region from pCHOI (Hirata *et al.*, FEBS Letter (1994) 356, 244-248) and inserting the expression region of the Zeocin resistance gene. The full-length human IL-6R cDNA was amplified by PCR and cloned into pcDNA3.1(+) (Invitrogen) to construct hIL-6R/pcDNA3.1(+).

10 μg of pCOS2Zeo/gp130 was mixed with BaF3 cells (0.8×10^7 cells) suspended in PBS, and then pulsed at 0.33 kV and 950 μFD using Gene Pulser (Bio-Rad). The BaF3 cells having the gene introduced by electroporation were cultured for one whole day and night in RPMI 1640 medium (Invitrogen) supplemented with 0.2 ng/ml mouse interleukin-3 (Peprotech) and 10% Fetal Bovine Serum (hereinafter FBS; HyClone), and selected by adding RPMI 1640 medium supplemented with 100 ng/ml human interleukin-6 (R&D systems), 100 ng/ml human interleukin-6 soluble receptor (R&D systems), and 10% FBS to establish a human gp130-expressing BaF3 cell line (hereinafter BaF3/gp130). This BaF/gp130 proliferates in the presence of human interleukin-6 (R&D systems) and SR344, and thus can be used to assess the growth inhibition activity (or IL-6 receptor neutralizing activity) of an anti-IL-6 receptor antibody.

Construction of a library of altered CDRs

First, a humanized PM-1 antibody (Cancer Res. 1993 Feb 15;53(4):851-6) was converted into scFv. The VH and VL regions were amplified by PCR to prepare a humanized PM-1 HL scFv having the linker sequence GGGGSGGGGSGGGGS (SEQ ID NO: 106) between VH and VL.

Two types of libraries were constructed by PCR using the prepared humanized PM-1 HL scFv-encoding DNA as a template. One was a target library where one of the amino acids in a CDR is designed as X, and the other was a library where only the hot spot sequences in a CDR are substituted with random sequences. The target library where one of the amino acids in each CDR is designed as X was constructed as follows. The library portion was constructed by PCR using a primer containing NNS for the amino acids to be incorporated into the library, while the remaining was prepared by standard PCR. The two were linked together by assembly PCR. In this construction, only one CDR was diversified as a library (see J. Mol. Biol. (1996) 256, 77-88). Likewise, the library where only the hot spot sequences were substituted with random sequences was constructed by PCR using a primer containing NNS for all hot spot amino acids. In this construction, two libraries were constructed: one was a library where only the hot spot in VH was diversified, and the other was a library where only the hot spot in VL was diversified (see Nature Biotechnology 1999 June;17:568-572).

A ribosome display library was constructed using the above-described libraries

according to J. Immunological Methods (1999) 231, 119-135. To perform *in vitro* translation based on the cell-free *E. coli* system, an SDA sequence (ribosome binding site) and T7 promoter were attached to the 5' end and a partial gene3 sequence was ligated as a ribosome display linker to the 3' end using *Sfi*I.

Selection of high affinity scFv by ribosome display

Ribosome display-based panning was carried out (Nature Biotechnology 2000 Dec;18:1287-1292). The prepared SR344 was biotinylated using NHS-PEO4-Biotin (Pierce) and then used as an antigen. Off-rate selection was performed to obtain high affinity scFv with high efficiency (JBC (2004) 279(18), 18870-18877). The concentrations of biotinylated antigen and competitor antigen were 1 nM and 1 μM, respectively. The time of competition in the fourth round was 10 O/N.

scFv: insertion into phagemid, antigen binding and sequence analysis

PCR was performed to reconstruct HL scFv using the template DNA pool obtained in the fourth round and specific primers. After digestion with *Sfi*I, the fragment was inserted into the phagemid vector pELBG lacI predigested with *Sfi*I. XL1-Blue (Stratagene) was transformed with the resulting construct. Using the yielded colonies, antigen binding was assessed by phage ELISA and the HL scFv sequence was analyzed. The phage ELISA was carried out using plates coated with SR344 at 1 μg/ml (J. Mol. Biol. (1992) 227, 381-388). Clones exhibiting SR344 binding were analyzed for their sequences using specific primers.

Conversion of scFv into IgG, and expression and purification of IgG

IgG expression was conducted using animal cell expression vectors. Clones enriched with a particular mutation were subjected to PCR to amplify their VLs and VHs. After *Xho*I/*Nhe*I digestion and *Eco*RI digestion, the amplified DNAs were inserted into an animal cell expression vector. The nucleotide sequence of each DNA fragment was determined using a DNA sequencer (ABI PRISM 3730xL DNA Sequencer or ABI PRISM 3700 DNA Sequencer (Applied Biosystems)) using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) according to the method described in the attached instruction manual.

Expression of IgG-converted antibodies

Antibody expression was performed by the method described below. Human embryonic kidney cancer-derived HEK293H cells (Invitrogen) were suspended in DMEM (Invitrogen) supplemented with 10% Fetal Bovine Serum (Invitrogen). The cells (10-ml/plate; cell density of 5 to 6 x 10^5 cells/ml) were plated on dishes for adherent cells (10 cm in diameter;

CORNING) and cultured in a CO_2 incubator (37°C, 5% CO_2) for one whole day and night. Then, the medium was removed by aspiration, and 6.9 ml of CHO-S-SFM-II medium (Invitrogen) was added. The prepared plasmid DNA mixture (13.8 μg in total) was combined with 20.7 μl of 1 μg/ml Polyethylenimine (Polysciences Inc.) and 690 μl of CHO-S-SFMII medium. The resulting mixture was incubated at room temperature for 10 minutes, and then added to the cells in each dish. The cells were incubated in a CO_2 incubator (at 37°C under 5% CO_2) for 4 to 5 hours. Then, 6.9 ml of CHO-S-SFM-II medium (Invitrogen) was added to the dishes, and the cells were incubated in a CO_2 incubator for three days. The culture supernatants were collected and centrifuged (approx. 2000 g, 5 min, room temperature) to remove the cells, and sterilized through 0.22-μm filter MILLEX(R)-GV (Millipore). The samples were stored at 4°C until use.

Purification of IgG-converted antibodies

50 μl of rProtein A Sepharose™ Fast Flow (Amersham Biosciences) suspended in TBS was added to the obtained culture supernatants, and the combined solutions were mixed by inversion at 4°C for four hours or more. The solutions were transferred into 0.22-μm filter cups of Ultrafree(R)-MC (Millipore). After washing three times with 500 μl of TBS, the rProtein A Sepharose™ resin was suspended in 100 μl of 50 mM sodium acetate (pH 3.3) aqueous solution, and the mixture was incubated for two minutes to elute the antibody. Immediately, the eluate was neutralized by adding 6.7 μl of 1.5 M Tris-HCl (pH 7.8). Elution was carried out twice, yielding 200 μl of purified antibody. The absorbance at 280 nm was determined using ND-1000 Spectrophotometer (NanoDrop) or spectrophotometer DU-600 (BECKMAN) using 2 or 50 μl of the antibody solution, respectively. The antibody concentration was calculated from the obtained value according to the following formula:

Antibody concentration (mg/ml) = [absorbance x dilution fold] / 14.6 x 10

Assessment of the IgG-converted clones for human IL-6 receptor-neutralizing activity

The IL-6 receptor neutralizing activity was assessed using BaF3/gp130 which proliferates in an IL-6/IL-6 receptor-dependent manner. After three washes with RPMI1640 supplemented with 10% FBS, BaF3/gp130 cells were suspended at 5 x 10^4 cells/ml in RPMI1640 supplemented with 60 ng/ml human interleukin-6 (TORAY), 60 ng/ml recombinant soluble human IL-6 receptor (SR344), and 10% FBS. The cell suspensions were dispensed (50 μl/well) into 96-well plates (CORNING). Then, the purified antibodies were diluted with RPMI1640 containing 10% FBS, and added to each well (50 μl/well). The cells were cultured at 37°C under 5% CO_2 for three days. WST-8 Reagent (Cell Counting Kit-8; Dojindo Laboratories) was diluted two-fold with PBS. Immediately after 20 μl of the reagent was added

to each well, the absorbance at 450 nm (reference wavelength: 620 nm) was measured using SUNRISE CLASSIC (TECAN). After culturing for two hours, the absorbance at 450 nm (reference wavelength: 620 nm) was measured again. The IL-6 receptor neutralizing activity was assessed using the change of absorbance during two hours as an indicator.

As a result, a number of antibodies whose activities were higher than that of the humanized PM-1 antibody (wild type (WT)) were obtained. Mutations in the antibodies whose activities were higher than that of WT are shown in Fig. 4. For example, as shown in Fig. 1, the neutralizing activity of RD_6 was about 50 times higher than WT in terms of 100% inhibitory concentration.

Biacore-based affinity analysis of the IgG-converted clones

The clones whose activities were higher than that of the wild type were analyzed for antigen-antibody reaction kinetics using Biacore T100 (BIACORE). The antigen-antibody interaction was measured by immobilizing 1800 to 2600 RU (resonance units) of rec-Protein A (ZYMED) (hereinafter Protein A) onto a sensor chip, binding various antibodies onto the chip, and then running the antigen over the chip as an analyte. Various concentrations of recombinant human IL-6R sR (R&D systems) (hereinafter rhIL-6sR) were used as the antigen. All measurements were carried out at 25°C. The kinetic parameters, association rate constant k_a (1/Ms) and dissociation rate constant k_d (1/s) were calculated from the sensorgrams obtained by measurement. Then, K_D (M) was determined based on the rate constants. The respective parameters were determined using Biacore T100 Evaluation Software (BIACORE).

As a result, a number of antibodies exhibiting higher affinity than the humanized PM-1 antibody (wild type (WT)) were obtained. As an example, sensorgrams of the wild type (WT) and RD_6 are shown in Figs. 2 and 3, respectively. The result of kinetic parameter analysis revealed that RD_6 had about 50 times higher affinity than WT (Table 1). In addition to RD_6, antibodies exhibiting affinity dozens of times higher than WT were also obtained. Mutations that result in higher affinity than WT are shown in Fig. 4.

Table 1

SAMPLE	k_a (1/Ms)	k_d (1/s)	K_D (M)
WT	2.8E+6	1.8E-3	6.5E-10
RD_6	2.3E+6	2.8E-5	1.2E-11

[Example 2] Improvement of antigen binding activity through various combinations of CDR alterations

Mutations associated with strong activity or high affinity were combined to create antibodies with stronger activity and higher affinity.

Production, expression, and purification of altered antibodies

Amino acids at selected sites were altered to produce altered antibodies. Specifically, mutations were introduced into the prepared H(WT) variable region (H(WT), SEQ ID NO: 107) and L(WT) variable region (L(WT), SEQ ID NO: 108) using the QuikChange Site-Directed Mutagenesis Kit (Stratagene) by the method described in the attached instruction manual. After it was confirmed that the antibody H chain gene fragment inserted into a plasmid was the humanized antibody variable region gene sequence of interest, the plasmid was digested with *Xho*I and *Not*I. A plasmid containing the antibody L chain gene fragment as an insert was digested with *Eco*RI. Then, the reaction mixtures were subjected to electrophoresis in 1% agarose gel. A DNA fragment of the expected size (about 400 bp) was purified using the QIAquick Gel Extraction Kit (QIAGEN) by the method described in the attached instruction manual. The DNA was eluted with 30 μl of sterile water. Then, the antibody H chain gene fragment was inserted into an animal cell expression vector to construct the H chain expression vector of interest. An expression vector for the L chain was also constructed in the same way. Ligation was carried out using the Rapid DNA Ligation Kit (Roche Diagnostics). The *E. coli* strain DH5α (Toyobo) was transformed with the plasmids. The nucleotide sequence of each DNA fragment was determined with a DNA sequencer (ABI PRISM 3730xL DNA Sequencer or ABI PRISM 3700 DNA Sequencer (Applied Biosystems)) using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) according to the method described in the attached instruction manual. The antibodies were expressed using the constructed expression vectors and purified by the method described in Example 1.

Assessment for the activity of neutralizing human IL-6 receptor

The purified antibodies were assessed for their neutralizing activity by the method described in Example 1. The neutralizing activity was assessed using 600 ng/ml human interleukin-6 (TORAY). A number of novel antibodies with stronger activity than WT were obtained. The CDR sequences of the antibodies are shown in Fig. 5. Of them, the antibody with the strongest activity (referred to as RDC_23) has RDC_5H as an H chain and RDC_11L as an L chain. The neutralizing activity of RDC_23 is shown in Fig. 6. The activity of RDC_23 was demonstrated to be about 100 times higher than WT in terms of 100% inhibitory concentration. Improved neutralizing activity was observed not only in RDC_23, which is an

antibody having RDC_5H as an H chain and RDC_11L as an L chain, but also in antibodies RDC_2, RDC_3, RDC_4, RDC_5, RDC_6, RDC_7, RDC_8, RDC_27, RDC_28, RDC_29, RDC_30, and RDC_32, which all have L(WT) as an L chain, and RDC_2H, RDC_3H, RDC_4H, RDC_5H, RDC_6H, RDC_7H, RDC_8H, RDC_27H, RDC_28H, RDC_29H, RDC_30H, and RDC_32H as an H chain, respectively, as well as in an antibody referred to as RDC_11, which has H(WT) and RDC_11L as H and L chains, respectively. It was thus shown that antibodies having stronger neutralizing activity could be obtained by combining mutations discovered by affinity maturation. Furthermore, since antibodies containing such a combination of mutations had improved neutralizing activity, they were also expected to have improved affinity.

Biacore-based affinity analysis using Protein A

Thus, of the antibodies with improved neutralizing activity, RDC_2, RDC_3, RDC_4, RDC_5, RDC_6, RDC_7, RDC_8, RDC_11, and RDC_23 were analyzed for antigen-antibody reaction kinetics using Biacore T100 (BIACORE). The antigen-antibody interaction was measured by immobilizing 4400 to 5000 RU of rec-Protein A (ZYMED) immobilized onto a sensor chip by the amine coupling method, binding various antibodies onto the chip, and then running the antigen over the chip as an analyte. For the antigen, various concentrations of rhIL-6sR were used. All measurements were carried out at 25°C. The kinetic parameters, association rate constant k_a (1/Ms) and dissociation rate constant k_d (1/s) were calculated from the sensorgrams obtained by measurement. Then, K_D (M) was determined based on the rate constants. The respective parameters were determined using Biacore T100 Evaluation Software (BIACORE). The result showed that RDC_2, RDC_3, RDC_4, RDC_5, RDC_6, RDC_7, RDC_8, RDC_11, and RDC_23, all of which contained a combination of mutations, had a smaller K_D value than RD_28 which contains a single mutation (Table 2). The sensorgram for RDC_23 which has a higher affinity than others is shown in Fig. 7.

Table 2

SAMPLE	ka (1/Ms)	kd (1/s)	KD (M)
RD_28	9.4E+05	1.1E-04	1.2E-10
RDC_2	1.1E+06	2.5E-05	2.2E-11
RDC_3	1.0E+06	3.7E-05	3.7E-11
RDC_4	1.1E+06	2.9E-05	2.7E-11
RDC_5	1.2E+06	2.8E-05	2.2E-11
RDC_6	1.2E+06	3.5E-05	2.9E-11
RDC_7	1.1E+06	4.2E-05	3.8E-11
RDC_8	1.4E+06	3.6E-05	2.5E-11
RDC_11	1.1E+06	7.0E-05	6.5E-11
RDC_23	1.2E+06	3.1E-05	2.5E-11

This finding suggests that these antibodies have higher affinities than the parental antibodies that do not have the combinations of mutations. As in the case of the neutralizing activity, this indicates that antibodies having greater affinity can be obtained by combining mutations discovered by affinity maturation. The amino acid sequences of variants having higher activity or affinity than WT are shown below (mutations relative to WT are underlined).

(HCDR2)

SEQ ID NO: 45 YISYSGITNYNPSLKS

(HCDR3)

SEQ ID NO: 57 LLARATAMDY

SEQ ID NO: 58 VLARATAMDY

SEQ ID NO: 59 ILARATAMDY

SEQ ID NO: 60 TLARATAMDY

SEQ ID NO: 61 VLARITAMDY

SEQ ID NO: 62 ILARITAMDY

SEQ ID NO: 63 TLARITAMDY

SEQ ID NO: 64 LLARITAMDY

(LCDR3)

SEQ ID NO: 79 GQGNRLPYT

Specifically, an anti-IL-6 receptor antibody with markedly improved affinity and neutralizing activity as compared to WT can be produced by designing the antibody to have Asn at amino acid position 9 in HCDR2, Leu, Val, Ile, or Thr at amino acid position 1 in HCDR3, Ala or Ile at amino acid position 5 in HCDR3, Gly at amino acid position 1 in LCDR3, and Arg at amino acid position 5 in LCDR3.

Biacore-based affinity analysis using Protein A/G

WT and RDC_23 were analyzed for antigen-antibody reaction kinetics using Biacore T100 (BIACORE). The antigen-antibody interaction was measured by immobilizing purified Recomb Protein A/G (Pierce) (hereinafter Protein A/G) onto a sensor chip, binding various antibodies onto the chip, and then running the antigen as an analyte over the chip. Various concentrations of rhIL-6sR (R&D systems) and recombinant soluble IL-6 receptor (SR344 prepared in Example 1 were used as the antigen. The sugar chain structure of rhIL-6sR produced by baculovirus-infected insect cells is of high-mannose type. On the other hand, the sugar chain structure of SR344 produced by CHO cells is assumed to be of the complex sugar chain type with sialic acid at its end. Since the sugar chain structure of soluble IL-6 receptor in an actual human body is assumed to be of the complex sugar chain type with sialic acid at its end, SR344 is expected to have a structure closer to that of soluble IL-6 receptor in the human body. Thus, a comparison test between rhIL-6sR and SR344 was carried out in this experiment.

The kinetic parameters, association rate constant k_a (1/Ms) and dissociation rate constant k_d (1/s) were calculated from the sensorgrams obtained by measurement. Then, K_D (M) was determined based on the rate constants. The respective parameters were determined using Biacore T100 Evaluation Software (BIACORE).

A sensor chip was prepared by immobilizing about 3000 RU of Protein A/G onto CM5 (BIACORE) with the amine coupling method. The kinetics of the interaction between the two types of soluble IL-6 receptors (rhIL-6sR and SR344) and the antibodies (WT and RDC_23) bound to Protein A/G was analyzed using the prepared sensor chip. The running buffer used was HBS-EP+, and the flow rate was 20 μl/min. Each antibody was prepared so that about 100 RU of the antibody was bound to Protein A/G. For the analyte, rhIL-6sR was prepared at 0, 0.156, 0.313, and 0.625 μg/ml using HBS-EP+, while SR344 was adjusted to 0, 0.0654, 0.131, and 0.261 μg/ml. In the first step of the measurement, the antibodies of interest, WT and RDC_23, were bound to Protein A/G, and an analyte solution was added thereto. After three minutes of interaction, the solution was switched with HBS-EP+ (BIACORE), and the dissociation phase was monitored for ten minutes. After measurement of the dissociation phase, the sensor chip was regenerated by washing with 10 μl of 10 mM glycine-HCl (pH 1.5). The association, dissociation, and regeneration constitute one analytic cycle. All experiments were carried out at 37°C.

WT and RDC_23 were measured according to the above cycle. The resulting sensorgrams for the two types of soluble IL-6 receptors, rhIL-6sR and SR344, are shown in Figs. 8, 9, 10, and 11. The obtained sensorgrams were kinetically analyzed using Biacore T100 Evaluation Software, which is a data analysis software specific for Biacore (Table 3). The result showed that when comparing rhIL-6sR and SR344, the affinities of both WT and RDC_23 for SR344 were two- to three-fold weaker For both rhIL-6sR and SR344, RDC_23 had

affinities that are about 40 to 60 times improved as compared to WT. Thus, it was demonstrated that because of the combination of respective CDR alterations obtained by affinity maturation, RDC_23 also had a markedly higher affinity than WT for SR344 whose structure is presumably close to that of soluble IL-6 receptor in the human body. All measurements described hereinafter in the Examples were carried out at 37°C to kinetically analyze the antigen-antibody reaction using SR344 and protein A/G.

Table 3

SAMPLE	ANALYTE	k_a (1/Ms)	k_d (1/s)	K_D (M)
WT	rhIL-6sR	1.3E+6	1.5E-3	1.2E-9
	SR344	4.9E+5	2.0E-3	4.0E-9
RDC_23	rhIL-6sR	1.6E+6	4.5E-5	2.8E-11
	SR344	6.4E+5	4.3E-5	6.7E-11

[Example 3] Generation of H53/L28 with improved pharmacokinetics and reduced immunogenicity risk through alterations of CDR and framework

The antibody obtained by humanizing a mouse PM-1 antibody (hereinafter referred to as wild type or WT; the WT H and L chains are referred to as H(WT) and L(WT), respectively) as described in Cancer Res. 1993 Feb 15;53(4):851-6, was altered to improve the pharmacokinetics, reduce the immunogenicity risk, and increase the stability. The alterations are described below. For the purpose of improving the pharmacokinetics, the H and L chain variable region sequences of WT were altered to lower the isoelectric point.

Creation of a three-dimensional structure model for the humanized PM-1 antibody

First, to identify amino acid residues exposed on the surface of the variable regions of the humanized PM-1 antibody (H(WT)/L(WT)), a model for the Fv domain of the antibody obtained by humanizing a mouse PM-1 antibody was created by homology modeling using the MOE software (Chemical Computing Group Inc.).

Selection of alteration sites to reduce the isoelectric point of the humanized PM-1 antibody

A detailed analysis of the model created suggested that of the surface exposed amino acids in the FR sequence, H16, H43, H81, H105, L18, L45, L79, and L107 (in Kabat's numbering system; Kabat EA *et al.*, 1991, Sequences of Proteins of Immunological Interest, NIH), and of those in the CDR sequence, H31, H64, H65, L24, L27, L53, and L55, were potential candidates for the sites of alteration to reduce the isoelectric point without decreasing

the activity or stability.

Removal of remaining mouse sequences from the humanized PM-1 antibody

The humanized PM-1 antibody is an antibody whose sequence was obtained by humanizing the mouse PM-1 antibody (Cancer Res. 1993 Feb 15;53(4):851-6). The H chain of the humanized PM-1 antibody was obtained by grafting CDR onto the NEW framework which is a human antibody variable region. However, mouse sequences remain at H27, H28, H29, H30, and H71 in the H chain to maintain the activity. From the perspective of immunogenicity risk, the best result is expected when the number of mouse sequences is minimized. Thus, the present inventors searched for sequences for converting H27, H28, H29, and H30 into human sequences.

Selection of alteration sites to improve the stability of the humanized PM-1 antibody

The present inventors speculated that it might be possible to improve the stability of the humanized PM-1 antibody (H(WT)/L(WT)) by substituting glycine for serine at H65 (stabilization of the turn structure; stabilization through conversion into an HCDR2 consensus sequence), isoleucine for methionine at H69 (stabilization of the hydrophobic core structure), serine for leucine at H70 (stabilization through replacement of the surface exposed residue with a hydrophilic residue), asparagine for threonine at H58 (stabilization through conversion into an HCDR2 consensus sequence), serine for threonine at L93 (stabilization through replacement of the surface exposed residue with a hydrophilic residue), and isoleucine for serine at H107 (stabilization of the β sheet) in its variable regions, and considered these alterations as candidates for increasing stability.

Removal of *in silico* predicted T-cell epitopes from the humanized PM-1 antibody

First, the variable regions of the humanized PM-1 antibody (H(WT)/L(WT)) were analyzed using TEPITOPE (Methods 2004 Dec;34(4):468-75). The result showed that the L chain CDR2 contained many T-cell epitopes that bind to HLA. Thus, TEPITOPE analysis was carried out to find alterations that would reduce the immunogenicity risk of the L chain CDR2 without decreasing the stability, binding activity, or neutralizing activity. The result demonstrated that HLA-binding T-cell epitopes can be removed without decreasing the stability, binding activity, or neutralizing activity by substituting glycine for threonine at L51 in the L chain CDR2.

Selection of respective framework sequences

Homology search can be performed for the individual frames by using a database

constructed with the data of human antibody amino acid sequences available from the public databases: Kabat Database (ftp://ftp.ebi.ac.uk/pub/databases/kabat/) and IMGT Database (http://imgt.cines.fr/). From the perspectives of reducing the isoelectric point, removing remaining mouse sequences, and improving the stability, human frameworks were selected by searching the database for human framework sequences containing the alterations described above. The result showed that the altered antibody H53/L28 met the requirements described above without decreasing the binding activity or neutralizing activity when its respective frameworks were constituted of the sequences indicated below. SOURCE indicates origins of the human sequences. Underlined amino acid residues in each sequence represent altered amino acids relative to WT.

Table 4

H53	SOURCE	SEQUENCE
FR1	Germline : IMGT_hVH_4_b	QVQLQESGPGLVKPSETLSLTCAVSGYSIS
FR2	Blood 1996 88: 4620-4629	WVRQPPGEGLEWIG
FR3	Germline : IMGT_hVH_4_b (EXCEPT BOLD-INDICATED H71 & H89)	RVTISRDTSKNQFSLKLSSVTAADTAAYYCAR
FR4	J IMMUNOL 142: 4027-4033 (1989)	WGEGTLVTVSS

L28	SOURCE	SEQUENCE
FR1	Immunology. 1988 Aug;64(4):573-9	DIQMTQSPSSLSASVGDSVTITC
FR2	Germline : IMGT_hVk_1D_8	WYQQKPGKAPELLIY
FR3	Germline : IMGT_hVk_8D_41	GVPSRFSGSGSGTDFTFTISSLEAEDAATYYC
FR4	J. Exp. Med. 1997 185: 1435-1446	FGQGTKVEIE

Furthermore, the above-described FR3 of H53 contains a non-human sequence; thus, it is preferable to further reduce the immunogenicity risk. A possible alteration for reducing the immunogenicity risk is a sequence substitution resulting in an exchange of Ala at H89 to Val (SEQ ID NO: 127). Moreover, since Arg at H71 in FR3 of H53 is important for the binding activity (Cancer Res. 1993 Feb 15;53(4):851-6), anti-human IL-6 receptor antibodies containing H and L chains whose frameworks consist of a fully human sequence may be produced by using an FR3 sequence of the human VH1 subclass (SEQ ID NO: 128) or the human VH3 subclass (SEQ ID NO: 129) where Arg at H71 is conserved.

Selection of respective CDR sequences

The respective CDR sequences of H53/L28 were selected as shown below, from the perspectives of reducing the isoelectric point, improving the stability, and removing T-cell

epitopes, and most importantly, not decreasing the binding activity or neutralizing activity.

Table 5

H53	SEQUENCE
CDR1	DDHAWS
CDR2	YISYSGITNYNPSLKG
CDR3	SLARTTAMDY

L28	SEQUENCE
CDR1	QASQDISSYLN
CDR2	YGSELHS
CDR3	QQGNSLPYT

Construction of expression vector for altered antibody, expression and purification of the antibody

An expression vector for altered antibody was constructed, and the antibody was expressed and purified by the method described in Example 1. The humanized mouse PM-1 antibody was successively altered to have the framework and CDR sequences selected for mutagenesis vectors for H(WT) and L(WT) of the antibody. Using the finally obtained H53/L28-encoding animal cell expression vector (antibody amino acid sequences: H53, SEQ ID NO: 104; and L28, SEQ ID NO: 105) having the selected framework and CDR sequences, H53/L28 was expressed and purified, and then used in the assessment described below.

Assessment of altered antibody H53/L28 for the isoelectric point by isoelectric focusing

WT and the altered antibody H53/L28 were analyzed by isoelectric focusing to assess the change in the isoelectric point of the whole antibody caused by the amino acid alterations in the variable regions. The procedure of isoelectric focusing is described below. Using Phastsystem Cassette (Amersham Biosciences), Phast-Gel Dry IEF gel (Amersham Biosciences) was rehydrated for about 30 minutes in the rehydration solution indicated below.

Milli-Q water	1.5 ml
Pharmalyte 5-8 for IEF (Amersham Biosciences)	50 μl
Pharmalyte 8-10.5 for IEF (Amersham Biosciences)	50 μl

Electrophoresis was carried out in PhastSystem (Amersham Biosciences) using the rehydrated gel according to the program indicated below. The samples were loaded onto the gel in Step 2. Calibration Kit for pI (Amersham Biosciences) was used as the pI markers.

Step 1:	2000 V	2.5 mA	3.5 W	15°C	75 Vh
Step 2:	200 V	2.5 mA	3.5 W	15°C	15 Vh
Step 3:	2000 V	2.5 mA	3.5 W	15°C	410 Vh

After electrophoresis, the gel was fixed with 20% TCA, and then silver-stained using the Silver Staining Kit, protein (Amersham Biosciences), according to the protocol attached to the kit. After staining, the isoelectric point of the sample (the whole antibody) was calculated from the known isoelectric points of pI markers. The result showed that the isoelectric point of WT was about 9.3, and the isoelectric point of the altered antibody H53/L28 was about 6.5 to 6.7. The amino acid substitution in WT yielded H53/L28 whose isoelectric point is about 2.7 lowered. The theoretical isoelectric point of the variable regions of H53/L28 (VH and VL sequences) was calculated by GENETYX (GENETYX CORPORATION). The determined theoretical isoelectric point was 4.52. Meanwhile, the theoretical isoelectric point of WT was 9.20. Thus, the amino acid substitution in WT yielded H53/L28 having a variable region whose theoretical isoelectric point is about 4.7 lowered.

Assessment of H53/L28 for the human IL-6 receptor-neutralizing activity

WT and H53/L28 were assessed by the method described in Example 1. The result is shown in Fig. 12. The activity of altered antibody H53/L28 to neutralize BaF/gp130 improved several fold in comparison to WT. Specifically, the comparison of H53/L28 with WT revealed that the isoelectric point could be reduced while improving the neutralizing activity.

Biacore-based analysis of H53/L28 for the affinity for human IL-6 receptor

The affinities of WT and H53/L28 for human IL-6 receptor were assessed by kinetic analysis using Biacore T100 (BIACORE). The antigen-antibody interaction was measured by immobilizing purified Recomb Protein A/G (Pierce) (hereinafter Protein A/G) onto a sensor chip, binding various antibodies onto the chip, and then running the antigen over the chip as an analyte. Various concentrations of recombinant soluble IL-6 receptor (SR344) were used as the antigen. The measurement conditions were the same as described in Example 2.

The sensorgrams obtained for WT and H53/L28 are shown in Fig. 13. Kinetic analysis was carried out using Biacore-specific data analysis software Biacore T100 Evaluation Software. The result is shown in Table 6. The result showed that K_D in H53/L28 was reduced about six-fold compared to WT, and this means the affinity was improved about six-fold. Specifically, the comparison of H53/L28 with WT revealed that the affinity could be improved six-fold while reducing the isoelectric point at the same time. A detailed analysis suggested that the amino acid mutation that contributed to the affinity improvement was the substitution of glycine for threonine at L51. In other words, it is thought that the affinity can be improved by

substituting glycine for threonine at L51.

Table 6

SAMPLE	k_a (1/Ms)	k_d (1/s)	K_D (M)
WT	4.9E+5	2.0E-3	4.0E-9
H53/L28	7.6E+5	5.2E-4	6.8E-10

Prediction of T-cell epitopes in H53/L28 using TEPITOPE

H53/L28 was analyzed by TEPITOPE (Methods. 2004 Dec;34(4):468-75). The result showed that the number of potential HLA-binding peptides was significantly reduced in H53/L28 as compared to WT. This suggests reduction of the immunogenicity risk in human.

[Example 4] Assessment of the plasma retention of H53/L28

Assessment of the altered antibody H53/L28 for its plasma pharmacokinetics in normal mice

To assess the retention in plasma of the altered antibody H53/L28 with reduced isoelectric point, the plasma pharmacokinetics was compared between WT and the altered antibody H53/L28 using normal mice.

A single dose of WT or H53/L28 was intravenously or subcutaneously administered at 1 mg/kg to mice (C57BL/6J; Charles River Japan, Inc.). The blood was collected before administration and 15 minutes, two hours, eight hours, one day, two days, five days, seven days, 14 days, 21 days, and 28 days after administration. Note that the blood was collected at 15 minutes after administration only from the intravenous administration groups. The collected blood was immediately centrifuged at 4°C and 15,000 rpm for 15 minutes to obtain plasma. The separated blood plasma was stored until use in a freezer at -20°C or below.

The concentration in the mouse plasma was determined by ELISA. First, Recombinant Human IL-6 sR (R&D Systems) was biotinylated using EZ-LinkTM Sulfo-NFS-Biotinylation Kit (PIERCE). The biotinylated human-sIL-6R was dispensed into Reacti-Bind Streptavidin High Binding Capacity (HBC) Coated Plates (PIERCE), and then incubated at room temperature for one hour or more. Thus, human-sIL-6R-immobilized plates were prepared as described above. Mouse plasma samples and standard samples (plasma concentrations: 3.2, 1.6, 0.8, 0.4, 0.2, 0.1, and 0.05 μg/ml) were prepared and dispensed into the human-sIL-6R-immobilized plates. The samples were incubated at room temperature for one hour, and then anti-human IgG-AP (SIGMA) was added for reaction. After color development using the BluePhos Microwell Phosphatase Substrates System (Kirkegaard & Perry

Laboratories) as a substrate, the absorbance at 650 nm was measured with a microplate reader. The plasma concentrations in the mice were determined based on the absorbance of the calibration curve using the analytical software SOFTmax PRO (Molecular Devices). The time courses for the plasma concentrations of WT and H53/L28 after intravenous administration and subcutaneous administration are shown in Figs. 14 and 15, respectively.

The obtained plasma concentration-time data were evaluated by a model-independent analysis using the pharmacokinetic analysis software WinNonlin (Pharsight) to estimate pharmacokinetic parameters (AUC, clearance (CL), and half-life (T1/2)). T1/2 was estimated from the plasma concentrations at the last three points or those in the terminal phase automatically selected by WinNonlin. BA was calculated from the ratio of AUC after subcutaneous administration versus AUC after intravenous administration. The determined pharmacokinetic parameters are shown in Table 7.

Table 7

	iv		sc		
	CL mL/h/kg	T1/2 day	CL/F mL/h/kg	T1/2 day	BA %
WT	0.177	18.5	0.180	14.7	113
H53/L28	0.102	23.5	0.086	29.7	121

The half-life (T1/2) of H53/L28 in plasma after intravenous administration was prolonged to about 1.3 times that of WT, while the clearance was reduced about 1.7 times. T1/2 of H53/L28 after subcutaneous administration was prolonged to about twice that of WT, while the clearance was reduced about 2.1 times. Thus, the pharmacokinetics of H53/L28 could be significantly improved by lowering the isoelectric point of WT.

H53/L28 is a humanized anti-IL-6 receptor antibody with improved binding activity and neutralizing activity, reduced immunogenicity risk, and significantly improved pharmacokinetics as compared to the humanized PM-1 antibody (WT). Therefore, the alterations used to create H53/L28 may be very useful in the development of pharmaceuticals.

[Example 5] Preparation of the PF1 antibody

Construction of expression and mutagenesis vectors for the humanized PM-1 antibody

A total of four CDR mutations discovered in Example 2which improve the affinity of RDC_23 (two each in the H and L chains) were introduced into H53/L28 created in Example 4. The H and L chains obtained by introducing the mutations of RDC_23 into H53/L28 were named PF1_H and PF1_L, respectively. The altered antibody was prepared, expressed, and

purified by the method described in Example 1. The amino acid sequences of PF1_H and PF1_L are shown in SEQ ID NOs: 22 and 23, respectively.

Assessment for the human IL-6 receptor-neutralizing activity

The neutralizing activity of the purified PF1 antibody was assessed by the method described in Example 1. The neutralizing activity assessment was carried out using 600 ng/ml human interleukin-6 (TORAY). The neutralizing activities of WT and PF1 are shown in Fig. 16. PF1 was demonstrated to have an activity about 100 to 1000 times higher than WT in terms of 100% inhibitory concentration.

Biacore-based analysis of the PF1 antibody for the affinity for human IL-6 receptor

This measurement was carried out under the same conditions described in Example 2. The running buffer used was HBS-EP+, and the flow rate was 20 μl/min. Each antibody was prepared so that about 100 RU of the antibody was bound to Protein A/G. SR344 was prepared at 0, 0.065, 0.131, and 0.261 μg/ml using HBS-EP+ and used as an analyte. In the first step of the measurement, the antibody in solution was bound to Protein A/G, and the analyte solution was allowed to interact therewith. After three minutes of interaction, the solution was switched to HBS-EP+, and the dissociation phase was monitored for 10 or 15 minutes. After measurement of the dissociation phase, the sensor chip was regenerated by washing with 10 μl of 10 mM glycine-HCl (pH 1.5). The association, dissociation, and regeneration constitute one analysis cycle. Each antibody was measured according to this cycle.

The obtained sensorgram for PF1 is shown in Fig. 17. The sensorgram was kinetically analyzed using the Biacore-specific data analysis software, Biacore T100 Evaluation Software. The result is shown along with those for WT and H53/L28 in Table 8. The result showed that the affinity of PF1 was about 150 times improved as compared to WT. RDC_23 has a high affinity as a result of combination through affinity maturation, and H53/L28 has an enhanced pharmacokinetics and improved affinity. Through combination of both, PF1 obtained a higher affinity than RDC_23 or H53/L28 by an additive effect.

Table 8

SAMPLE	k_a (1/Ms)	k_d (1/s)	K_D (M)
WT	4.9E+05	2.0E-03	4.0E-09
RDC_23	6.4E+05	4.3E-05	6.7E-11
H53/L28	7.6E+05	5.2E-04	6.8E-10
PF1	1.3E+06	3.5E-05	2.7E-11

Assessment of the PF1 antibody for thermal stability by differential scanning calorimetry (DSC)

To assess the thermal stability of the PF1 antibody, the midpoint of thermal denaturation (Tm value) was determined by differential scanning calorimetry (DSC). The purified antibodies of WT and PF1 were dialyzed against a solution of 20 mM sodium acetate, 150 mM NaCl, pH 6.0 (EasySEP, TOMY). DSC measurement was carried out at a heating rate of 1°C/min from 40 to 100°C at a protein concentration of about 0.1 mg/ml. The result showed that the Tm of the WT Fab domain was about 94°C and that of the PF1 Fab domain was 91°C. The Tm of the Fab domain of an IgG1 type antibody molecule is generally within the range of about 60 to 85°C (Biochem. Biophys. Res. Commun. 2007 Apr 13;355(3):751-7; Mol Immunol. 2007 Apr;44(11):3049-60). Thus, the observed thermal stability of the PF1 antibody was extremely high as compared to those of typical IgG1 molecules.

Assessment of the PF1 antibody for stability at high concentrations

The PF1 antibody was assessed for stability in high concentration formulations. Purified WT and PF1 antibodies were dialyzed against a solution of 20 mM histidine chloride, 150 mM NaCl, pH 6.5 (EasySEP, TOMY), and then concentrated by ultrafilters. The antibodies were tested for stability at high concentrations. The conditions were as follows.

Antibodies: WT and PF1

Buffer: 20 mM histidine chloride, 150 mM NaCl, pH 6.0

Concentration: 145 mg/ml

Storage temperature and time period: 25°C for two weeks, 25°C for four weeks, or 25°C for seven weeks

Aggregation assessment method:

System: Waters Alliance

Column: G3000SWxl (TOSOH)

Mobile phase: 50 mM sodium phosphate, 300 mM KCl, pH 7.0

Flow rate, wavelength: 0.5 ml/min, 220 nm

100 times diluted samples were analyzed

The contents of aggregate in the initial formulations (immediately after preparation) and formulations stored under various conditions were evaluated by the gel filtration chromatography described above. Differences (amounts increased) in the content of aggregate relative to the initial formulations are shown in Fig. 18. As a result, the following findings were obtained: (1) both WT and PF1 were very stable; (2) the amount of aggregate increased during seven weeks at 25°C was about 0.7% for WT and about 0.3% for PF1, which means that the amount of aggregate increased per month at 25°C was about 0.4% and about 0.17%, respectively; and (3) PF1 was markedly stable at high concentrations. WO 2003/039485 has disclosed data on the stability of Daclizumab, which is available as a high concentration IgG formulation on the market, at 25°C in a 100 mg/ml preparation. The amount of aggregate increased per month at 25°C is about 0.3% in the formulation of 100 mg/ml Daclizumab. Even when compared to Daclizumab, PF1 exhibits an excellent stability at high concentrations. The increase of the number of aggregates is very problematic in developing high-concentration liquid formulations as pharmaceuticals. The increase of PF1 antibody aggregate was demonstrated to be very small even when the concentration of the PF1 antibody was high.

PF1 is a molecule resulting from alteration of WT. The purposes of the alteration include improvement of the antigen-binding activity, improvement of the pharmacokinetics by lowering its isoelectric point, reduction of the immunogenicity risk by removing T-cell epitopes and remaining mouse sequences, and improvement of the stability. Indeed, the stability of PF1 in 100 mg/ml or higher concentration preparations was demonstrated to be very high even when compared to WT. Stable and highly convenient high-concentration formulations for subcutaneous administration can be provided by using such molecules.

[Example 6] PK/PD test of the PF1 antibody using human IL-6 receptor transgenic mice

Test for pharmacokinetics (*in vivo* kinetics) using human IL-6 receptor transgenic mice

WT and PF1 prepared in Example 5 were assessed for their pharmacokinetics (*in vivo* kinetics) in human IL-6 receptor transgenic mice (hIL-6R tg mice; Proc. Natl. Acad. Sci. U S A. 1995 May 23;92(11):4862-6) and their human soluble IL-6 receptor-neutralizing activity *in vivo*. WT and PF1 were intravenously administered once at 10 mg/kg into hIL-6R tg mice. Blood was collected before administration and 15 minutes, two, four, and eight hours, one day, two, four, and seven days after administration. The blood collected was immediately centrifuged at 4°C and 15,000 rpm for 15 minutes to obtain blood plasma. The separated plasma was stored in a freezer at -20°C or below until use.

The concentrations in the mouse plasma were determined by ELISA. Standard samples were prepared at 6.4, 3.2, 1.6, 0.8, 0.4, 0.2, and 0.1 μg/ml as concentrations in plasma.

Mouse plasma samples and standard samples were dispensed into immunoplates (Nunc-Immuno Plate, MaxiSorp (Nalge nunc International)) coated with Anti-human IgG (γ-chain specific) F(ab')2 (Sigma). The samples were incubated at room temperature for one hour, and then Goat Anti-Human IgG-BIOT (Southern Biotechnology Associates) and Streptavidin-alkaline phosphatase conjugate (Roche Diagnostics) were subsequently added for reaction. After color development using the BluePhos Microwell Phosphatase Substrates System (Kirkegaard & Perry Laboratories) as a substrate, the absorbance at 650 nm was measured with a microplate reader. The concentrations in the mouse plasma were determined based on the absorbance of the calibration curve using the analytical software SOFTmax PRO (Molecular Devices). The time courses for the plasma concentrations of WT and PF1 are shown in Fig. 19. The plasma PF1 concentration four days after administration was about five times higher than WT. This suggests that the pharmacokinetics of PF1 of human IL-6 receptor transgenic mice is improved as compared to WT.

The human IL-6 receptor transgenic mice have been demonstrated to produce plasma circulating human soluble IL-6 receptor. Thus, the human soluble IL-6 receptor-neutralizing efficacy in plasma can be assessed by administering anti-human IL-6 receptor antibodies to human IL-6 receptor transgenic mice.

The concentration of free human soluble IL-6 receptor in mouse plasma was determined to assess the degree of neutralization of human soluble IL-6 receptor by administration of WT or PF1. Six microliters of the mouse plasma was diluted two-fold with a dilution buffer containing BSA. The diluted plasma was loaded onto an appropriate amount of rProtein A Sepharose Fast Flow resin (GE Healthcare) dried in 0.22-μm filter cup (Millipore), and all IgG type antibodies (mouse IgG, anti-human IL-6 receptor antibody, and anti-human IL-6 receptor antibody-human soluble IL-6 receptor complex) in the plasma were adsorbed by Protein A. Then, the solution in the cup was spinned down using a high-speed centrifuge to collect the solution that passed through. Since the solution that passed through does not contain Protein A-bound anti-human IL-6 receptor antibody-human soluble IL-6 receptor complex, the concentration of free soluble IL-6 receptor can be determined by measuring the concentration of human soluble IL-6 receptor in the passed solution. The concentration of soluble IL-6 receptor was determined using Quantikine Human IL-6 sR (R&D Systems). The concentration of free soluble IL-6 receptor in mice was measured 4, 8, 24, 48, 96, and 168 hours after administration of WT or PF1 according to the attached instruction manual.

The result is shown in Fig. 20. In both cases of WT and PF1, the concentration of free soluble IL-6 receptor was 10 ng/ml or less, four hours and up to eight hours after intravenous administration of a single dose of WT or PF1 at 10 mg/kg, indicating that the human soluble IL-6 receptor was neutralized. However, while the concentration of free soluble IL-6 receptor

was about 500 ng/ml 24 hours after WT administration, it was 10 ng/ml or less after PF1 administration. This indicates that PF1 neutralizes human soluble IL-6 receptor in a more sustainable way than WT.

PF1 was created by combining RDC_23 discovered through affinity maturation and H53/L28 exhibiting improved properties such as improved pharmacokinetics, and thus predicted to be able to exhibit prolonged retention in plasma and high neutralizing activity *in vivo*. Indeed, as compared to WT, PF1 was demonstrated to be more sustainable in plasma and to exhibit a prolonged neutralizing effect in human IL-6 receptor transgenic mice producing human soluble IL-6 receptor.

PF1 is more superior than WT (humanized PM-1 antibody) in terms of immunogenicity risk and stability in high concentration preparations, as well as retention in plasma and IL-6 receptor-neutralizing effect in human IL-6 receptor transgenic mice. Thus, the alterations made to create PF1 may be very useful in the development of pharmaceuticals.

[Example 7] Improvement of the stability of IgG2 and IgG4 under acidic condition

Construction of expression vectors for IgG2- or IgG4-converted humanized IL-6 receptor antibodies and expression of the antibodies

To reduce the Fcγ receptor binding, the constant region of humanized PM-1 antibody (Cancer Res. 1993 Feb 15;53(4):851-6), which is of the IgG1 isotype, was substituted with IgG2 or IgG4 (Mol. Immunol. 1993 Jan;30(1):105-8) to generate molecules WT-IgG2 (SEQ ID NO: 109) and WT-IgG4 (SEQ ID NO: 110). An animal cell expression vector was used to express the IgGs. An expression vector, in which the constant region of humanized PM-1 antibody (IgG1) used in Example 1 was digested with *NheI*/*NotI* and then substituted with the IgG2 or IgG4 constant region by ligation, was constructed. The nucleotide sequence of each DNA fragment was determined with a DNA sequencer (ABI PRISM 3730xL DNA Sequencer or ABI PRISM 3700 DNA Sequencer (Applied Biosystems)) using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) according to the attached instruction manual. Using the WT L chain, WT-IgG1, WT-IgG2, and WT-IgG4 were expressed by the method described in Example 1.

(1) Humanized PM-1 antibody (WT-IgG1) H chain, SEQ ID NO: 15 (amino acid sequence)
(2) WT-IgG2 H chain, SEQ ID NO: 109 (amino acid sequence)
(3) WT-IgG4 H chain, SEQ ID NO: 110 (amino acid sequence)

Purification of WT-IgG1, WT-IgG2, and WT-IgG4 through elution from Protein A using hydrochloric acid

50 μl of rProtein A Sepharose™ Fast Flow (Amersham Biosciences) suspended in TBS was added to the obtained culture supernatants, and the combined solutions were mixed by inversion at 4°C for four hours or more. The solutions were transferred into 0.22-μm filter cups of Ultrafree(R)-MC (Millipore). After washing three times with 500 μl of TBS, the rProtein A Sepharose™ resins were suspended in 100 μl of 10 mM HCl/150 mM NaCl (pH 2.0) and the mixtures were incubated for two minutes to elute the antibodies (hydrochloric acid elution). Immediately, the eluates were neutralized by adding 6.7 μl of 1.5 M Tris-HCl (pH 7.8). The elution was carried out twice, yielding 200 μl of purified antibodies.

Gel filtration chromatography analysis of WT-IgG1, WT-IgG2, and WT-IgG4 purified by hydrochloric acid elution

The contents of aggregate in the purified samples obtained by hydrochloric acid elution were assessed by gel filtration chromatography analysis.

Aggregation assessment method:

System: Waters Alliance
Column: G3000SWxl (TOSOH)
Mobile phase: 50 mM sodium phosphate, 300 mM KCl, pH 7.0
Flow rate, wavelength: 0.5 ml/min, 220 nm

The result is shown in Fig. 21. While the content of aggregate in WT-IgG1 after purification was about 2%, those of WT-IgG2 and WT-IgG4 after purification were about 25%. This suggests that IgG1 is stable to acid during hydrochloric acid elution, and by contrast, IgG2 and IgG4 are unstable and underwent denaturation/aggregation. Thus, the stability of IgG2 and IgG4 under acidic condition was demonstrated to be lower than that of IgG1. Protein A has been frequently used to purify IgG molecules, and the IgG molecules are eluted from Protein A under acidic condition. In addition, virus inactivation, which is required when developing IgG molecules as pharmaceuticals, is generally carried out under acidic condition. It is thus desirable that the stability of IgG molecules under acidic condition is higher. However, the stability of IgG2 and IgG4 molecules under acidic condition was found to be lower than that of IgG1, and suggests for the first time that there is a problem of denaturation/aggregation under acidic condition in developing IgG2 and IgG4 molecules as pharmaceuticals. It is desirable that this problem of denaturation/aggregation be overcome when developing them as pharmaceuticals. To date, however, no report has been published on a method for solving this problem through amino acid substitution.

Preparation and assessment of WT-IgG2 and WT-IgG4 having an altered CH3 domain

The stability of IgG2 and IgG4 molecules under acidic condition was demonstrated to

be lower than that of IgG1. Thus, altered forms of IgG2 and IgG4 molecules were tested to improve the stability under acidic condition. According to models for the constant regions of IgG2 and IgG4 molecules, one of the potential destabilizing factors under acidic condition was thought to be the instability at the CH3-CH3 domain interface. As a result of various examinations, methionine at position 397 in the EU numbering system in IgG2, or arginine at position 409 in the EU numbering system in IgG4 was thought to destabilize the CH3/CH3 interface. Then, altered IgG2 and IgG4 antibodies were prepared. An altered IgG2 antibody comprises the substitution of valine for methionine at position 397 in the EU numbering system (IgG2-M397V, SEQ ID NO: 111 (amino acid sequence)) and an altered IgG4 antibody comprises the substitution of lysine for arginine at position 409 in the EU numbering system (IgG4-R409K, SEQ ID NO: 112 (amino acid sequence)).

The methods used for constructing expression vectors for the antibodies of interest, and expressing and purifying the antibodies, were the same as those used for the hydrochloric acid elution described above. Gel filtration chromatography analysis was carried out to estimate the contents of aggregate in the purified samples obtained by hydrochloric acid elution from Protein A.

Aggregation assessment method:

System: Waters Alliance
Column: G3000SWxl (TOSOH)
Mobile phase: 50 mM sodium phosphate, 300 mM KCl, pH 7.0
Flow rate, wavelength: 0.5 ml/min, 220 nm

The result is shown in Fig. 21. While the content of aggregate in WT-IgG1 after purification was about 2%, those in WT-IgG2 and WT-IgG4 after purification were about 25%. By contrast, the contents of aggregate in variants with altered CH3 domain, IgG2-M397V and IgG4-R409K, were comparable (approx. 2%) to that in IgG1. This finding demonstrates that the stability of an IgG2 or IgG4 antibody under acidic condition can be improved by substituting valine for methionine of IgG2 at position 397 in the EU numbering system or lysine for arginine of IgG4 at position 409 in the EU numbering system, respectively. Furthermore, the midpoint temperatures of thermal denaturation of WT-IgG2, WT-IgG4, IgG2-M397V, and IgG4-R409K were determined by the same method as described in Example 5. The result showed that the Tm value for the altered CH3 domain was higher in IgG2-M397V and IgG4-R409K as compared to WT-IgG2 and WT-IgG4, respectively. This suggests that IgG2-M397V and IgG4-R409K are also superior in terms of thermal stability as compared to WT-IgG2 and WT-IgG4, respectively.

IgG2 and IgG4 are exposed to acidic condition in virus inactivation process and in the purification process using Protein A. Thus, denaturation/aggregation in the above processes

was problematic. However, it was discovered that the problem could be solved by using IgG2-M397V and IgG4-R409K for the sequences of IgG2 and IgG4 constant regions. Thus, these alterations were revealed to be very useful in developing IgG2 and IgG4 antibody pharmaceuticals. Furthermore, the usefulness of IgG2-M397V and IgG4-R409K was also demonstrated by the finding that they are superior in thermal stability.

[Example 8] Improvement of heterogeneity derived from disulfide bonds in IgG2

Purification of WT-IgG1, WT-IgG2, and WT-IgG4 through acetic acid elution from Protein A

50 µl of rProtein A Sepharose™ Fast Flow (Amersham Biosciences) suspended in TBS was added to the culture supernatants obtained in Example 7, and the combined solutions were mixed by inversion at 4°C for four hours or more. The solutions were transferred into 0.22-µm filter cups of Ultrafree®-MC (Millipore). After washing three times with 500 µl of TBS, the rProtein A Sepharose™ resins were suspended in 100 µl of aqueous solution of 50 mM sodium acetate (pH 3.3) and the mixtures were incubated for two minutes to elute the antibodies. Immediately, the eluates were neutralized by adding 6.7 µl of 1.5 M Tris-HCl (pH 7.8). The elution was carried out twice, yielding 200 µl of purified antibodies.

Analysis of WT-IgG1, WT-IgG2, and WT-IgG4 by cation exchange chromatography (IEC)

Purified WT-IgG1, WT-IgG2, and WT-IgG4 were analyzed for homogeneity by cation exchange chromatography.

Assessment method using IEC:

System: Waters Alliance

Column: ProPac WCX-10 (Dionex)

Mobile phase A: 25 mM MES-NaOH, pH 6.1

B: 25 mM MES-NaOH, 250 mM Na-Acetate, pH 6.1

Flow rate, wavelength: 0.5 ml/min, 280 nm

GradientB: 50%-75% (75 min) in the analysis of WT-IgG1

B: 30%-55% (75 min) in the analysis of WT-IgG2 and WT-IgG4

The result is shown in Fig. 22. WT-IgG2 showed more than one peak in the ion exchange analysis while WT-IgG1 and WT-IgG4 exhibited a single peak. This suggests that the IgG2 molecule is more heterogeneous as compared to IgG1 and IgG4. Indeed, IgG2 isotypes have been reported to have heterogeneity derived from disulfide bonds in the hinge region (Non-patent Document 30). Thus, the hetero-peaks of IgG2 shown in Fig. 22 are also assumed to be objective substance/related substances derived from the disulfide bonds. It is not easy to manufacture them as a pharmaceutical in large-scale while maintaining the objective

substances/related substances related heterogeneity between productions. Thus, homogeneous (less heterogeneous) substances are desirable as much as possible for antibody molecules to be developed as pharmaceuticals. For wild type IgG2, there is a problem of homogeneity which is important in developing antibody pharmaceuticals. Indeed, US20060194280 (A1) has shown that natural IgG2 gives various hetero-peaks as a result of the disulfide bonds in ion exchange chromatography analysis, and that the biological activity varies among these peaks. US20060194280 (A1) reports refolding in the purification process as a method for combining the hetero-peaks into a single one, but use of such a process in the production is costly and complicated. Thus, a preferred method for combining the hetero-peaks into a single one is based on amino acid substitution. Although the heterogeneity originated from disulfide bonds in the hinge region should be overcome to develop IgG2 as pharmaceuticals, no report has been published to date on a method for solving this problem through amino acid substitution.

Preparation and assessment of altered WT-IgG2 CH1 domain and hinge region

As shown in Fig. 23, there are various potential disulfide bond patterns for an IgG2 molecule. Possible causes of the heterogeneity derived from the hinge region of IgG2 were differential pattern of disulfide bonding and free cysteines. IgG2 has two cysteines (at positions 219 and 220 in the EU numbering system) in the upper hinge region, and cysteines adjacent to the two upper-hinge cysteines include cysteine at position 131 in the EU numbering system in the H chain CH1 domain and L chain C-terminal cysteine, and two corresponding cysteines in the H chain upper hinge of the dimerization partner. Specifically, there are eight cysteines in total in the vicinity of the upper hinge region of IgG2 when the antibody is in the associated form of H2L2. This may be the reason for the various heterogeneous patterns due to wrong disulfide bonding and free cysteines.

The hinge region sequence and CH1 domain of IgG2 were altered to reduce the heterogeneity originated from the IgG2 hinge region. Examinations were conducted to avoid the heterogeneity of IgG2 due to differential pattern of disulfide bonding and free cysteines. The result of examining various altered antibodies suggested that the heterogeneity could be avoided without decreasing the thermal stability by substituting serine and lysine for cysteine and arginine at positions 131 and 133 in the EU numbering system, respectively, in the H chain CH1 domain, and substituting serine for cysteine at position 219, EU numbering, in the upper hinge of H chain of the wild type IgG2 constant region sequence (hereinafter IgG2-SKSC) (IgG2-SKSC, SEQ ID NO: 120). These substitutions would enable IgG2-SKSC to form a homogenous covalent bond between H and L chains, which is a disulfide bond between the C-terminal cysteine of the L chain and cysteine at position 220 in the EU numbering system (Fig. 24).

The methods described in Example 1 were used to construct an expression vector for IgG2-SKSC and to express and purify IgG2-SKSC. The purified IgG2-SKSC and wild type IgG2 (WT-IgG2) were analyzed for homogeneity by cation exchange chromatography.
Assessment method using IEC:

System: Waters Alliance
Column: ProPac WCX-10 (Dionex)
Mobile phase A: 25 mM MES-NaOH, pH 5.6
B: 25 mM MES-NaOH, 250 mM Na-Acetate, pH 5.6
Flow rate, wavelength: 0.5 ml/min, 280 nm
Gradient B: 50%-100% (75 min)

The result is shown in Fig. 25. As expected above, IgG2-SKSC was shown to be eluted at a single peak while WT-IgG2 gave multiple peaks. This suggests that the heterogeneity derived from disulfide bonds in the hinge region of IgG2 can be avoided by using alterations such as those used to generate IgG2-SKSC, which allow formation of a single disulfide bond between the C-terminal cysteine of the L chain and cysteine at position 220 in the EU numbering system. The midpoint temperatures of thermal denaturation of WT-IgG1, WT-IgG2, and IgG2-SKSC were determined by the same methods as described in Example 5. The result showed that WT-IgG2 gave a peak for Fab domain which has a lower Tm value than WT-IgG1, while IgG2-SKSC did not give such a peak. This suggests that IgG2-SKSC is also superior in thermal stability as compared to WT-IgG2.

Although wild type IgG2 was thought to have a homogeneity problem which is important in developing antibody pharmaceuticals, it was found that this problem could be solved by using IgG2-SKSC for the constant region sequence of IgG2. Thus, IgG2-SKSC is very useful in developing IgG2 antibody pharmaceuticals. Furthermore, the usefulness of IgG2-SKSC was also demonstrated by the finding that it is superior in thermal stability.

[Example 9] Improvement of C-terminal heterogeneity in IgG molecules

Construction of an expression vector for H chain C-terminal ΔGK antibody from WT-IgG1

For heterogeneity of the C-terminal sequences of an antibody, deletion of C-terminal amino acid lysine residue, and amidation of the C-terminal amino group due to deletion of both of the two C-terminal amino acids, glycine and lysine, have been reported (Non-patent Document 32). The absence of such heterogeneity is preferred when developing antibody pharmaceuticals. Actually, in humanized PM-1 antibody TOCILIZUMAB, the major component is the sequence that lacks the C-terminal amino acid lysine, which is encoded by the nucleotide sequence but deleted in post-translational modification, and the minor component

having the lysine also coexists as heterogeneity. Thus, the C-terminal amino acid sequence was altered to reduce the C-terminal heterogeneity. Specifically, the present inventors altered the nucleotide sequence of wild type IgG1 to delete the C-terminal lysine and glycine from the H chain constant region of the IgG1, and assessed whether the amidation of the C-terminal amino group could be suppressed by deleting the two C-terminal amino acids glycine and lysine.

Mutations were introduced into the C-terminal sequence of the H chain using pB-CH vector encoding the humanized PM-1 antibody (WT) obtained in Example 1. The nucleotide sequence encoding Lys at position 447 and/or Gly at position 446 in the EU numbering system was converted into a stop codon by introducing a mutation using the QuikChange Site-Directed Mutagenesis Kit (Stratagene) according to the method described in the attached instruction manual. Thus, expression vectors for antibody engineered to lack the C-terminal amino acid lysine (position 447 in the EU numbering system) and antibody engineered to lack the two C-terminal amino acids glycine and lysine (positions 446 and 447 in the EU numbering system, respectively) were constructed. H chain C-terminal ΔK and ΔGK antibodies were obtained by expressing the engineered H chains and the L chain of the humanized PM-1 antibody. The antibodies were expressed and purified by the method described in Example 1.

Purified H chain C-terminal ΔGK antibody was analyzed by cation exchange chromatography according to the following procedure. The effect of the C-terminal deletion on heterogeneity was assessed by cation exchange chromatography analysis using the purified H chain C-terminal ΔGK antibody according to the method described below. The conditions of cation exchange chromatography analysis are described below. Chromatograms for humanized PM-1 antibody, H chain C-terminal ΔK antibody, and H chain C-terminal ΔGK antibody were compared.

Column: ProPac WCX-10, 4 x 250 mm (Dionex)
Mobile phase A: 25 mmol/l MES/NaOH, pH 6.1
B: 25 mmol/l MES/NaOH, 250 mmol/l NaCl, pH 6.1
Flow rate: 0.5 ml/min
Gradient: 25% B (5 min) -> (105 min) -> 67% B -> (1 min) -> 100% B (5 min)
Detection: 280 nm

The analysis result for the non-altered humanized PM-1 antibody, H chain C-terminal ΔK antibody, and H chain C-terminal ΔGK antibody is shown in Fig. 26. According to Non-patent Document 30, a basic peak with more prolonged retention time than that of the main peak contains an H chain C terminus with Lys at position 449 and an H chain C terminus with amidated Pro at position 447. The intensity of the basic peak was significantly reduced in the H chain C-terminal ΔGK antibody, while no such significant reduction was observed in the H chain C-terminal ΔK antibody. This suggests that the C-terminal heterogeneity of the H chain can be

reduced only when the two C-terminal amino acids are deleted from the H chain.

The temperature of thermal denaturation of the H chain C-terminal ΔGK antibody was determined by DSC to assess the effect of the deletion of the two residues at the H chain C terminus on thermal stability. For the DSC measurement, the antibody was dialyzed against 20 mM acetic acid buffer (pH 6.0) containing 150 mM NaCl to change the buffer. After thorough deaeration, the humanized PM-1 antibody and H chain C-terminal ΔGK antibody solutions, and the reference solution (outer dialysate) were enclosed in calorimetric cells, and thoroughly thermally equilibrated at 40°C. Then, the samples were scanned at from 40 to 100°C with a rate of about 1K/min. The resulting denaturation peaks were assigned (Rodolfo *et al.*, Immunology Letters, 1999, p 47-52). The result showed that the C-terminal deletion had no effect on the thermal denaturation temperature of CH3 domain.

Thus, the heterogeneity originated from the C-terminal amino acid can be reduced without affecting the thermal stability of antibody by deleting the C-terminal lysine and glycine from the H chain constant region at the nucleotide sequence level. Since all of the constant regions of human antibodies IgG1, IgG2, and IgG4 contain Gly and Lys at positions 446 and 447 in the EU numbering system in their C-terminal sequences, the method for reducing the C-terminal amino acid heterogeneity discovered in this example and others is also expected to be applicable to IgG2 and IgG4 constant regions and variants thereof.

[Example 10] Construction of M14ΔGK with a novel optimized constant region sequence

When an antibody pharmaceutical is aimed at neutralizing an antigen, effector functions such as ADCC of Fc domain are unnecessary and therefore the binding to Fcγ receptor is unnecessary. The binding to Fcγ receptor is assumed to be unfavorable from the perspectives of immunogenicity and adverse effect (Non-patent Documents 24 and 25). The humanized anti-IL-6 receptor IgG1 antibody TOCILIZUMAB does not need to bind to Fcγ receptor, because it only needs to specifically bind to IL-6 receptor and neutralize its biological activity in order to be used as a therapeutic agent for diseases associated with IL-6, such as rheumatoid arthritis.

Construction and assessment of M14ΔGK, a Fcγ receptor-nonbinding, optimized constant region

A possible method for impairing the Fcγ receptor binding is to convert the IgG antibody from IgG1 isotype to IgG2 or IgG4 isotype (Ann. Hematol. 1998 Jun;76(6):231-48). As a method for completely eliminating the binding to Fcγ receptor, a method of introducing an artificial alteration into Fc domain has been reported. For example, since the effector functions of anti-CD3 antibody and anti-CD4 antibody cause adverse effects, amino acid mutations that are not present in the wild type sequence have been introduced into the Fcγ receptor-binding

region of Fc domain (Non-patent Documents 26 and 27), and the resulting Fcγ receptor-nonbinding anti-CD3 and anti-CD4 antibodies are under clinical trials (Non-patent Documents 24 and 28). According to another report (Patent Document 6), Fcγ receptor-nonbinding antibodies can be prepared by converting the FcγR-binding domain of IgG1 (at positions 233, 234, 235, 236, 327, 330, and 331 in the EU numbering system) into the sequence of IgG2 (at positions 233, 234, 235, and 236 in the EU numbering system) or IgG4 (at positions 327, 330, and 331 in the EU numbering system). However, if all of the above mutations are introduced into IgG1, novel peptide sequences of nine amino acids, which potentially serve as non-natural T-cell epitope peptides, will be generated, and this increases the immunogenicity risk. The immunogenicity risk should be minimized in developing antibody pharmaceuticals.

To overcome the above problem, alterations in the IgG2 constant region were considered. In the FcγR-binding domain of IgG2 constant region, residues at positions 327, 330, and 331 in the EU numbering system are different from the nonbinding sequence of IgG4 while those at positions 233, 234, 235, and 236 in the EU numbering system are amino acids of nonbinding type. Thus, it is necessary to alter the amino acids at positions 327, 330, and 331 in the EU numbering system to the sequence of IgG4 (G2Δa described in Eur. J. Immunol. 1999 Aug;29(8):2613-24). However, since the amino acid at position 339 in the EU numbering system in IgG4 is alanine while the corresponding residue in IgG2 is threonine, a simple alteration of the amino acids at positions 327, 330, and 331 in the EU numbering system to the sequence of IgG4 unfavorably generates a novel peptide sequence of 9 amino acids, potentially serving as a non-natural T-cell epitope peptide, and thus increases the immunogenicity risk, which is unpreferable. Then, the present inventors found that the generation of novel peptide sequence could be prevented by introducing the substitution of alanine for threonine at position 339 in the EU numbering system in IgG2, in addition to the alteration described above.

In addition to the mutations described above, other mutations were introduced, and they were the substitution of valine for methionine at position 397 in the EU numbering system in IgG2, which was discovered in Example 7 to improve the stability of IgG2 under acidic condition; and the substitution of serine for cysteine at position 131 in the EU numbering system, the substitution of lysine for arginine at position 133 in the EU numbering system, and the substitution of serine for cysteine at position 219 in the EU numbering system, which were discovered in Example 8 to improve the heterogeneity originated from disulfide bonds in the hinge region. Furthermore, since the mutations at positions 131 and 133 generate a novel peptide sequence of 9 amino acids, potentially serving as a non-natural T-cell epitope peptide, and thus generate the immunogenicity risk, the peptide sequence around positions 131 to 139 was converted into a natural human sequence by introducing the substitution of glycine for

glutamic acid at position 137 in the EU numbering system and the substitution of glycine for serine at position 138 in the EU numbering system. Furthermore, glycine and lysine at positions 446 and 447 in the EU numbering system were deleted from the C terminus of H chain to reduce the C-terminal heterogeneity. The constant region sequence having all of the mutations introduced was named M14ΔGK (M14ΔGK, SEQ ID NO: 24). Although there is a mutation of cysteine at position 219 to serine in M14ΔGK as a novel 9-amino acid peptide sequence which potentially serves as a T-cell epitope peptide, the immunogenicity risk was considered very low since the amino acid property of serine is similar to that of cysteine. The immunogenicity prediction by TEPITOPE also suggested that there was no difference in immunogenicity.

An expression vector for the antibody H chain sequence whose variable region was WT and constant region was M14ΔGK (M14ΔGK, SEQ ID NO: 24; WT-M14ΔGK, SEQ ID NO: 113) was constructed by the method described in Example 1. An antibody having WT-M14ΔGK as H chain and WT as L chain was expressed and purified by the method described in Example 1.

Furthermore, WT-M17ΔGK (M17ΔGK, SEQ ID NO: 116; WT-M17ΔGK, SEQ ID NO: 115) was constructed with the same method by introducing mutations into the IgG1 constant region at positions 233, 234, 235, 236, 327, 330, 331, and 339 in the EU numbering system (G1Δab described in Eur. J. Immunol. 1999 Aug;29(8):2613-24) to impair the Fcγ receptor binding and by deleting the amino acids at positions 446 and 447 in the EU numbering system to reduce the C-terminal heterogeneity (Example 9). An expression vector for WT-M11ΔGK (M11ΔGK, SEQ ID NO: 25; WT-M11ΔGK, SEQ ID NO: 114) was constructed. In WT-M11ΔGK, mutations were introduced into the IgG4 constant region at positions 233, 234, 235, and 236 in the EU numbering system (G4Δb described in Eur. J. Immunol. 1999 Aug;29(8):2613-24; this alteration newly generates non-human sequence and thus increases the immunogenicity risk) to reduce the Fcγ receptor binding. In addition to the above alteration, to reduce the immunogenicity risk, mutations were introduced at positions 131, 133, 137, 138, 214, 217, 219, 220, 221, and 222 in the EU numbering system so that the pattern of disulfide bonding in the hinge region was the same as that of M14ΔGK; a mutation was introduced at position 409 in the EU numbering system (Example 7) to improve the stability under acidic condition; and the amino acids at positions 446 and 447 in the EU numbering system were deleted (Example 9) to reduce the C-terminal heterogeneity. WT-M17ΔGK or WT-M11ΔGK was used as the H chain, and WT was used as the L chain. These antibodies were expressed and purified by the method described in Example 1.

Assessment of WT-M14ΔGK, WT-M17ΔGK, and WT-M11ΔGK for Fcγ receptor binding

The FcγRI binding was assessed by the procedure described below. Using Biacore T100, human-derived Fcγ receptor I (hereinafter FcγRI) immobilized onto a sensor chip was allowed to interact with IgG1, IgG2, IgG4, M11ΔGK, M14ΔGK, or M1ΔGK 7 as an analyte. The amounts of bound antibody were compared. The measurement was conducted using Recombinant Human FcRIA/CD64 (R&D systems) as human-derived FcγRI, and IgG1, IgG2, IgG4, M11ΔGK, M14ΔGK, and M17ΔGK as samples. FcγRI was immobilized onto the sensor chip CM5 (BIACORE) by the amine coupling method. The final amount of immobilized hFcγRI was about 13000 RU. The running buffer used was HBS-EP+, and the flow rate was 20 μl/min. The sample concentration was adjusted to 100 μg/ml using HBS-EP+. The analysis included two steps: two minutes of association phase where 10 μl of an antibody solution was injected and the subsequent four minutes of dissociation phase where the injection was switched with HBS-EP+. After the dissociation phase, the sensor chip was regenerated by injecting 20 μl of 5 mM sodium hydroxide. The association, dissociation, and regeneration constitute one analysis cycle. Various antibody solutions were injected to obtain sensorgrams. As analytes, IgG4, IgG2, IgG1, M11, M14, and M17 were injected in this order. This series of injection was repeated twice. The result of comparison of data on the determined amounts of bound antibody is shown in Fig. 27. The comparison shows that the amount of bound antibody is reduced in the order of: IgG1 > IgG4 >> IgG2 = M11ΔGK = M14ΔGK = M17ΔGK. Thus, it was revealed that the FcγRI binding of wild type IgG2, M11ΔGK, M14ΔGK, and M17ΔGK was weaker than that of wild type IgG1 and IgG4.

The FcγRIIa binding was assessed by the procedure described below. Using Biacore T100, human-derived Fcγ receptor IIa (hereinafter FcγRIIa) immobilized onto a sensor chip was allowed to interact with IgG1, IgG2, IgG4, M11ΔGK, M14ΔGK, or M17ΔGK as an analyte. The amounts of bound antibody were compared. The measurement was conducted using Recombinant Human FcRIIA/CD32a (R&D systems) as human-derived FcγRIIa, and IgG1, IgG2, IgG4, M11ΔGK, M14ΔGK, and M17ΔGK as samples. FcγRIIa was immobilized onto the sensor chip CM5 (BIACORE) by the amine coupling method. The final amount of immobilized FcγRIIa was about 3300 RU. The running buffer used was HBS-EP+, and the flow rate was 20 μl/min. Then, the running buffer was injected until the baseline was stabilized. The measurement was carried out after the baseline was stabilized. The immobilized FcγRIIa was allowed to interact with an antibody of each IgG isotype (IgG1, IgG2, or IgG4) or antibody introduced with mutations (M11ΔGK, M14ΔGK, or M17ΔGK) as an analyte. The amount of bound antibody was observed. The running buffer used was HBS-EP+, and the flow rate was 20 μl/min. The measurement temperature was 25°C. The concentration of each IgG or altered form thereof was adjusted to 100 μg/ml. 20 μl of an analyte was injected and allowed to interact with the immobilized FcγRIIa. After interaction, the analyte was dissociated from

FcγRIIa and the sensor chip was regenerated by injecting 200 μl of the running buffer. As analytes, IgG4, IgG2, IgG1, M11ΔGK, M14ΔGK, and M17ΔGK were injected in this order. This series of injection was repeated twice. The result of comparison of data on the amounts of bound antibody determined is shown in Fig. 28. The comparison shows that the amount of bound antibody is reduced in the order of: IgG1 > IgG2 = IgG4 > M11ΔGK = M14ΔGK = M17ΔGK. Thus, it was revealed that the FcγRIIa binding of M11ΔGK, M14ΔGK, and M17ΔGK was weaker than that of wild type IgG1, IgG2, and IgG4.

The FcγRIIb binding was assessed by the procedure described below. Using Biacore T100, human-derived Fcγ receptor IIb (hereinafter FcγRIIb) immobilized onto a sensor chip was allowed to interact with IgG1, IgG2, IgG4, M11ΔGK, M14ΔGK, or M17ΔGK as an analyte. The amounts of bound antibody were compared. The measurement was conducted using Recombinant Human FcRIIB/C (R&D systems) as human-derived FcγRIIb, and IgG1, IgG2, IgG4, M11ΔGK, M14ΔGK, and M17ΔGK as samples. FcγRIIb was immobilized onto the sensor chip CM5 (BIACORE) by the amine coupling method. The final amount of immobilized FcγRIIb was about 4300 RU. Then, the running buffer was injected until the baseline was stabilized. The measurement was carried out after the baseline was stabilized. The immobilized FcγRIIb was allowed to interact with an antibody of each IgG isotype (IgG1, IgG2, or IgG4) or antibody introduced with mutations (M11ΔGK, M14ΔGK, or M17ΔGK) as an analyte. The amount of bound antibody was observed. The running buffer used was HBS-EP+ (10 mM HEPES, 0.15 M NaCl, 3 mM EDTA, 0.05% v/v Surfactant P20), and the flow rate was 20 μl/min. The measurement temperature was 25°C. The concentration of each IgG or altered form thereof was adjusted to 200 μg/ml. 20 μl of an analyte was injected and allowed to interact with the immobilized FcγRIIb. After interaction, the analyte was dissociated from FcγRIIb and the sensor chip was regenerated by injecting 200 μl of the running buffer. As analytes, IgG4, IgG2, IgG1, M11ΔGK, M14ΔGK, and M17ΔGK were injected in this order. This series of injection was repeated twice. The result of comparison of data on the amounts of bound antibody determined is shown in Fig. 29. The comparison shows that the amount of bound antibody is reduced in the order of: IgG4 > IgG1 > IgG2 > M11ΔGK = M14ΔGK = M17ΔGK. Thus, it was revealed that the FcγRIIb binding of M11ΔGK, M14ΔGK, and M17ΔGK was weaker than that of wild type IgG1, IgG2, and IgG4.

The FcγRIIIa binding was assessed by the procedure described below. Using Biacore T100, human-derived Fcγ receptor IIIa (hereinafter FcγRIIIa) immobilized onto a sensor chip was allowed to interact with IgG1, IgG2, IgG4, M11ΔGK, M14ΔGK, or M17ΔGK as an analyte. The amounts of bound antibody were compared. The measurement was conducted using hFcγRIIIaV-His6 (recombinant hFcγRIIIaV-His6 prepared in the applicants' company) as human-derived FcγRIIIa, and IgG1, IgG2, IgG4, M11ΔGK, M14ΔGK, and M17ΔGK as samples.

FcγRIIIa was immobilized onto the sensor chip CM5 (BIACORE) by the amine coupling method. The final amount of immobilized hFcγRIIIaV-His6 was about 8200 RU. The running buffer used was HBS-EP+, and the flow rate was 5 μl/min. The sample concentration was adjusted to 250 μg/ml using HBS-EP+. The analysis included two steps: two minutes of association phase where 10 μl of an antibody solution was injected and the subsequent four minutes of dissociation phase where the injection was switched with HBS-EP+. After the dissociation phase, the sensor chip was regenerated by injecting 20 μl of 5 mM hydrochloric acid. The association, dissociation, and regeneration constitute one analysis cycle. Various antibody solutions were injected to obtain sensorgrams. As analytes, IgG4, IgG2, IgG1, M11ΔGK, M14ΔGK, and M17ΔGK were injected in this order. The result of comparison of data on the determined amounts of bound antibody is shown in Fig. 30. The comparison shows that the amount of bound antibody is reduced in the order of: IgG1 >> IgG4 > IgG2 > M17ΔGK > M11ΔGK = M14ΔGK. Thus, it was revealed that the FcγRIIIa binding of M11ΔGK, M14ΔGK, and M17ΔGK was weaker than that of wild type IgG1, IgG2, and IgG4. Furthermore, the FcγRIIIa binding of M11ΔGK and M14ΔGK was found to be weaker than that of M17ΔGK containing the mutation G1Δab reported in Eur. J. Immunol. 1999 Aug;29(8):2613-24.

The finding described above demonstrates that the Fcγ receptor binding of WT-M14ΔGK, WT-M17ΔGK, and WT-M11ΔGK is markedly reduced as compared to wild type IgG1. The immunogenicity risk due to Fcγ receptor-mediated internalization into APC and adverse effects caused by the effector function such as ADCC can be avoided by using WT-M14ΔGK, WT-M17ΔGK, or WT-M11ΔGK as a constant region. Thus, WT-M14ΔGK, WT-M17ΔGK, and WT-M11ΔGK are useful as constant region sequence of antibody pharmaceuticals aimed at neutralizing antigens.

Assessment of WT-M14ΔGK, WT-M17ΔGK, and WT-M11ΔGK for stability at high concentrations

WT-M14ΔGK, WT-M17ΔGK, and WT-M11ΔGK were assessed for stability at high concentrations. The purified antibodies of WT-IgG1, WT-M14ΔGK, WT-M17ΔGK, and WT-M11ΔGK were dialyzed against a solution of 20 mM histidine chloride, 150 mM NaCl, pH 6.5 (EasySEP, TOMY), and then concentrated by ultrafilters. The antibodies were tested for stability at high concentrations. The conditions were as follows.

Antibodies: WT-IgG1, WT-M14ΔGK, WT-M17ΔGK, and WT-M11ΔGK

Buffer: 20 mM histidine chloride, 150 mM NaCl, pH 6.5

Concentration: 61 mg/ml

Storage temperature and time period: 40°C for two weeks, 40°C for one month, 40°C for two months

Aggregation assessment method:

System: Waters Alliance

Column: G3000SWxl (TOSOH)

Mobile phase: 50 mM sodium phosphate, 300 mM KCl, pH 7.0

Flow rate, wavelength: 0.5 ml/min, 220 nm

100 times diluted samples were analyzed

The contents of aggregate in the initial formulations (immediately after preparation) and formulations stored under various conditions were estimated by gel filtration chromatography described above. Differences (amounts increased) in the content of aggregate relative to the initial formulations are shown in Fig. 31. The result showed that the amounts of aggregate in WT-M14ΔGK, WT-M17ΔGK, and WT-M11ΔGK increased only slightly as compared to WT-IgG1 and were about half of the content in WT. Furthermore, as shown in Fig. 32, the amount of increased Fab fragment was comparable between WT-IgG1 and WT-M17ΔGK, while the amounts increased in WT-M14ΔGK and WT-M11ΔGK were about one quarter of the amount in WT. Degeneration pathways of IgG type antibody formulations include formation of aggregate and generation of Fab degradate as described in WO 2003/039485. Based on the two criteria, aggregation and Fab fragment generation, WT-M14ΔGK and WT-M11ΔGK were demonstrated to have a superior stability in formulations as compared to WT-IgG1. Thus, even for antibodies that have an IgG1 constant region with poor stability and could not be prepared as antibody pharmaceuticals in high-concentration liquid formulations, the use of WT-M14ΔGK, WT-M17ΔGK, or WT-M11ΔGK as a constant region was expected to allow production of more stable high-concentration liquid formulations.

In particular, M14ΔGK was expected to be very useful as a novel constant region sequence that would (1) overcome the instability of the original IgG2 molecule under acidic condition; (2) improve the heterogeneity originated from disulfide bonds in the hinge region; (3) not bind to Fcγ receptor; (4) have a minimized number of novel peptide sequences of 9 amino acids which potentially serve as T-cell epitope peptides; and (5) have a better stability than IgG1 in high-concentration formulations.

[Example 11] Preparation of PF1-M14ΔGK antibody

The variable region of PF1 (whose constant region is IgG1) constructed in Example 5 was excised using *Xho*I and *Nhe*I. The constant region of M14ΔGK (whose variable region is WT) constructed in Example 7 was excised using *Nhe*I and *Not*I. The two antibody H chain gene fragments were inserted into an animal cell expression vector to construct an expression vector for the H chain of interest, PF1-M14ΔGK (PF1_H-M14ΔGK, SEQ ID NO: 117). The L chain used was PF1_L. The antibody PF1-M14ΔGK was expressed and purified by the method

described in Example 1.

The antibody PF1-M14ΔGK was superior in various aspects as compared to WT (humanized PM-1 antibody) and thus expected to be very useful as anti-IL-6 receptor antibody pharmaceuticals.

[Example 12] Preparation and assessment of M31ΔGK

M14ΔGK prepared in Example 10 was altered by substituting the IgG2 sequence for the amino acids at positions 330, 331, and 339 in the EU numbering system to construct M31ΔGK (M31ΔGK, SEQ ID NO: 118). An expression vector for a sequence of antibody H chain whose variable region is WT and constant region sequence is M31ΔGK (WT-M31ΔGK, SEQ ID NO: 119) was constructed by the method described in Example 1. Using WT-M31ΔGK H chain and WT L chain, WT-M31 was expressed and purified by the method described in Example 1.

In addition to WT-M31, WT-IgG2 and WT-M14ΔGK were expressed and purified at the same time, and analyzed by cation exchange chromatography by the procedure described below. The conditions used in the cation exchange chromatography analysis were as follows. Chromatograms for WT-IgG2, WT-M14ΔGK, and WT-M31ΔGK were compared.

Column: ProPac WCX-10, 4 x 250 mm (Dionex)
Mobile phase A: 25 mmol/l MES/NaOH, pH 6.1
B: 25 mmol/l MES/NaOH, 250 mmol/l NaCl, pH 6.1
Flow rate: 0.5 ml/min
Gradient: 0% B (5 min) -> (65 min) -> 100% B -> (1 min)
Detection: 280 nm

The analysis result for WT-IgG2, WT-M14ΔGK, and WT-M31ΔGK is shown in Fig. 33. Like WT-M14ΔGK, WT-M31ΔGK was demonstrated to be eluted as a single peak, while WT-IgG2 gave multiple peaks. This indicates that the heterogeneity derived from disulfide bonds in the hinge region of IgG2 can also be avoided in WT-M31ΔGK.

[Example 13] Preparation of a fully humanized antibody F2H/L39-IgG1

Full humanization of the framework sequence of the PF1 antibody

Arginine at position 71 (Kabat's numbering system; Kabat EA *et al.* 1991. Sequences of Proteins of Immunological Interest. NIH) is the only mouse sequence that remains in PF1_H prepared in Example 5. This is unpreferable from the perspective of immunogenicity. In general, the residue at position 71 in the H chain is an important sequence for the conformation of HCDR2. In fact, it has been reported that during generation of the humanized PM1 antibody, the residue at position 71 is essential for the binding activity of mouse PM1 antibody. The

binding activity was demonstrated to be significantly reduced by substituting valine at position 71 (Cancer Research 53, 851-856, 1993). Meanwhile, PF1_H is classified into the VH4 family of human germ line genes, and valine at position 71 is highly conserved in the VH4 family. The neutralizing activity was also demonstrated to be significantly reduced by substituting valine for arginine at position 71.

Thus, to completely remove the mouse sequence while maintaining the arginine at position 71, the present inventors searched among sequences of human germ line genes and reported human antibodies for sequences that have arginine at position 71 and share conserved residues important for the maintenance of antibody tertiary structure. As a result, the inventors discovered a candidate sequence which contains important conserved residues although its homology to PF1_H is low as shown in Table 9.

Table 9

KABAT NUMBERING	66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 82a 82b 82c 83 84 85 86 87 88 89 90 91 92 93 94	source
PF1_H CANDIDATE SEQUENCE	R V T I S R D T S K N Q F S L K L S S V T A A D T A A Y Y C A R	Germline: IMGT_hVH_4_b (EXCEPT H71&H89)
	R V T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A R	Mol. Immunol. 44(4):412-422 (2007)

H96-IgG1 (amino acid sequence of SEQ ID NO: 134) was designed by substituting the above-described candidate sequence for the region of positions 66 to 94 in PF1_H-IgG1, Kabat's numbering. The antibody variable region was prepared by PCR (assembly PCR) using a combination of synthetic oligo-DNAs. The constant region was amplified from an expression vector for IgG1 by PCR. The antibody variable region and constant region were linked together by assembly PCR, and then inserted into an animal cell expression vector. H96/PF1L-IgG1 was expressed and purified by the method described in Example 1.

Assessment of H96/PF1L-IgG1, an antibody with fully humanized framework

The Tm of purified H96/PF1L-IgG1 was determined by the method described in Example 5. The affinity measurement was carried out under essentially the same conditions used in Example 5. Note that the concentration of SR344 was adjusted to 0, 0.36, and 1.4 μg/ml, and the dissociation phase was monitored for 15 minutes. The result showed that the Tm and affinity of H96/PF1L-IgG1 were almost the same as those of PF1-IgG1 (Table 10).

Table 10

	Tm (°C)	k_a (1/Ms)	k_d (1/s)	KD (M)
PF1 ANTIBODY	91.3	1.4E+06	4.2E-05	3.1E-11
H96/PF1L-IgG1	89.8	1.2E+06	4.8E-05	3.9E-11

As described above, the present inventors generated an antibody with a fully humanized PF1 antibody framework, using H96 for the PF1 antibody H chain to completely remove the remaining mouse sequence from the PF1 antibody while maintaining its Tm and affinity. Since the framework sequence of H96/PF1L-IgG1 has no mouse-derived sequence, H96/PF1L-IgG1 is expected to be superior, especially from the perspective of immunogenicity.

Construction of F2H/L39-IgG1 with lowered pI and attenuated immunogenicity risk

As demonstrated in Example 4, the pharmacokinetics can be enhanced by lowering pI through alteration of amino acids in the antibody variable region. Thus, the amino acid substitutions shown below were further introduced into H96-IgG1 constructed above. To lower pI, glutamine was substituted for lysine at position 64, and aspartic acid was substituted for glycine at position 65. Furthermore, to reduce the immunogenicity risk, glutamine was substituted for glutamic acid at position 105 and isoleucine was substituted for threonine at position 107. In addition, to achieve affinity enhancement such as that in Example 2, alternation was introduced where leucine was substituted for valine at position 95 and alanine was substituted for isoleucine at position 99. To prepare F2H-IgG1 (amino acid sequence of SEQ ID NO: 135), these amino acid substitutions were introduced into H96-IgG1 by the method described in Example 1.

Furthermore, the following amino acid substitutions were introduced into PF1L. To lower pI, glutamic acid was substituted for glutamine at position 27 and glutamic acid was substituted for leucine at position 55. To prepare L39 (amino acid sequence of SEQ ID NO: 136), these amino acid substitutions were introduced into PF1L by the method described in Example 1. Using F2H-IgG1 as heavy chain and L39 as light chain, F2H/L39-IgG1 was expressed and purified by the method described in Example 1.

Biacore-based analysis of F2H/L39-IgG1 for the affinity for human IL-6 receptor

Humanized PM1 antibody (wild type (WT)), PF1 antibody (constructed in Example 5), and F2H/L39-IgG1 were analyzed for affinity. This measurement was carried out under essentially the same conditions used in Example 4. Note that the concentration of SR344 was adjusted to 0, 0.36, and 1.4 μg/ml, and the dissociation phase was monitored for 15 minutes (Table 11).

Table 11

SAMPLE	k_a (1/Ms)	k_d (1/s)	KD (M)
PF1-IgG1	1.5E+06	4.4E-05	3.0E-11
F2H/L39-IgG1	7.7E+05	4.0E-05	5.2E-11

The result showed that F2H/L39-IgG1 had very strong affinity (maintaining a KD in the order of 10^{-11}) but its k_a was decreased to about half of that of PF1-IgG1.

Assessment of F2H/L39-IgG1 for its human IL-6 receptor-neutralizing activity

The neutralizing activities of humanized PM1 antibody (wild type (WT)) and F2H/L39-IgG1 were assessed by the method described in Example 1. The assessment of neutralizing activity was carried out using 600 ng/ml human interleukin-6 (TORAY). As shown in Fig. 34, F2H/L39-IgG1 was demonstrated to have a very strong activity, 100 or more times higher than WT in terms of 100% inhibitory concentration.

Assessment of F2H/L39-IgG1 for its isoelectric point by isoelectric focusing

The isoelectric point of F2H/L39-IgG1 was determined by the method described in Example 3. The isoelectric point of F2H/L39-IgG1 was 5.5, suggesting that its pharmacokinetics was improved due to a lower isoelectric point relative to the PF1 antibody prepared in Example 5.

The theoretical isoelectric point of the variable regions of F2H/L39 (VH and VL sequences) was calculated to be 4.3 by using GENETYX (GENETYX CORPORATION). Meanwhile, the theoretical isoelectric point of WT was 9.20. Thus, WT has been converted through amino acid substitution into F2H/L39 which has a variable region with a theoretical isoelectric point decreased by about 4.9.

PK/PD test of F2H/L39-IgG1 using cynomolgus monkeys

The humanized PM1 antibody (wild type (WT)), PF1 antibody, and F2H/L39-IgG1 were assessed for their pharmacokinetics (PK) and pharmacodynamics (PD) in cynomolgus monkeys. WT, PF1, and F2H/L39-IgG1 were subcutaneously administered once at 1.0 mg/kg, and blood was collected before administration and over the time course. The concentration of each antibody in plasma was determined in the same way as described in Example 6. The plasma concentration time courses of WT, PF1, and F2H/L39-IgG1 are shown in Fig. 35. The efficacy of each antibody to neutralize membrane-bound cynomolgus monkey IL-6 receptor was assessed. Cynomolgus monkey IL-6 was administered subcutaneously in the lower back at 5

μg/kg every day from Day 3 to Day 10 after antibody administration, and the CRP concentration in each animal was determined 24 hours later. The time courses of CRP concentration after administration of WT or F2H/L39 are shown in Fig. 36. To assess the efficacy of each antibody to neutralize soluble cynomolgus monkey IL-6 receptor, the concentration of free soluble cynomolgus monkey IL-6 receptor in the plasma of cynomolgus monkeys was determined. The time courses of free soluble cynomolgus monkey IL-6 receptor concentration after administration of WT or F2H/L39 are shown in Fig. 37.

These results showed that the plasma concentration time courses of WT and PF1 were comparable to each other; however, the plasma concentration of F2H/L39-IgG1, which has a lowered pI, was maintained higher than that of these two antibodies. Meanwhile, when compared to WT, F2H/L39-IgG1 which has a high affinity for IL-6 receptor was found to maintain lower concentrations of CRP and free soluble cynomolgus monkey IL-6 receptor.

[Example 14] Assessment of the plasma retention of WT-M14

Method for estimating the retention in human plasma

The prolonged retention (slow elimination) of IgG molecule in plasma is known to be due to the function of FcRn which is known as a salvage receptor of IgG molecule (Nat. Rev. Immunol. 2007 Sep;7(9):715-25). When taken up into endosomes via pinocytosis, under the acidic conditions within endosome (approx. pH 6.0), IgG molecules bind to FcRn expressed in endosomes. While IgG molecules that do not bind to FcRn are transferred and degraded in lysosomes, those bound to FcRn are translocated to the cell surface and then released from FcRn back into plasma again under the neutral conditions in plasma (approx. pH 7.4).

Known IgG-type antibodies include the IgG1, IgG2, IgG3, and IgG4 isotypes. The plasma half-lives of these isotypes in human are reported to be about 36 days for IgG1 and IgG2; about 29 days for IgG3; and 16 days for IgG4 (Nat. Biotechnol. 2007 Dec; 25(12):1369-72). Thus, the retention of IgG1 and IgG2 in plasma is believed to be the longest. In general, the isotypes of antibodies used as pharmaceutical agents are IgG1, IgG2, and IgG4. Methods reported for further improving the pharmacokinetics of these IgG antibodies include methods for improving the above-described binding to human FcRn, and this is achieved by altering the sequence of IgG constant region (J. Biol. Chem. 2007 Jan 19;282(3):1709-17; J. Immunol. 2006 Jan 1;176(1):346-56).

There are species-specific differences between mouse FcRn and human FcRn (Proc. Natl. Acad. Sci. USA. 2006 Dec 5;103(49):18709-14). Therefore, to predict the plasma retention of IgG antibodies that have an altered constant region sequence in human, it is desirable to assess the binding to human FcRn and retention in plasma in human FcRn transgenic

mice (Int. Immunol. 2006 Dec;18(12):1759-69).

Assessment of the binding to human FcRn

FcRn is a complex of FcRn and β2-microglobulin. Oligo-DNA primers were prepared based on the human FcRn gene sequence disclosed (J. Exp. Med. (1994) 180 (6), 2377-2381). A DNA fragment encoding the whole gene was prepared by PCR using human cDNA (Human Placenta Marathon-Ready cDNA, Clontech) as a template and the prepared primers. Using the obtained DNA fragment as a template, a DNA fragment encoding the extracellular domain containing the signal region (Met1-Leu290) was amplified by PCR, and inserted into an animal cell expression vector (the amino acid sequence of human FcRn as set forth in SEQ ID NO: 140). Likewise, oligo-DNA primers were prepared based on the human β2-microglobulin gene sequence disclosed (Proc. Natl. Acad. Sci. USA. (2002) 99 (26), 16899-16903). A DNA fragment encoding the whole gene was prepared by PCR using human cDNA (Hu-Placenta Marathon-Ready cDNA, CLONTECH) as a template and the prepared primers. Using the obtained DNA fragment as a template, a DNA fragment encoding the whole β2-microglobulin containing the signal region (Met1-Met119) was amplified by PCR and inserted into an animal cell expression vector (the amino acid sequence of human β2-microglobulin as set forth in SEQ ID NO: 141).

Soluble human FcRn was expressed by the following procedure. The plasmids constructed for human FcRn and β2-microglobulin were introduced into cells of the human embryonic kidney cancer-derived cell line HEK293H (Invitrogen) using 10% Fetal Bovine Serum (Invitrogen) by lipofection. The resulting culture supernatant was collected, and FcRn was purified using IgG Sepharose 6 Fast Flow (Amersham Biosciences) by the method described in J. Immunol. 2002 Nov 1;169(9):5171-80, followed by further purification using HiTrap Q HP (GE Healthcare).

The binding to human FcRn was assessed using Biacore 3000. An antibody was bound to Protein L or rabbit anti-human IgG Kappa chain antibody immobilized onto a sensor chip, human FcRn was added as an analyte for interaction with the antibody, and the affinity (KD) was calculated from the amount of bound human FcRn. Specifically, Protein L or rabbit anti-human IgG Kappa chain antibody was immobilized onto sensor chip CM5 (BIACORE) by the amine coupling method using 50 mM Na-phosphate buffer (pH 6.0) containing 150 mM NaCl as the running buffer. Then, an antibody was diluted with a running buffer containing 0.02% Tween20, and injected to be bound to the chip. Human FcRn was then injected and the binding of the human FcRn to antibody was assessed.

The affinity was computed using BIAevaluation Software. The obtained sensorgram was used to calculate the amount of hFcRn bound to the antibody immediately before the end of

human FcRn injection. The affinity of the antibody for human FcRn was calculated by fitting with the steady state affinity method.

Assessment for the plasma retention in human FcRn transgenic mice

The pharmacokinetics in human FcRn transgenic mice (B6.mFcRn-/-.hFcRn Tg line 276 +/+ mice; Jackson Laboratories) was assessed by the following procedure. An antibody was intravenously administered once at a dose of 1 mg/kg to mice, and blood was collected at appropriate time points. The collected blood was immediately centrifuged at 15,000 rpm and 4°C for 15 minutes to obtain blood plasma. The separated plasma was stored in a freezer at -20°C or below until use. The plasma concentration was determined by ELISA.

Predictive assessment of the plasma retention of WT-M14 in human

The bindings of WT-IgG1 and WT-M14 to bind to human FcRn were assessed by BIAcore. As shown in Table 12, the result indicated that the binding of WT-M14 was slightly greater than that of WT-IgG1.

Table 12

	KD(μM)
WT-IgG1	2.07
WT-M14	1.85

As shown in Fig. 38, however, the retention in plasma was comparable between WT-IgG1 and WT-M14 when assessed using human FcRn transgenic mice. This finding suggests that the plasma retention of the M14 constant region in human is comparable to that of the IgG1 constant region.

[Example 15] Preparation of WT-M44, WT-M58, and WT-M73 which have improved pharmacokinetics

Preparation of the WT-M58 molecule

As described in Example 14, the plasma retention of WT-M14 in human FcRn transgenic mice was comparable to that of WT-IgG1. Known methods to improve pharmacokinetics include those to lower the isoelectric point of an antibody and those to enhance the binding to FcRn. Here, the modifications described below were introduced to improve the pharmacokinetics of WT-M14. Specifically, the following substitutions were introduced into WT-M31ΔGK, which was prepared from WT-M14 as described in Example 4: substitution of methionine for valine at position 397; substitution of glutamine for histidine at position 268;

substitution of glutamine for arginine at position 355; and substitution of glutamic acid for glutamine at position 419 in the EU numbering system. These four substitutions were introduced into WT-M31ΔGK to generate WT-M58 (amino acid sequence of SEQ ID NO: 142). Expression vectors were prepared by the same method described in Example 1. WT-M58 and L(WT) were used as H chain and L chain, respectively. WT-M58 was expressed and purified by the method described in Example 1.

Construction of the WT-M73 molecule

On the other hand, WT-M44 (amino acid sequence of SEQ ID NO: 143) was generated by introducing into IgG1 a substitution of alanine for the amino acid at position 434, EU numbering. WT-M83 (amino acid sequence of SEQ ID NO: 185) was also generated by deletions of glycine at position 446, EU numbering and lysine at position 447, EU numbering to reduce H chain C-terminal heterogeneity. Furthermore, WT-M73 (amino acid sequence of SEQ ID NO: 144) was generated by introducing into WT-M58 a substitution of alanine at position 434, EU numbering.

Expression vectors for the above antibodies were constructed by the method described in Example 1. WT-M44, WT-M58, or WT-M73 was used as H chain, while L (WT) was used as L chain. WT-M44, WT-M58, and WT-M73 were expressed and purified by the method described in Example 1.

Predictive assessment of the plasma retention of WT-M44, WT-M58, and WT-M73 in human

The bindings of WT-IgG1, WT-M44, WT-M58, and WT-M73 to human FcRn were assessed by BIAcore. As shown in Table 13, the result indicates that the bindings of WT-M44, WT-M58, and WT-M73 are greater than WT-IgG1, and about 2.7, 1.4, and 3.8 times of that of WT-IgG1, respectively.

Table 13

	KD(μM)
WT-IgG1	1.62
WT-M44	0.59
WT-M58	1.17
WT-M73	0.42

As a result of assessing WT-IgG1, WT-M44, and WT-M58 for their plasma retention in human FcRn transgenic mice, as shown in Fig. 39, WT-M58 was confirmed to have increased retention in plasma relative to WT-IgG1 and WT-M44. Furthermore, WT-IgG1, WT-M44, WT-M58, and WT-M73 were assessed for their plasma retention in human FcRn transgenic mice.

As shown in Fig. 40, all of WT-M44, WT-M58, and WT-M73 were confirmed to have improved pharmacokinetics relative to WT-IgG1. The pharmacokinetics-improving effect correlated with the binding activity to human FcRn. In particular, the plasma level of WT-M73 at Day 28 was improved to about 16 times of that of WT-IgG1. This finding suggests that the pharmacokinetics of antibodies with the M73 constant region in human is also significantly enhanced when compared to antibodies with the IgG1 constant region.

[Example 16] Effect of the novel constant regions M14 and M58 in reducing heterogeneity in various antibodies

As described in Example 8, it was demonstrated that the heterogeneity originated from the hinge region of IgG2 could be reduced by converting the IgG2 constant region to M14 in the humanized anti-IL-6 receptor PM1 antibody (WT). IgG2 type antibodies other than the humanized PM1 antibody were also tested to assess whether the heterogeneity can be reduced by converting their constant regions into M14 or M58.

Antibodies other than the humanized PM1 antibody were: the anti IL-6 receptor antibody F2H/L39 (the amino acid sequences of F2H/L39_VH and F2H/L39 VL as set forth in SEQ ID NOs: 145 and 146, respectively); anti-IL-31 receptor antibody H0L0 (the amino acid sequences of H0L0_VH and H0L0_VL as set forth in SEQ ID NOs: 147 and 148, respectively); and anti-RANKL antibody DNS (the amino acid sequences of DNS_VH and DNS_VL as set forth in SEQ ID NOs: 149 and 150, respectively). For each of these antibodies, antibodies with IgG1 constant region (SEQ ID NO: 19), IgG2 constant region (SEQ ID NO: 20), or M14 (SEQ ID NO: 24) or M58 (SEQ ID NO: 151) were generated.

The generated antibodies were assessed for heterogeneity by cation exchange chromatography using an adequate gradient and an appropriate flow rate on a ProPac WCX-10 (Dionex) column (mobile phase A: 20 mM sodium acetate (pH 5.0), mobile phase B: 20 mM sodium acetate/1M NaCl (pH 5.0)). The assessment result obtained by cation exchange chromatography (IEC) is shown in Fig. 41.

As shown in Fig. 41, conversion of the constant region from an IgG1 type into an IgG2 type was demonstrated to increase heterogeneity not only in the humanized anti-IL-6 receptor PM1 antibody (WT), but also in the anti-IL-6 receptor antibody F2H/L39, anti-IL-31 receptor antibody H0L0, and anti-RANKL antibody DNS. In contrast, heterogeneity could be decreased in all of these antibodies by converting their constant region into M14 or M58. Thus, it was demonstrated that, regardless of the type of antigen or antibody variable region sequence, the heterogeneity originated from natural IgG2 could be reduced by substituting serines for cysteines at position 131, EU numbering, in the H-chain CH1 domain and at position 219, EU numbering, in the upper hinge of H chain.

[Example 17] Effect of the novel constant region M58 to improve the pharmacokinetics in various antibodies

As described in Example 15, it was demonstrated that conversion of the constant region from IgG1 into M58 in the humanized anti-IL-6 receptor PM1 antibody (WT) improved the binding to human FcRn and pharmacokinetics in human FcRn transgenic mice. So, IgG1 type antibodies other than the humanized PM1 antibody were also tested to assess whether their pharmacokinetics can be improved by converting their constant region into M58.

Antibodies other than the humanized PM1 antibody (WT) were the anti-IL-31 receptor antibody H0L0 (the amino acid sequences of H0L0_VH and H0L0_VL as set forth in SEQ ID NOs: 147 and 148, respectively) and anti-RANKL antibody DNS (the amino acid sequences of DNS_VH and DNS_VL as set forth in SEQ ID NOs: 149 and 150, respectively). For each of these antibodies, antibodies with IgG1 constant region (SEQ ID NO: 19) or M58 (SEQ ID NO: 151) were generated, and assessed for their binding to human FcRn by the method described in Example 14. The result is shown in Table 14.

Table 14

	KD (μM)		
	WT	H0L0	DNS
IgG1	1.42	1.07	1.36
M58	1.03	0.91	1.03

As shown in Table 14, it was demonstrated that as a result of conversion of the constant region from the IgG1 type to M58, as with anti-IL-6 receptor antibody WT, the bindings of both the anti-IL-31 receptor antibody H0L0 and anti-RANKL antibody DNS to human FcRn were improved. This suggests the possibility that regardless of the type of antigen or sequence of antibody variable region, the pharmacokinetics in human is improved by converting the constant region from the IgG1 type to M58.

[Example 18] Effect of cysteine in the CH1 domain on heterogeneity and stability

As described in Example 8, cysteines in the hinge region and CH1 domain of IgG2 were substituted to decrease the heterogeneity of natural IgG2. Assessment of various altered antibodies revealed that heterogeneity could be reduced without decreasing stability by using SKSC (SEQ ID NO: 154). SKSC (SEQ ID NO: 154) is an altered constant region obtained by substituting serine for cysteine at position 131 and lysine for arginine at position 133, EU numbering, in the H-chain CH1 domain, and serine for cysteine at position 219, EU numbering,

in the H-chain upper hinge of the wild type IgG2 constant region sequence.

Meanwhile, another possible method for decreasing heterogeneity is a single substitution of serine for cysteine at position 219, or serine for cysteine at position 220, EU numbering, in the H-chain upper hinge. The altered IgG2 constant region SC (SEQ ID NO: 155) was prepared by substituting serine for cysteine at position 219 and CS (SEQ ID NO: 156) was prepared by substituting serine for cysteine at position 220, EU numbering, in IgG2. WT-SC (SEQ ID NO: 157) and WT-CS (SEQ ID NO: 158) were prepared to have SC and CS, respectively, and compared with WT-IgG1, WT-IgG2, WT-SKSC, and WT-M58 in terms of heterogeneity and thermal stability. Furthermore, F2H/L39-IgG1, F2H/L39-IgG2, F2H/L39-SC, F2H/L39-CS, F2H/L39-SKSC, and F2H/L39-M14, which have the constant region of IgG1 (SEQ ID NO: 19), IgG2 (SEQ ID NO: 20), SC (SEQ ID NO: 155), CS (SEQ ID NO: 156), SKSC (SEQ ID NO: 154), or M14 (SEQ ID NO: 24), respectively, were prepared from F2H/L39 (the amino acid sequences of F2H/L39_VH and F2H/L39_VL as set forth in SEQ ID NOs: 145 and 146, respectively), which is an anti IL-6 receptor antibody different from WT. The antibodies were compared with regard to heterogeneity and stability.

WT-IgG1, WT-IgG2, WT-SC, WT-CS, WT-SKSC, WT-M58, F2H/L39-IgG1, F2H/L39-IgG2, F2H/L39-SC, F2H/L39-CS, F2H/L39-SKSC, and F2H/L39-M14 were assessed for heterogeneity by cation exchange chromatography using an adequate gradient and an appropriate flow rate on a ProPac WCX-10 (Dionex) column (mobile phase A: 20 mM sodium acetate (pH 5.0), mobile phase B: 20 mM sodium acetate/1M NaCl (pH 5.0)). The assessment result obtained by cation exchange chromatography is shown in Fig. 42.

As shown in Fig.42, conversion of the constant region from an IgG1 type to an IgG2 type was demonstrated to increase heterogeneity in both WT and F2H/L39. In contrast, heterogeneity was significantly decreased by converting the constant region into SKSC and M14 or M58. Meanwhile, conversion of the constant region into SC significantly decreased heterogeneity, as in the case of SKSC. However, conversion into CS did not sufficiently improve heterogeneity.

In addition to low heterogeneity, high stability is generally desired when preparing stable formulations in development of antibody pharmaceuticals. Thus, to assess stability, the midpoint temperature of thermal denaturation (Tm value) was determined by differential scanning calorimetry (DSC) (VP-DSC; Microcal). The midpoint temperature of thermal denaturation (Tm value) serves as an indicator of stability. In order to prepare stable formulations as pharmaceutical agents, a higher midpoint temperature of thermal denaturation (Tm value) is preferred (J. Pharm. Sci. 2008 Apr;97(4):1414-26). WT-IgG1, WT-IgG2, WT-SC, WT-CS, WT-SKSC, and WT-M58 were dialyzed (EasySEP; TOMY) against a solution of 20 mM sodium acetate, 150 mM NaCl, pH 6.0. DSC measurement was carried out at a

heating rate of 1°C/min in a range of 40 to 100°C, and at a protein concentration of about 0.1 mg/ml. The denaturation curves obtained by DSC are shown in Fig. 43. The Tm values of the Fab domains are listed in Table 15 below.

Table 15

	Tm/°C
WT-IgG1	94.8
WT-IgG2	93.9
WT-SC	86.7
WT-CS	86.4
WT-SKSC	93.7
WT-M58	93.7

The Tm values of WT-IgG1 and WT-IgG2 were almost the same (about 94°C; Tm of IgG2 was about 1°C lower). Meawhile, the Tm values of WT-SC and WT-CS were about 86°C, and thus significantly lower than those of WT-IgG1 and WT-IgG2. On the other hand, the Tm values of WT-M58 and WT-SKSC were about 94°C, and comparable to those of WT-IgG1 and WT-IgG2. This suggests that WT-SC and WT-CS are markedly unstable as compared to IgG2, and thus, WT-SKSC and WT-M58, both of which also comprise substituion of serine for cysteine in the CH1 domain, are preferred in the development of antibody pharmaceuticals. The reason for the significant decrease of Tm in WT-SC and WT-CS relative to IgG2 is thought to be differences in the disulfide-bonding pattern between WT-SC or WT-CS and IgG2.

Furthermore, comparison of DSC denaturation curves showed that WT-IgG1, WT-SKSC, and WT-M58 each gave a sharp and single denaturation peak for the Fab domain. In contrast, WT-SC and WT-CS each gave a broader denaturation peak for the Fab domain. WT-IgG2 also gave a shoulder peak on the lower temperature side of the Fab domain denaturation peak. In general, it is considered that a single component gives a sharp DSC denaturation peak, and when two or more components with different Tm values (namely, heterogeneity) are present, the denaturation peak becomes broader. Specifically, the above-described result suggests the possibility that each of WT-IgG2, WT-SC, and WT-CS contains two or more components, and thus the natural-IgG2 heterogeneity has not been sufficiently reduced in WT-SC and WT-CS. This finding suggests that not only cysteines in the hinge region but also those in the CH1 domain are involved in the wild type-IgG2 heterogeneity , and it is necessary to alter not only cysteines in the hinge region but also those in the CH1 domain to decrease the DSC heterogeneity. Furthermore, as described above, stability comparable to that of wild type IgG2 can be acheived only when cysteines in both the hinge region and CH1 domain are substituted.

The above finding suggests that from the perspective of heterogeneity and stability, SC

and CS, which are constant regions introduced with serine substitution for only the hinge region cysteine, are insufficient as constant regions to decrease heterogeneity originated from the hinge region of IgG2. It was thus discovered that the heterogeneity could be significantly decreased while maintaining an IgG2-equivalent stability, only when the cysteine at position 131, EU numbering, in the CH1 domain was substituted with serine in addition to cysteine at hinge region. Such constant regions include M14, M31, M58, and M73 described above. In particular, M58 and M73 are stable and less heterogeneous, and exhibit improved pharmacokinetics, and therefore are expected to be very useful as constant regions for antibody pharmaceuticals.

[Example 19] Generation of fully humanized anti-IL-6 receptor antibodies with improved PK/PD

To generate a fully humanized anti-IL-6 receptor antibody with improved PK/PD, the molecules described below were created by altering TOCILIZUMAB (H chain, WT-IgG1 (SEQ ID NO: 15); L chain, WT (SEQ ID NO: 105).

To improve the ka of F2H-IgG1, substitutions of valine for tryptophan at position 35, phenylalanine for tyrosine at position 50, and threonine for serine at position 62, which are the affinity enhancing substitution obtained in Example 2, were carried out. Furthermore, to lower pI without increasing immunogenicity risk, substitutions of valine for tyrosine at position 102, glutamic acid for glutamine at position 105, and threonine for isoleucine at position 107 were carried out, and conversion of the constant region from an IgG1 type to an M83 type was carried out and generated VH5-M83 (amino acid sequence of SEQ ID NO: 139). In addition, to improve the ka of L39, VL5-kappa (amino acid sequence of SEQ ID NO: 181) was prepared and it comprises a substitution of glutamine for glutamic acid at position 27. Furthermore, TOCILIZUMAB variants were prepared by combining two or more of the mutations in variable and constant regions described in the above examples and newly discovered mutations. The following fully humanized IL-6 receptor antibodies were discovered using various screening tests: Fv3-M73 (H chain, VH4-M73, SEQ ID NO: 182; L chain, VL1-kappa, SEQ ID NO: 183), Fv4-M73 (H chain, VH3-M73, SEQ ID NO: 180; L chain, VL3-kappa, SEQ ID NO: 181), and Fv5-M83 (H chain, VH5-M83, SEQ ID NO: 139; L chain, VL5-kappa, SEQ ID NO: 138).

The affinities of prepared Fv3-M73, Fv4-M73, and Fv5-M83 against IL-6 receptor were compared to that of TOCILIZUMAB (see Reference Example for method). The affinities of these anti- IL-6 receptor antibodies determined are shown in Table 16. Furthermore, their BaF/gp130-neutralizing activities were compared to those of TOCILIZUMAB and the control (the known high affinity anti-IL-6 receptor antibody described in Reference Example, and VQ8F11-21 hIgG1 described in US 2007/0280945; see Reference Example for method). The results obtained by determining the biological activities of these antibodies using BaF/gp130 are shown in Fig. 44 (TOCILIZUMAB, the control, and Fv5-M83 with a final IL-6 concentration of

300 ng/ml) and Fig. 45 (TOCILIZUMAB, Fv3-M73, and Fv4-M73 with a final IL-6 concentration of 30 ng/ml). As shown in Table 16, Fv3-M73 and Fv4-M73 have about two to three times higher affinity than TOCILIZUMAB, while Fv5-M83 exhibits about 100 times higher affinity than TOCILIZUMAB (since it was difficult to measure the affinity of Fv5-M83, instead the affinity was determined using Fv5-IgG1, which has an IgG1-type constant region; the constant region is generally thought to have no effect on affinity). As shown in Fig. 45, Fv3-M73 and Fv4-M73 exhibit slightly stronger activities than TOCILIZUMAB. As shown in Fig. 44, Fv5-M83 has a very strong activity, which is more than 100 times greater than that of TOCILIZUMAB in terms of 50% inhibitory concentration. Fv5-M83 also exhibits about 10 times higher neutralizing activity in terms of 50% inhibitory concentration than the control (the known high-affinity anti-IL-6 receptor antibody).

Table 16

	k_a(1/Ms)	k_d(1/s)	KD (M)
TOCILIZUMAB	4.0E+05	1.1E-03	2.7E-09
Fv3-M73	8.5E+05	8.7E-04	1.0E-09
Fv4-M73	7.5E+05	1.0E-03	1.4E-09
Fv5-M83	1.1E+06	2.8E-05	2.5E-11

The isoelectric points of TOCILIZUMAB, the control, Fv3-M73, Fv4-M73, and Fv5-M83 were determined by isoelectric focusing using a method known to those skilled in the art. The result showed that the isoelectric point was about 9.3 for TOCILIZUMAB; about 8.4 to 8.5 for the control; about 5.7 to 5.8 for Fv3-M73; about 5.6 to 5.7 for Fv4-M73; and 5.4 to 5.5 for Fv5-M83. Thus, each antibody had a significantly lowered isoelectric point when compared to TOCILIZUMAB and the control. Furthermore, the theoretical isoelectric point of the variable regions VH/VL was calculated by GENETYX (GENETYX CORPORATION). The result showed that the theoretical isoelectric point was 9.20 for TOCILIZUMAB; 7.79 for the control; 5.49 for Fv3-M73; 5.01 for Fv4-M73; and 4.27 for Fv5-M83. Thus, each antibody had a significantly lowered isoelectric point when compared to TOCILIZUMAB and the control. Accordingly, the pharmacokinetics of Fv3-M73, Fv4-M73, and Fv5-M83 was thought to be improved when compared to TOCILIZUMAB and the control.

T-cell epitopes in the variable region sequence of TOCILIZUMAB, Fv3-M73, Fv4-M73, or Fv5-M83 were analyzed using TEPITOPE (Methods. 2004 Dec;34(4):468-75). As a result, TOCILIZUMAB was predicted to have T-cell epitopes, of which many could bind to HLA. In contrast, the number of sequences that were predicted to bind to T-cell epitopes was significantly reduced in Fv3-M73, Fv4-M73, and Fv5-M83. In addition, the framework of Fv3-M73, Fv4-M73, or Fv5-M83 has no mouse sequence and is thus fully humanized. These suggest the

possibility that immunogenicity risk is significantly reduced in Fv3-M73, Fv4-M73, and Fv5-M83 when compared to TOCILIZUMAB.

[Example 20] PK/PD test of fully humanized anti-IL-6 receptor antibodies in monkeys

Each of TOCILIZUMAB, the control, Fv3-M73, Fv4-M73, and Fv5-M83 was intravenously administered once at a dose of 1 mg/kg to cynomolgus monkeys to assess the time courses of their plasma concentrations (see Reference Example for method). The plasma concentration time courses of TOCILIZUMAB, Fv3-M73, Fv4-M73, and Fv5-M83 after intravenous administration are shown in Fig. 46. The result showed that each of Fv3-M73, Fv4-M73, and Fv5-M83 exhibited significantly improved plasma retention in cynomolgus monkeys when compared to TOCILIZUMAB and the control. Of them, Fv3-M73 and Fv4-M73 exhibited substantially improved plasma retention when compared to TOCILIZUMAB.

The efficacy of each antibody to neutralize membrane-bound cynomolgus monkey IL-6 receptor was assessed. Cynomolgus monkey IL-6 was administered subcutaneously in the lower back at 5 μg/kg every day from Day 6 to Day 18 after antibody administration (Day 3 to Day 10 for TOCILIZUMAB), and the CRP concentration in each animal was determined 24 hours later (see Reference Example for method). The time course of CRP concentration after administration of each antibody is shown in Fig. 47. To assess the efficacy of each antibody to neutralize soluble cynomolgus monkey IL-6 receptor, the plasma concentration of free soluble cynomolgus monkey IL-6 receptor in the cynomolgus monkeys was determined and percentage of soluble IL-6 receptor neutralization were calculated (see Reference Example for method). The time course of percentage of soluble IL-6 receptor neutralization after administration of each antibody is shown in Fig. 48.

Each of Fv3-M73, Fv4-M73, and Fv5-M83 neutralized membrane-bound cynomolgus monkey IL-6 receptor in a more sustainable way, and suppressed the increase of CRP over a longer period when compared to TOCILIZUMAB and the control (the known high-affinity anti-IL-6 receptor antibody). Furthermore, each of Fv3-M73, Fv4-M73, and Fv5-M83 neutralized soluble cynomolgus monkey IL-6 receptor in a more sustainable way, and suppressed the increase of free soluble cynomolgus monkey IL-6 receptor over a longer period when compared to TOCILIZUMAB and the control. These findings demonstrate that all of Fv3-M73, Fv4-M73, and Fv5-M83 are superior in sustaining the neutralization of membrane-bound and soluble IL-6 receptors than TOCILIZUMAB and the control. Of them, Fv3-M73 and Fv4-M73 are remarkably superior in sustaining the neutralization. Meanwhile, Fv5-M83 suppressed CRP and free soluble cynomolgus monkey IL-6 receptor more strongly than Fv3-M73 and Fv4-M73. Thus, Fv5-M83 is considered to be stronger than Fv3-M73, Fv4-M73, and the control (the

known high-affinity anti-IL-6 receptor antibody) in neutralizing membrane-bound and soluble IL-6 receptors. It was considered that results in *in vivo* of cynomolgus monkeys reflect the stronger affinity of Fv5-M83 for IL-6 receptor and stronger biological activity of Fv5-M83 in the BaF/gp130 assay system relative to the control.

These findings suggest that Fv3-M73 and Fv4-M73 are highly superior in sustaining their activities as an anti-IL-6 receptor-neutralizing antibody when compared to TOCILIZUMAB and the control, and thus enable to significantly reduce the dosage and frequency of administration. Furthermore, Fv5-M83 was demonstrated to be remarkably superior in terms of the strength of activity as an anti-IL-6 receptor-neutralizing antibody as well as sustaining their activity. Thus, Fv3-M73, Fv4-M73, and Fv5-M83 are expected to be useful as pharmaceutical IL-6 antagonists.

[Reference Example]

Preparation of soluble recombinant cynomolgus monkey IL-6 receptor (cIL-6R)

Oligo-DNA primers were prepared based on the disclosed gene sequence for Rhesus monkey IL-6 receptor (Birney *et al.*, Ensembl 2006, Nucleic Acids Res. 2006 Jan 1;34 (Database issue):D556-61). A DNA fragment encoding the whole cynomolgus monkey IL-6 receptor gene was prepared by PCR using the primers, and as a template, cDNA prepared from the pancreas of cynomolgus monkey. The resulting DNA fragment was inserted into an animal cell expression vector, and a stable expression CHO line (cyno.sIL-6R-producing CHO cell line) was prepared using the vector. The culture medium of cyno.sIL-6R-producing CHO cells was purified using a HisTrap column (GE Healthcare Bioscience) and then concentrated with Amicon Ultra-15 Ultracel-10k (Millipore). A final purified sample of soluble cynomolgus monkey IL-6 receptor (hereinafter cIL-6R) was obtained through further purification on a Superdex200pg16/60 gel filtration column (GE Healthcare Bioscience).

Preparation of recombinant cynomolgus monkey IL-6 (cIL-6)

Cynomolgus monkey IL-6 was prepared by the procedure described below. The nucleotide sequence encoding 212 amino acids deposited under SWISSPROT Accession No. P79341 was prepared and cloned into an animal cell expression vector. The resulting vector was introduced into CHO cells to prepare a stable expression cell line (cyno.IL-6-producing CHO cell line). The culture medium of cyno.IL-6-producing CHO cells was purified using a SP-Sepharose/FF column (GE Healthcare Bioscience) and then concentrated with Amicon Ultra-15 Ultracel-5k (Millipore). A final purified sample of cynomolgus monkey IL-6 (hereinafter cIL-6) was obtained through further purification on a Superdex75pg26/60 gel

filtration column (GE Healthcare Bioscience), followed by concentration with Amicon Ultra-15 Ultracel-5k (Millipore).

Preparation of a known high-affinity anti-IL-6 receptor antibody

An animal cell expression vector was constructed to express VQ8F11-21 hIgG1, a known high-affinity anti-IL-6 receptor antibody. VQ8F11-21 hIgG1 is described in US 2007/0280945 A1 (US 2007/0280945 A1; the amino acid sequences of H chain and L chain as set forth in SEQ ID NOs: 19 and 27, respectively). The antibody variable region was constructed by PCR using a combination of synthetic oligo DNAs (assembly PCR). IgG1 was used as the constant region. The antibody variable and constant regions were combined together by assembly PCR, and then inserted into an animal cell expression vector to construct expression vectors for the H chain and L chain of interest. The nucleotide sequences of the resulting expression vectors were determined by a method known to those skilled in the art. The high-affinity anti-IL-6 receptor antibody (hereinafter abbreviated as "control") was expressed and purified using the constructed expression vectors by the method described in Example 1.

Biacore-based analysis of binding to IL-6 receptor

Antigen-antibody reaction kinetics was analyzed using Biacore T100 (GE Healthcare). The SR344-antibody interaction was measured by immobilizing appropriate amounts of anti-IgG (γ-chain specific) $F(ab')_2$ onto a sensor chip by amine coupling method, binding antibodies of interest onto the chip at pH7.4, and then running IL-6 receptor SR344 adjusted to be various concentrations at pH7.4 over the chip as an analyte. All measurements were carried out at 37°C. The kinetic parameters, association rate constant k_a (1/Ms) and dissociation rate constant k_d (1/s) were calculated from the sensorgrams obtained by measurement. Then, K_D (M) was determined based on the rate constants. The respective parameters were determined using Biacore T100 Evaluation Software (GE Healthcare).

PK/PD test to determine the plasma concentrations of antibodies, CRP, and free soluble IL-6 receptor in monkeys

The plasma concentrations in cynomolgus monkeys were determined by ELISA using a method known to those skilled in the art.

The concentration of CRP was determined with an automated analyzer (TBA-120FR; Toshiba Medical Systems Co.) using Cias R CRP (KANTO CHEMICAL CO., INC.).

The plasma concentration of free soluble cynomolgus monkey IL-6 receptor in cynomolgus monkeys was determined by the procedure described below. All IgG antibodies

(cynomolgus monkey IgG, anti-human IL-6 receptor antibody, and anti-human IL-6 receptor antibody-soluble cynomolgus monkey IL-6 receptor complex) in the plasma were adsorbed onto Protein A by loading 30 μl of cynomolgus monkey plasma onto an appropriate amount of rProtein A Sepharose Fast Flow resin (GE Healthcare) dried in a 0.22-μm filter cup (Millipore). Then, the solution in cup was spinned down using a high-speed centrifuge to collect the solution that passed through. The solution that passed through does not contain Protein A-bound anti-human IL-6 receptor antibody-soluble cynomolgus monkey IL-6 receptor complex. Therefore, the concentration of free soluble IL-6 receptor can be determined by measuring the concentration of soluble cynomolgus monkey IL-6 receptor in the solution that passed through Protein A. The concentration of soluble cynomolgus monkey IL-6 receptor was determined using a method known to those skilled in the art for measuring the concentrations of soluble human IL-6 receptor. Soluble cynomolgus monkey IL-6 receptor (cIL-6R) prepared as described above was used as a standard.

Then the percentage of soluble IL-6 receptor neutralization was calculated by following formula.

$$\frac{\text{Free soluble IL-6 receptor concentration after antibody administration}}{\text{Soluble IL-6 receptor concentration before antibody administration}} \times 100$$

Industrial Applicability

The present invention provides second-generation molecules, which are more superior than the humanized anti-IL-6 receptor IgG1 antibody TOCILIZUMAB, and have been improved to exhibit enhanced antigen-neutralizing activity and pharmacokinetics, and thus produce a prolonged therapeutic effect even when the frequency of administration is reduced. They have also been improved to have reduced immunogenicity, and improved safety and physiochemical properties, by altering amino acid sequences of the variable and constant regions of TOCILIZUMAB. Furthermore, the present invention also provides antibody constant regions suitable for pharmaceuticals.

CLAIMS

1. An anti-IL-6 receptor antibody comprising a heavy chain variable region of SEQ ID NO: 17 and a light chain variable region of SEQ ID NO: 18, in which
   - Ser at position 1 in the CDR1 of the heavy chain has been substituted with Asp,
   - Thr at position 9 and Ser at position 16 in the CDR2 of the heavy chain have been substituted with Asn and Gly, respectively,
   - Arg at position 13, Gln at position 16, Thr at position 23 and Thr at position 30 in the FR1 of the heavy chain have been substituted with Lys, Glu, Ala and Ser, respectively,
   - Arg at position 8 in the FR2 of the heavy chain has been substituted with Glu,
   - Met at position 4, Leu at position 5, Arg at position 16 and Val at position 27 in the FR3 of the heavy chain have been substituted with Ile, Ser, Lys and Ala, respectively,
   - Gln at position 3 and Ser at position 5 in the FR4 of the heavy chain have been substituted with Glu and Thr, respectively,
   - Arg at position 1 in the CDR1 of the light chain has been substituted with Gln,
   - Thr at position 2 and Arg at position 4 in the CDR2 of the light chain have been substituted with Gly and Glu, respectively,
   - Thr at position 5 in the CDR3 of the light chain has been substituted with Ser,
   - Arg at position 18 in the FR1 of the light chain has been substituted with Ser,
   - Lys at position 11 in the FR2 of the light chain has been substituted with Glu,
   - Gln at position 23, Pro at position 24 and Ile at position 27 in the FR3 of the light chain have been substituted with Glu, Ala and Ala, respectively,
   - Lys at position 10 in the FR4 of the light chain has been substituted with Glu.

2. An anti-IL-6 receptor antibody comprising a heavy chain variable region of SEQ ID NO: 17 and a light chain variable region of SEQ ID NO: 18, in which
   - Ser at position 1 in the CDR1 of the heavy chain has been substituted with Asp,
   - Thr at position 9 and Ser at position 16 in the CDR2 of the heavy chain have been substituted with Asn and Gly, respectively,
   - Ser at position 1 and Thr at position 5 in the CDR3 of the heavy chain have been substituted with Val and Ile, respectively,
   - Arg at position 13, Gln at position 16, Thr at position 23 and Thr at position 30 in the FR1 of the heavy chain have been substituted with Lys, Glu, Ala and Ser, respectively,
   - Arg at position 8 in the FR2 of the heavy chain has been substituted with Glu,
   - Met at position 4, Leu at position 5, Arg at position 16 and Val at position 27 in the

FR3 of the heavy chain have been substituted with Ile, Ser, Lys and Ala, respectively,
- Gln at position 3 and Ser at position 5 in the FR4 of the heavy chain have been substituted with Glu and Thr, respectively,
- Arg at position 1 in the CDR1 of the light chain has been substituted with Gln,
- Thr at position 2 and Arg at position 4 in the CDR2 of the light chain have been substituted with Gly and Glu, respectively,
- Gln at position 1 and Thr at position 5 in the CDR3 of the light chain have been substituted with Gly and Arg, respectively,
- Arg at position 18 in the FR1 of the light chain has been substituted with Ser,
- Lys at position 11 in the FR2 of the light chain has been substituted with Glu,
- Gln at position 23, Pro at position 24 and Ile at position 27 in the FR3 of the light chain have been substituted with Glu, Ala and Ala, respectively,
- Lys at position 10 in the FR4 of the light chain has been substituted with Glu.

3. An anti-IL-6 receptor antibody comprising a heavy chain variable region of SEQ ID NO: 17 and a light chain variable region of SEQ ID NO: 18, in which
   - Ser at position 1 in the CDR1 of the heavy chain has been substituted with Asp,
   - Thr at position 9 and Ser at position 16 in the CDR2 of the heavy chain have been substituted with Asn and Gly, respectively,
   - Ser at position 1 and Thr at position 5 in the CDR3 of the heavy chain have been substituted with Val and Ile, respectively,
   - Arg at position 13, Gln at position 16, Thr at position 23 and Thr at position 30 in the FR1 of the heavy chain have been substituted with Lys, Glu, Ala and Ser, respectively,
   - Arg at position 8 in the FR2 of the heavy chain has been substituted with Glu,
   - the amino acid sequence of the FR3 of the heavy chain has been substituted with that of SEQ ID NO: 184,
   - Gln at position 3 and Ser at position 5 in the FR4 of the heavy chain have been substituted with Glu and Thr, respectively,
   - Arg at position 1 in the CDR1 of the light chain has been substituted with Gln,
   - Thr at position 2 and Arg at position 4 in the CDR2 of the light chain have been substituted with Gly and Glu, respectively,
   - Gln at position 1 and Thr at position 5 in the CDR3 of the light chain have been substituted with Gly and Arg, respectively,
   - Arg at position 18 in the FR1 of the light chain has been substituted with Ser,
   - Lys at position 11 in the FR2 of the light chain has been substituted with Glu,
   - Gln at position 23, Pro at position 24 and Ile at position 27 in the FR3 of the light

chain have been substituted with Glu, Ala and Ala, respectively,
- Lys at position 10 in the FR4 of the light chain has been substituted with Glu.

4. An anti-IL-6 receptor antibody comprising a heavy chain variable region of SEQ ID NO: 17 and a light chain variable region of SEQ ID NO: 18, in which
   - Ser at position 1 in the CDR1 of the heavy chain has been substituted with Asp,
   - the amino acid sequence of the CDR2 of the heavy chain has been substituted with that of SEQ ID NO: 122,
   - Ser at position 1 and Thr at position 5 in the CDR3 of the heavy chain have been substituted with Leu and Ala, respectively,
   - Arg at position 13, Gln at position 16, Thr at position 23 and Thr at position 30 in the FR1 of the heavy chain have been substituted with Lys, Glu, Ala and Ser, respectively,
   - Arg at position 8 in the FR2 of the heavy chain has been substituted with Glu,
   - the amino acid sequence of the FR3 of the heavy chain has been substituted with that of SEQ ID NO: 184,
   - Ser at position 5 in the FR4 of the heavy chain has been substituted with Ile,
   - Arg at position 1 and Gln at position 4 in the CDR1 of the light chain have been substituted with Gln and Glu respectively,
   - the amino acid sequence of the CDR2 of the light chain has been substituted with that of SEQ ID NO: 126,
   - Gln at position 1 and Thr at position 5 in the CDR3 of the light chain have been substituted with Gly and Arg, respectively,
   - Arg at position 18 in the FR1 of the light chain has been substituted with Ser,
   - Lys at position 11 in the FR2 of the light chain has been substituted with Glu,
   - Gln at position 23, Pro at position 24 and Ile at position 27 in the FR3 of the light chain have been substituted with Glu, Ala and Ala, respectively,
   - Lys at position 10 in the FR4 of the light chain has been substituted with Glu.

5. An anti-IL-6 receptor antibody comprising a heavy chain variable region of SEQ ID NO: 17 and a light chain variable region of SEQ ID NO: 18, in which
   - the amino acid sequence of the CDR1 of the heavy chain has been substituted with that of SEQ ID NO: 165,
   - the amino acid sequence of the CDR2 of the heavy chain has been substituted with that of SEQ ID NO: 166,
   - the amino acid sequence of the CDR3 of the heavy chain has been substituted with that of SEQ ID NO: 167,

- Arg at position 13, Gln at position 16, Thr at position 23 and Thr at position 30 in the FR1 of the heavy chain have been substituted with Lys, Glu, Ala and Ser, respectively,
- Arg at position 8 in the FR2 of the heavy chain has been substituted with Glu,
- the amino acid sequence of the FR3 of the heavy chain has been substituted with that of SEQ ID NO: 184,
- Gln at position 3 and Ser at position 5 in the FR4 of the heavy chain have been substituted with Glu and Thr, respectively,
- the amino acid sequence of the CDR1 of the light chain has been substituted with that of SEQ ID NO: 101,
- the amino acid sequence of the CDR2 of the light chain has been substituted with that of SEQ ID NO: 168,
- Gln at position 1 and Thr at position 5 in the CDR3 of the light chain have been substituted with Gly and Arg, respectively,
- Arg at position 18 in the FR1 of the light chain has been substituted with Ser,
- Lys at position 11 in the FR2 of the light chain has been substituted with Glu,
- Gln at position 23, Pro at position 24 and Ile at position 27 in the FR3 of the light chain have been substituted with Glu, Ala and Ala, respectively,
- Lys at position 10 in the FR4 of the light chain has been substituted with Glu.

6. An anti-IL-6 receptor antibody comprising a heavy chain variable region of SEQ ID NO: 17 and a light chain variable region of SEQ ID NO: 18, in which
   - the amino acid sequence of the CDR1 of the heavy chain has been substituted with that of SEQ ID NO: 169,
   - the amino acid sequence of the CDR2 of the heavy chain has been substituted with that of SEQ ID NO: 174,
   - the amino acid sequence of the FR1 of the heavy chain has been substituted with that of SEQ ID NO: 186,
   - Arg at position 8 in the FR2 of the heavy chain has been substituted with Glu,
   - the amino acid sequence of the FR3 of the heavy chain has been substituted with that of SEQ ID NO: 184,
   - Gln at position 3 and Ser at position 5 in the FR4 of the heavy chain have been substituted with Glu and Thr, respectively,
   - the amino acid sequence of the CDR1 of the light chain has been substituted with that of SEQ ID NO: 175,
   - the amino acid sequence of the CDR2 of the light chain has been substituted with that of SEQ ID NO: 173,

- Gln at position 1 and Thr at position 5 in the CDR3 of the light chain have been substituted with Gly and Arg, respectively,
- Arg at position 18 in the FR1 of the light chain has been substituted with Ser,
- Lys at position 11 in the FR2 of the light chain has been substituted with Glu,
- Gln at position 23, Pro at position 24 and Ile at position 27 in the FR3 of the light chain have been substituted with Glu, Ala and Ala, respectively,
- Lys at position 10 in the FR4 of the light chain has been substituted with Glu.

7. The pharmaceutical composition comprising the antibody of any one of claims 1-6.

ABSTRACT

The present inventors succeeded in discovering specific amino acid mutations in the variable region, framework region, and constant region of TOCILIZUMAB, and this enables to reduce immunogenicity risk and the heterogeneity originated from disulfide bonds in the hinge region, as well as to improve antigen binding activity, pharmacokinetics, stability under acidic conditions, and stability in high concentration preparations.

SEQUENCE LISTING

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<150> JP 2007-250165
<151> 2007-09-26

<160> 194

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20 25 30

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35 40 45

Ile Gly Tyr Ile Ser Tyr Ser Gly Ile Thr Thr Tyr Asn Pro Ser Leu
50 55 60

Lys Ser Arg Val Thr Met Leu Arg Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys

85 90 95

Ala Arg Ser Leu Ala Arg Thr Thr Ala Met Asp Tyr Trp Gly Gln Gly
100 105 110

Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
195 200 205

Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
210 215 220

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro

225 230 235 240

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
245 250 255

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
260 265 270

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
275 280 285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
290 295 300

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305 310 315 320

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
325 330 335

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
340 345 350

Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
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Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu

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Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
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35 40 45

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50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Gly Asn Thr Leu Pro Tyr
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190

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195 200 205

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20 25 30

His Ala Trp Ser Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp
35 40 45

Ile Gly Tyr Ile Ser Tyr Ser Gly Ile Thr Thr Tyr Asn Pro Ser Leu

50 55 60

Lys Ser Arg Val Thr Met Leu Arg Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
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Tyr Tyr Thr Ser Arg Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro
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20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65 70 75 80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
100 105 110

Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
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Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
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Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
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Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
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Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
180 185 190

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
195 200 205

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
210 215 220

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
225 230 235 240

Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
245 250 255

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
260 265 270

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
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Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
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Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
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Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
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Leu Ser Ser Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr
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Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
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Thr Val Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro

100 105 110

Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
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Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile

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20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr
65 70 75 80

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro
100 105 110

Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
115 120 125

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165 170 175

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
180 185 190

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu
195 200 205

Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
210 215 220

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys
225 230 235 240

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
245 250 255

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
260 265 270

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
275 280 285

Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser
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Leu Ser Leu Ser Leu Gly Lys
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His Ala Trp Ser Trp Val Arg Gln Pro Pro Gly Glu Gly Leu Glu Trp
35 40 45

Ile Gly Tyr Ile Ser Tyr Ser Gly Ile Thr Asn Tyr Asn Pro Ser Leu
50 55 60

Lys Gly Arg Val Thr Ile Ser Arg Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Ala Tyr Tyr Cys
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Asp Ser Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Ile Ser Ser Tyr
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Glu Leu Leu Ile
35 40 45

Tyr Tyr Gly Ser Glu Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Glu Ala
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Glu Asp Ala Ala Thr Tyr Tyr Cys Gly Gln Gly Asn Arg Leu Pro Tyr
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Glu
100 105

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Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr
65 70 75 80

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Thr Val Glu Arg Lys Ser Cys Val Glu Cys Pro Pro Cys Pro Ala Pro
100 105 110

Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
115 120 125

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
130 135 140

Val Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly
145 150 155 160

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn
165 170 175

Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp
180 185 190

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro
195 200 205

Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
210 215 220

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn
225 230 235 240

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
245 250 255

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
260 265 270

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
275 280 285

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
290 295 300

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
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Ser Leu Ser Pro

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<400> 25

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Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
            20                  25                  30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35                  40                  45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50                  55                  60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr
65                  70                  75                  80

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95

Thr Val Glu Arg Lys Ser Cys Val Glu Cys Pro Pro Cys Pro Ala Pro
            100                 105                 110
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Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
115 120 125

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
130 135 140

Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly
145 150 155 160

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn
165 170 175

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
180 185 190

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro
195 200 205

Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
210 215 220

Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn
225 230 235 240

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
245 250 255

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
260 265 270

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
275 280 285

Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys
290 295 300

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
305 310 315 320

Ser Leu Ser Leu

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Trp Asp His Ala Trp Ser
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Thr Asp His Ala Trp Ser
1 5

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Asp Asp His Ala Trp Ser
1 5

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Asn Asp His Ala Trp Ser
1 5

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Arg Asp His Ala Trp Ser
1 5

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Val Asp His Ala Trp Ser
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<400> 32

Phe Asp His Ala Trp Ser
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Ala Asp His Ala Trp Ser
1 5

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Gln Asp His Ala Trp Ser
1 5

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Tyr Asp His Ala Trp Ser
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Leu Asp His Ala Trp Ser
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His Asp His Ala Trp Ser
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Glu Asp His Ala Trp Ser
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Cys Asp His Ala Trp Ser
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Ser Asp His Ala Ile Ser
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Ser Asp His Ala Val Ser
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Tyr Ile Ser Tyr Ser Gly Ile Arg Thr Tyr Asn Pro Ser Leu Lys Ser
1 5 10 15

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<400> 44

Tyr Ile Ser Tyr Ser Gly Ile Thr Ser Tyr Asn Pro Ser Leu Lys Ser
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<400> 46

Ile Leu Ala Arg Thr Thr Ala Met Asp Tyr
1 5 10

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<400> 47

Val Leu Ala Arg Thr Thr Ala Met Asp Tyr
1 5 10

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<211> 10
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Thr Leu Ala Arg Thr Thr Ala Met Asp Tyr
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Leu Leu Ala Arg Thr Thr Ala Met Asp Tyr
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Ser Thr Ala Arg Thr Thr Ala Met Asp Tyr
1 5 10

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Ser Leu Ala Arg Ala Thr Ala Met Asp Tyr
1 5 10

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1 5 10

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Thr Leu Ala Arg Ile Thr Ala Met Asp Tyr
1 5 10

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Leu Leu Ala Arg Ile Thr Ala Met Asp Tyr
1 5 10

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Ser Thr Ala Arg Thr Thr Val Leu Asp Tyr
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<400> 66

Phe Ala Ser Gln Asp Ile Ser Ser Tyr Leu Asn
1 5 10

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<400> 67

Arg Ala Ser Arg Asp Ile Ser Ser Tyr Leu Asn
1 5 10

<210> 68
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<400> 68

Arg Ala Ser Thr Asp Ile Ser Ser Tyr Leu Asn
1 5 10

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<400> 69

Arg Ala Ser Gln Asp Ile Ser Ser Phe Leu Asn
1 5 10

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<400> 70

Arg Ala Ser Gln Asp Ile Ser Ser Tyr Leu Ser
1 5 10

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<400> 71

Tyr Gly Ser Arg Leu His Ser
1 5

<210> 72
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Gly Gln Gly Asn Thr Leu Pro Tyr Thr
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Asn Gln Gly Asn Thr Leu Pro Tyr Thr
1 5

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Ser Gln Gly Asn Thr Leu Pro Tyr Thr
1 5

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Gln Gln Ser Asn Thr Leu Pro Tyr Thr
1 5

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Gln Gln Gly Asn Arg Leu Pro Tyr Thr
1 5

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Gln Gln Gly Asn Ser Leu Pro Tyr Thr
1 5

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Gly Gln Gly Asn Ser Leu Pro Tyr Thr
1 5

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Gly Gln Gly Asn Arg Leu Pro Tyr Thr
1 5

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Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
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Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Ser Ile Thr
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Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Ser Ile Thr
20 25 30

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Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Ser Gly Tyr Ser Ile Thr
20 25 30

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Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Ser Ile Ser
20 25 30

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<400> 84

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Ser Gly Tyr Ser Ile Ser
20 25 30

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<400> 86

Arg Val Thr Ile Leu Arg Asp Thr Ser Lys Asn Gln Phe Ser Leu Arg
1 5 10 15

Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala Arg
20 25 30

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Arg Val Thr Met Ser Arg Asp Thr Ser Lys Asn Gln Phe Ser Leu Arg
1 5 10 15

Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala Arg
20 25 30

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<400> 88

Arg Val Thr Met Leu Arg Asp Thr Ser Lys Asn Gln Phe Ser Leu Lys
1 5 10 15

Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala Arg
20 25 30

<210> 89
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Arg Val Thr Met Leu Arg Asp Thr Ser Lys Asn Gln Phe Ser Leu Arg
1 5 10 15

Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Ala Tyr Tyr Cys Ala Arg
20 25 30

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<400> 90

Arg Val Thr Ile Ser Arg Asp Thr Ser Lys Asn Gln Phe Ser Leu Lys
1 5 10 15

Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Ala Tyr Tyr Cys Ala Arg
20 25 30

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Trp Gly Glu Gly Ser Leu Val Thr Val Ser Ser
1 5 10

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<212> PRT
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<400> 92

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Ser Val Thr Ile Thr Cys
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<210> 93
<211> 15
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<400> 93

Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Glu Leu Leu Ile Tyr
1 5 10 15

<210> 94
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<220>
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<400> 94

Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
1 5 10 15

Phe Thr Ile Ser Ser Leu Glu Pro Glu Asp Ile Ala Thr Tyr Tyr Cys
20 25 30

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Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
1 5 10 15

Phe Thr Ile Ser Ser Leu Gln Ala Glu Asp Ile Ala Thr Tyr Tyr Cys
20 25 30

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<400> 96

Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
1 5 10 15

Phe Thr Ile Ser Ser Leu Gln Pro Glu Asp Ala Ala Thr Tyr Tyr Cys
20 25 30

<210> 97
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<400> 97

Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
1 5 10 15

Phe Thr Ile Ser Ser Leu Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys
20 25 30

<210> 98
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<400> 98

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1 5 10

<210> 99
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<212> PRT
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<400> 99

Tyr Ile Ser Tyr Ser Gly Ile Thr Thr Tyr Asn Pro Ser Leu Lys Gly
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<400> 100

Tyr Ile Ser Tyr Ser Gly Ile Thr Asn Tyr Asn Pro Ser Leu Lys Gly
1 5 10 15

<210> 101
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Tyr Thr Ser Glu Leu His Ser
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<210> 103
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<400> 103

Tyr Gly Ser Glu Leu His Ser
1 5

<210> 104
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<400> 104

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Ser Gly Tyr Ser Ile Ser Asp Asp
20 25 30

His Ala Trp Ser Trp Val Arg Gln Pro Pro Gly Glu Gly Leu Glu Trp
35 40 45

Ile Gly Tyr Ile Ser Tyr Ser Gly Ile Thr Asn Tyr Asn Pro Ser Leu
50 55 60

Lys Gly Arg Val Thr Ile Ser Arg Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Ala Tyr Tyr Cys
85 90 95

Ala Arg Ser Leu Ala Arg Thr Thr Ala Met Asp Tyr Trp Gly Glu Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
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<210> 105
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<212> PRT
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<400> 105

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Ser Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Ile Ser Ser Tyr
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Glu Leu Leu Ile
35 40 45

Tyr Tyr Gly Ser Glu Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Glu Ala
65 70 75 80

Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Gly Asn Ser Leu Pro Tyr
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Glu
100 105

<210> 106
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<400> 106

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1 5 10 15

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tgcaccgtgt ctggctactc aattaccagc gatcatgcct ggagctgggt tcgccagcca 180

cctggacgag gtcttgagtg gattggatac attagttata gtggaatcac aacctataat 240

ccatctctca aatccagagt gacaatgctg agagacacca gcaagaacca gttcagcctg 300

agactcagca gcgtgacagc cgccgacacc gcggtttatt attgtgcaag atccctagct 360

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acctgtagag ccagccagga catcagcagt tacctgaatt ggtaccagca gaagccagga 180

aaggctccaa agctgctgat ctactacacc tccagactgc actctggtgt gccaagcaga 240

ttcagcggta gcggtagcgg taccgacttc accttcacca tcagcagcct ccagccagag 300

gacatcgcta cctactactg ccaacagggt aacacgcttc catacacgtt cggccaaggg 360

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Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Ser Ile Thr Ser Asp
20 25 30

His Ala Trp Ser Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp
35 40 45

Ile Gly Tyr Ile Ser Tyr Ser Gly Ile Thr Thr Tyr Asn Pro Ser Leu
50 55 60

Lys Ser Arg Val Thr Met Leu Arg Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Leu Ala Arg Thr Thr Ala Met Asp Tyr Trp Gly Gln Gly
100 105 110

Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125

Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu
130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190

Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro
195 200 205

Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys Val Glu
210 215 220

Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu
225 230 235 240

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
245 250 255

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln
260 265 270

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
275 280 285

Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu
290 295 300

Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
305 310 315 320

Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
325 330 335

Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
340 345 350

Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
355 360 365

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
370 375 380

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly
385 390 395 400

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
405 410 415

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
420 425 430

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440 445

<210> 110
<211> 446

<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 110

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Ser Ile Thr Ser Asp
20 25 30

His Ala Trp Ser Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp
35 40 45

Ile Gly Tyr Ile Ser Tyr Ser Gly Ile Thr Thr Tyr Asn Pro Ser Leu
50 55 60

Lys Ser Arg Val Thr Met Leu Arg Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Leu Ala Arg Thr Thr Ala Met Asp Tyr Trp Gly Gln Gly
100 105 110

Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125

Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu
130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190

Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro
195 200 205

Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro
210 215 220

Cys Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe
225 230 235 240

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
245 250 255

Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val
260 265 270

Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
275 280 285

Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val
290 295 300

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
305 310 315 320

Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser
325 330 335

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
340 345 350

Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
355 360 365

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
370 375 380

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
385 390 395 400

Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp
405 410 415

Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
420 425 430

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys
435 440 445

<210> 111
<211> 445
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 111

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Ser Ile Thr Ser Asp
20 25 30

His Ala Trp Ser Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp
35 40 45

Ile Gly Tyr Ile Ser Tyr Ser Gly Ile Thr Thr Tyr Asn Pro Ser Leu
50 55 60

Lys Ser Arg Val Thr Met Leu Arg Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Leu Ala Arg Thr Thr Ala Met Asp Tyr Trp Gly Gln Gly
100 105 110

Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125

Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu
130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190

Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro
195 200 205

Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys Val Glu
210 215 220

Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu
225 230 235 240

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
245 250 255

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln
260 265 270

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
275 280 285

Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu
290 295 300

Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
305 310 315 320

Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
325 330 335

Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
340 345 350

Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
355 360 365

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
370 375 380

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
385 390 395 400

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
405 410 415

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
420 425 430

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440 445

<210> 112
<211> 446
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 112

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Ser Ile Thr Ser Asp
20 25 30

His Ala Trp Ser Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp
35 40 45

Ile Gly Tyr Ile Ser Tyr Ser Gly Ile Thr Thr Tyr Asn Pro Ser Leu
50 55 60

Lys Ser Arg Val Thr Met Leu Arg Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Leu Ala Arg Thr Thr Ala Met Asp Tyr Trp Gly Gln Gly
100 105 110

Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125

Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu
130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190

Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro
195 200 205

Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro
210 215 220

Cys Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe
225 230 235 240

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
245 250 255

Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val
260 265 270

Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
275 280 285

Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val
290 295 300

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
305 310 315 320

Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser
325 330 335

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
340 345 350

Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
355 360 365

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
370 375 380

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
385 390 395 400

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
405 410 415

Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
420 425 430

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys
435 440 445

<210> 113
<211> 443
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 113

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Ser Ile Thr Ser Asp
20 25 30

His Ala Trp Ser Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp
35 40 45

Ile Gly Tyr Ile Ser Tyr Ser Gly Ile Thr Thr Tyr Asn Pro Ser Leu
50 55 60

Lys Ser Arg Val Thr Met Leu Arg Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Leu Ala Arg Thr Thr Ala Met Asp Tyr Trp Gly Gln Gly
100 105 110

Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190

Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro
195 200 205

Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Ser Cys Val Glu
210 215 220

Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu
225 230 235 240

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
245 250 255

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln
260 265 270

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
275 280 285

Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu
290 295 300

Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
305 310 315 320

Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys
325 330 335

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
340 345 350

Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
355 360 365

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
370 375 380

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
385 390 395 400

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
405 410 415

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
420 425 430

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
435 440

<210> 114
<211> 443
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 114

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Ser Ile Thr Ser Asp
20 25 30

His Ala Trp Ser Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp
35 40 45

Ile Gly Tyr Ile Ser Tyr Ser Gly Ile Thr Thr Tyr Asn Pro Ser Leu
50 55 60

Lys Ser Arg Val Thr Met Leu Arg Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Leu Ala Arg Thr Thr Ala Met Asp Tyr Trp Gly Gln Gly
100 105 110

Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190

Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro
195 200 205

Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Ser Cys Val Glu
210 215 220

Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu
225 230 235 240

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
245 250 255

Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln
260 265 270

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
275 280 285

Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu
290 295 300

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
305 310 315 320

Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys
325 330 335

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
340 345 350

Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
355 360 365

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
370 375 380

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
385 390 395 400

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
405 410 415

Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
420 425 430

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu
435 440

<210> 115
<211> 446
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 115

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Ser Ile Thr Ser Asp
20 25 30

His Ala Trp Ser Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp
35 40 45

Ile Gly Tyr Ile Ser Tyr Ser Gly Ile Thr Thr Tyr Asn Pro Ser Leu
50 55 60

Lys Ser Arg Val Thr Met Leu Arg Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Leu Ala Arg Thr Thr Ala Met Asp Tyr Trp Gly Gln Gly
100 105 110

Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
195 200 205

Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
210 215 220

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser
225 230 235 240

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
245 250 255

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
260 265 270

Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
275 280 285

Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
290 295 300

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
305 310 315 320

Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr
325 330 335

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
340 345 350

Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys
355 360 365

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
370 375 380

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
385 390 395 400

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
405 410 415

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
420 425 430

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
435 440 445

<210> 116
<211> 327
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 116

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1 5 10 15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65 70 75 80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
100 105 110

Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
115 120 125

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
130 135 140

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
145 150 155 160

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
165 170 175

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
180 185 190

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
195 200 205

Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
210 215 220

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu
225 230 235 240

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
245 250 255

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
260 265 270

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
275 280 285

Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
290 295 300

Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
305 310 315 320

Lys Ser Leu Ser Leu Ser Pro
325

<210> 117
<211> 443
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 117

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Ser Gly Tyr Ser Ile Ser Asp Asp
20 25 30

His Ala Trp Ser Trp Val Arg Gln Pro Pro Gly Glu Gly Leu Glu Trp
35 40 45

Ile Gly Tyr Ile Ser Tyr Ser Gly Ile Thr Asn Tyr Asn Pro Ser Leu
50 55 60

Lys Gly Arg Val Thr Ile Ser Arg Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Ala Tyr Tyr Cys
85 90 95

Ala Arg Val Leu Ala Arg Ile Thr Ala Met Asp Tyr Trp Gly Glu Gly
100 105 110

Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190

Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro
195 200 205

Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Ser Cys Val Glu
210 215 220

Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu
225 230 235 240

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
245 250 255

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln
260 265 270

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
275 280 285

Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu
290 295 300

Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
305 310 315 320

Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys
325 330 335

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
340 345 350

Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
355 360 365

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
370 375 380

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
385 390 395 400

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
405 410 415

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
420 425 430

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
435 440

<210> 118
<211> 324
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 118

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1 5 10 15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr
65 70 75 80

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Thr Val Glu Arg Lys Ser Cys Val Glu Cys Pro Pro Cys Pro Ala Pro
100 105 110

Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
115 120 125

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
130 135 140

Val Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly
145 150 155 160

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn
165 170 175

Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp
180 185 190

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro
195 200 205

Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu
210 215 220

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn
225 230 235 240

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
245 250 255

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
260 265 270

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
275 280 285

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
290 295 300

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
305 310 315 320

Ser Leu Ser Pro

<210> 119
<211> 443
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 119

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Ser Ile Thr Ser Asp
20 25 30

His Ala Trp Ser Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp
35 40 45

Ile Gly Tyr Ile Ser Tyr Ser Gly Ile Thr Thr Tyr Asn Pro Ser Leu
50 55 60

Lys Ser Arg Val Thr Met Leu Arg Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Leu Ala Arg Thr Thr Ala Met Asp Tyr Trp Gly Gln Gly
100 105 110

Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190

Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro
195 200 205

Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Ser Cys Val Glu
210 215 220

Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu
225 230 235 240

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
245 250 255

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln
260 265 270

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
275 280 285

Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu
290 295 300

Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
305 310 315 320

Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
325 330 335

Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
340 345 350

Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
355 360 365

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
370 375 380

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
385 390 395 400

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
405 410 415

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
420 425 430

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
435 440

<210> 120
<211> 445
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 120

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Ser Ile Thr Ser Asp
20 25 30

His Ala Trp Ser Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp
35 40 45

Ile Gly Tyr Ile Ser Tyr Ser Gly Ile Thr Thr Tyr Asn Pro Ser Leu
50 55 60

Lys Ser Arg Val Thr Met Leu Arg Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Leu Ala Arg Thr Thr Ala Met Asp Tyr Trp Gly Gln Gly
100 105 110

Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Glu Ser Thr Ala Ala Leu
130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190

Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro
195 200 205

Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Ser Cys Val Glu
210 215 220

Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu
225 230 235 240

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
245 250 255

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln
260 265 270

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
275 280 285

Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu
290 295 300

Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
305 310 315 320

Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
325 330 335

Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
340 345 350

Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
355 360 365

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
370 375 380

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly
385 390 395 400

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
405 410 415

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
420 425 430

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440 445

<210> 121
<211> 16
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 121

Tyr Ile Ser Tyr Ser Gly Ile Thr Thr Tyr Asn Pro Ser Leu Gln Asp
1 5 10 15

<210> 122
<211> 16
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 122

Tyr Ile Ser Tyr Ser Gly Ile Thr Asn Tyr Asn Pro Ser Leu Gln Asp
1 5 10 15

<210> 123
<211> 11
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 123

Arg Ala Ser Glu Asp Ile Ser Ser Tyr Leu Asn
1 5 10

<210> 124
<211> 11
<212> PRT
<213> Artificial

<220>

<223> An artificially synthesized peptide sequence

<400> 124

Gln Ala Ser Glu Asp Ile Ser Ser Tyr Leu Asn
1 5 10

<210> 125
<211> 7
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 125

Tyr Thr Ser Arg Leu Glu Ser
1 5

<210> 126
<211> 7
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 126

Tyr Gly Ser Glu Leu Glu Ser
1 5

<210> 127
<211> 32
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 127

Arg Val Thr Ile Ser Arg Asp Thr Ser Lys Asn Gln Phe Ser Leu Lys
1 5 10 15

Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala Arg
20 25 30

<210> 128
<211> 32
<212> PRT
<213> Homo sapiens

<400> 128

Arg Val Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
20 25 30

<210> 129
<211> 32
<212> PRT

<213> Homo sapiens

<400> 129

Arg Val Thr Ile Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr Met Glu
1 5 10 15

Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
20 25 30

<210> 130
<211> 11
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 130

Trp Gly Gln Gly Ile Leu Val Thr Val Ser Ser
1 5 10

<210> 131
<211> 11
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 131

Trp Gly Glu Gly Ile Leu Val Thr Val Ser Ser
1 5 10

<210> 132
<211> 11
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 132

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
1 5 10

<210> 133
<211> 11
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 133

Trp Gly Glu Gly Thr Leu Val Thr Val Ser Ser
1 5 10

<210> 134
<211> 449
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 134

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Ser Gly Tyr Ser Ile Ser Asp Asp
20 25 30

His Ala Trp Ser Trp Val Arg Gln Pro Pro Gly Glu Gly Leu Glu Trp
35 40 45

Ile Gly Tyr Ile Ser Tyr Ser Gly Ile Thr Asn Tyr Asn Pro Ser Leu
50 55 60

Lys Gly Arg Val Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Val Leu Ala Arg Ile Thr Ala Met Asp Tyr Trp Gly Glu Gly
100 105 110

Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
195 200 205

Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
210 215 220

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225 230 235 240

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
245 250 255

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
260 265 270

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
275 280 285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
290 295 300

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305 310 315 320

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
325 330 335

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
340 345 350

Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
355 360 365

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
370 375 380

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385 390 395 400

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
405 410 415

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
420 425 430

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445

Lys

<210> 135
<211> 449
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 135

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Ser Gly Tyr Ser Ile Ser Asp Asp
20 25 30

His Ala Trp Ser Trp Val Arg Gln Pro Pro Gly Glu Gly Leu Glu Trp
35 40 45

Ile Gly Tyr Ile Ser Tyr Ser Gly Ile Thr Asn Tyr Asn Pro Ser Leu
50 55 60

Gln Asp Arg Val Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Leu Leu Ala Arg Ala Thr Ala Met Asp Tyr Trp Gly Gln Gly
100 105 110

Ile Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
195 200 205

Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
210 215 220

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225 230 235 240

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
245 250 255

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
260 265 270

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
275 280 285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
290 295 300

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305 310 315 320

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
325 330 335

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
340 345 350

Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
355 360 365

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
370 375 380

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385 390 395 400

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
405 410 415

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
420 425 430

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445

Lys

<210> 136
<211> 214
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 136

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Ser Val Thr Ile Thr Cys Gln Ala Ser Glu Asp Ile Ser Ser Tyr
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Glu Leu Leu Ile
35 40 45

Tyr Tyr Gly Ser Glu Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Glu Ala
65 70 75 80

Glu Asp Ala Ala Thr Tyr Tyr Cys Gly Gln Gly Asn Arg Leu Pro Tyr
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Glu Arg Thr Val Ala Ala
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205

Phe Asn Arg Gly Glu Cys
210

<210> 137
<211> 449
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 137

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Ser Gly Tyr Ser Ile Ser Asp Asp
20 25 30

His Ala Val Ser Trp Val Arg Gln Pro Pro Gly Glu Gly Leu Glu Trp
35 40 45

Ile Gly Phe Ile Ser Tyr Ser Gly Ile Thr Asn Tyr Asn Pro Thr Leu
50 55 60

Gln Asp Arg Val Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Leu Leu Ala Arg Ala Thr Ala Met Asp Val Trp Gly Glu Gly
100 105 110

Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
195 200 205

Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
210 215 220

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225 230 235 240

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
245 250 255

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
260 265 270

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
275 280 285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
290 295 300

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305 310 315 320

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
325 330 335

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
340 345 350

Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
355 360 365

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
370 375 380

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385 390 395 400

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
405 410 415

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
420 425 430

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445

Lys

<210> 138
<211> 214
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 138

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Ser Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Ile Ser Ser Tyr
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Glu Leu Leu Ile
35 40 45

Tyr Tyr Gly Ser Glu Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Glu Ala
65 70 75 80

Glu Asp Ala Ala Thr Tyr Tyr Cys Gly Gln Gly Asn Arg Leu Pro Tyr
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Glu Arg Thr Val Ala Ala
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205

Phe Asn Arg Gly Glu Cys
210

<210> 139
<211> 447
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 139

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Ser Gly Tyr Ser Ile Ser Asp Asp
20 25 30

His Ala Val Ser Trp Val Arg Gln Pro Pro Gly Glu Gly Leu Glu Trp
35 40 45

Ile Gly Phe Ile Ser Tyr Ser Gly Ile Thr Asn Tyr Asn Pro Thr Leu
50 55 60

Gln Asp Arg Val Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Leu Leu Ala Arg Ala Thr Ala Met Asp Val Trp Gly Glu Gly
100 105 110

Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
195 200 205

Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
210 215 220

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225 230 235 240

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
245 250 255

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
260 265 270

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
275 280 285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
290 295 300

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305 310 315 320

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
325 330 335

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
340 345 350

Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
355 360 365

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
370 375 380

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385 390 395 400

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
405 410 415

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
420 425 430

Ala Leu His Ala His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
435 440 445

<210> 140
<211> 267
<212> PRT
<213> Homo sapiens

<400> 140

Ala Glu Ser His Leu Ser Leu Leu Tyr His Leu Thr Ala Val Ser Ser
1 5 10 15

Pro Ala Pro Gly Thr Pro Ala Phe Trp Val Ser Gly Trp Leu Gly Pro
20 25 30

Gln Gln Tyr Leu Ser Tyr Asn Ser Leu Arg Gly Glu Ala Glu Pro Cys
35 40 45

Gly Ala Trp Val Trp Glu Asn Gln Val Ser Trp Tyr Trp Glu Lys Glu

50 55 60

Thr Thr Asp Leu Arg Ile Lys Glu Lys Leu Phe Leu Glu Ala Phe Lys
65 70 75 80

Ala Leu Gly Gly Lys Gly Pro Tyr Thr Leu Gln Gly Leu Leu Gly Cys
85 90 95

Glu Leu Gly Pro Asp Asn Thr Ser Val Pro Thr Ala Lys Phe Ala Leu
100 105 110

Asn Gly Glu Glu Phe Met Asn Phe Asp Leu Lys Gln Gly Thr Trp Gly
115 120 125

Gly Asp Trp Pro Glu Ala Leu Ala Ile Ser Gln Arg Trp Gln Gln Gln
130 135 140

Asp Lys Ala Ala Asn Lys Glu Leu Thr Phe Leu Leu Phe Ser Cys Pro
145 150 155 160

His Arg Leu Arg Glu His Leu Glu Arg Gly Arg Gly Asn Leu Glu Trp
165 170 175

Lys Glu Pro Pro Ser Met Arg Leu Lys Ala Arg Pro Ser Ser Pro Gly
180 185 190

Phe Ser Val Leu Thr Cys Ser Ala Phe Ser Phe Tyr Pro Pro Glu Leu

195 200 205

Gln Leu Arg Phe Leu Arg Asn Gly Leu Ala Ala Gly Thr Gly Gln Gly
210 215 220

Asp Phe Gly Pro Asn Ser Asp Gly Ser Phe His Ala Ser Ser Ser Leu
225 230 235 240

Thr Val Lys Ser Gly Asp Glu His His Tyr Cys Cys Ile Val Gln His
245 250 255

Ala Gly Leu Ala Gln Pro Leu Arg Val Glu Leu
260 265

<210> 141
<211> 99
<212> PRT
<213> Homo sapiens

<400> 141

Ile Gln Arg Thr Pro Lys Ile Gln Val Tyr Ser Arg His Pro Ala Glu
1 5 10 15

Asn Gly Lys Ser Asn Phe Leu Asn Cys Tyr Val Ser Gly Phe His Pro
20 25 30

Ser Asp Ile Glu Val Asp Leu Leu Lys Asn Gly Glu Arg Ile Glu Lys
35 40 45

Val Glu His Ser Asp Leu Ser Phe Ser Lys Asp Trp Ser Phe Tyr Leu
50 55 60

Leu Tyr Tyr Thr Glu Phe Thr Pro Thr Glu Lys Asp Glu Tyr Ala Cys
65 70 75 80

Arg Val Asn His Val Thr Leu Ser Gln Pro Lys Ile Val Lys Trp Asp
85 90 95

Arg Asp Met

<210> 142
<211> 443
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 142

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Ser Ile Thr Ser Asp
20 25 30

His Ala Trp Ser Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp
35 40 45

Ile Gly Tyr Ile Ser Tyr Ser Gly Ile Thr Thr Tyr Asn Pro Ser Leu
50 55 60

Lys Ser Arg Val Thr Met Leu Arg Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Leu Ala Arg Thr Thr Ala Met Asp Tyr Trp Gly Gln Gly
100 105 110

Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190

Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro
195 200 205

Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Ser Cys Val Glu
210 215 220

Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu
225 230 235 240

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
245 250 255

Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln
260 265 270

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
275 280 285

Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu
290 295 300

Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
305 310 315 320

Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
325 330 335

Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
340 345 350

Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
355 360 365

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
370 375 380

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly
385 390 395 400

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
405 410 415

Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
420 425 430

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
435 440

<210> 143
<211> 449
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 143

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Ser Ile Thr Ser Asp
20 25 30

His Ala Trp Ser Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp
35 40 45

Ile Gly Tyr Ile Ser Tyr Ser Gly Ile Thr Thr Tyr Asn Pro Ser Leu
50 55 60

Lys Ser Arg Val Thr Met Leu Arg Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Leu Ala Arg Thr Thr Ala Met Asp Tyr Trp Gly Gln Gly
100 105 110

Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
195 200 205

Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
210 215 220

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225 230 235 240

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
245 250 255

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
260 265 270

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
275 280 285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
290 295 300

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305 310 315 320

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
325 330 335

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
340 345 350

Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
355 360 365

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
370 375 380

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385 390 395 400

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
405 410 415

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
420 425 430

Ala Leu His Ala His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445

Lys

<210> 144
<211> 443
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 144

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Ser Ile Thr Ser Asp
20 25 30

His Ala Trp Ser Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp
35 40 45

Ile Gly Tyr Ile Ser Tyr Ser Gly Ile Thr Thr Tyr Asn Pro Ser Leu
50 55 60

Lys Ser Arg Val Thr Met Leu Arg Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Leu Ala Arg Thr Thr Ala Met Asp Tyr Trp Gly Gln Gly
100 105 110

Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190

Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro
195 200 205

Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Ser Cys Val Glu
210 215 220

Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu
225 230 235 240

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
245 250 255

Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln
260 265 270

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
275 280 285

Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu
290 295 300

Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
305 310 315 320

Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
325 330 335

Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
340 345 350

Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
355 360 365

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
370 375 380

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly
385 390 395 400

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
405 410 415

Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Ala
420 425 430

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
435 440

<210> 145
<211> 119
<212> PRT
<213> Mus musculus

<400> 145

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu

1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Ser Gly Tyr Ser Ile Ser Asp Asp
20 25 30

His Ala Trp Ser Trp Val Arg Gln Pro Pro Gly Glu Gly Leu Glu Trp
35 40 45

Ile Gly Tyr Ile Ser Tyr Ser Gly Ile Thr Asn Tyr Asn Pro Ser Leu
50 55 60

Gln Asp Arg Val Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Leu Leu Ala Arg Ala Thr Ala Met Asp Tyr Trp Gly Gln Gly
100 105 110

Ile Leu Val Thr Val Ser Ser
115

<210> 146
<211> 107
<212> PRT
<213> Mus musculus

<400> 146

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Ser Val Thr Ile Thr Cys Gln Ala Ser Glu Asp Ile Ser Ser Tyr
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Glu Leu Leu Ile
35 40 45

Tyr Tyr Gly Ser Glu Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Glu Ala
65 70 75 80

Glu Asp Ala Ala Thr Tyr Tyr Cys Gly Gln Gly Asn Arg Leu Pro Tyr
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Glu
100 105

<210> 147
<211> 120
<212> PRT
<213> Mus musculus

<400> 147

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
20 25 30

Ile Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Leu Ile Asn Pro Tyr Asn Gly Gly Thr Ser Tyr Asn Gln Lys Phe
50 55 60

Lys Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Gly Tyr Asp Asp Gly Pro Tyr Thr Met Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Leu Val Thr Val Ser
115 120

<210> 148
<211> 107
<212> PRT

<213> Mus musculus

<400> 148

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Thr Ser Glu Asn Ile Tyr Ser Phe
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Asn Ala Lys Thr Leu Ala Lys Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln His His Tyr Glu Ser Pro Leu
85 90 95

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 149
<211> 122
<212> PRT
<213> Mus musculus

<400> 149

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Gly Ile Thr Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Asp Pro Gly Thr Thr Val Ile Met Ser Trp Phe Asp Pro Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 150

<211> 108
<212> PRT
<213> Mus musculus

<400> 150

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Arg Gly Arg
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Phe Tyr Cys Gln Gln Tyr Gly Ser Ser Pro
85 90 95

Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 151
<211> 324

<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 151

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Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1               5                  10                  15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
            20                  25                  30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35                  40                  45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50                  55                  60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr
65                  70                  75                  80

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95

Thr Val Glu Arg Lys Ser Cys Val Glu Cys Pro Pro Cys Pro Ala Pro
            100                 105                 110
```

Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
115 120 125

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
130 135 140

Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly
145 150 155 160

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn
165 170 175

Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp
180 185 190

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro
195 200 205

Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu
210 215 220

Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn
225 230 235 240

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
245 250 255

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
260 265 270

Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
275 280 285

Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys
290 295 300

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
305 310 315 320

Ser Leu Ser Pro

<210> 152
<211> 330
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 152

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1 5 10 15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65 70 75 80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
100 105 110

Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
115 120 125

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
130 135 140

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145 150 155 160

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
165 170 175

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
180 185 190

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
195 200 205

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
210 215 220

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
225 230 235 240

Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
245 250 255

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
260 265 270

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
275 280 285

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
290 295 300

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Ala His Tyr Thr
305 310 315 320

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
325 330

<210> 153
<211> 324
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 153

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1 5 10 15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr
65 70 75 80

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Thr Val Glu Arg Lys Ser Cys Val Glu Cys Pro Pro Cys Pro Ala Pro
100 105 110

Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
115 120 125

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
130 135 140

Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly
145 150 155 160

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn
165 170 175

Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp
180 185 190

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro
195 200 205

Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu
210 215 220

Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn
225 230 235 240

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
245 250 255

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
260 265 270

Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
275 280 285

Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys
290 295 300

Ser Val Met His Glu Ala Leu His Ala His Tyr Thr Gln Lys Ser Leu
305 310 315 320

Ser Leu Ser Pro

<210> 154
<211> 326
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 154

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1 5 10 15

Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr
65 70 75 80

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Thr Val Glu Arg Lys Ser Cys Val Glu Cys Pro Pro Cys Pro Ala Pro
100 105 110

Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
115 120 125

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
130 135 140

Val Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly
145 150 155 160

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn
165 170 175

Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp
180 185 190

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro
195 200 205

Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu
210 215 220

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn
225 230 235 240

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
245 250 255

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
260 265 270

Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
275 280 285

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
290 295 300

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
305 310 315 320

Ser Leu Ser Pro Gly Lys
325

<210> 155
<211> 326
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 155

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
1 5 10 15

Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr
65 70 75 80

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Thr Val Glu Arg Lys Ser Cys Val Glu Cys Pro Pro Cys Pro Ala Pro
100 105 110

Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
115 120 125

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
130 135 140

Val Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly
145 150 155 160

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn
165 170 175

Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp
180 185 190

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro
195 200 205

Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu
210 215 220

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn
225 230 235 240

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
245 250 255

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
260 265 270

Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
275 280 285

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
290 295 300

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
305 310 315 320

Ser Leu Ser Pro Gly Lys
325

<210> 156
<211> 326
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 156

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
1 5 10 15

Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr
65 70 75 80

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Thr Val Glu Arg Lys Cys Ser Val Glu Cys Pro Pro Cys Pro Ala Pro
100 105 110

Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
115 120 125

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
130 135 140

Val Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly
145 150 155 160

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn
165 170 175

Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp
180 185 190

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro
195 200 205

Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu
210 215 220

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn
225 230 235 240

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
245 250 255

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
260 265 270

Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
275 280 285

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
290 295 300

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
305 310 315 320

Ser Leu Ser Pro Gly Lys
325

<210> 157
<211> 445
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 157

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Ser Ile Thr Ser Asp
20 25 30

His Ala Trp Ser Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp
35 40 45

Ile Gly Tyr Ile Ser Tyr Ser Gly Ile Thr Thr Tyr Asn Pro Ser Leu
50 55 60

Lys Ser Arg Val Thr Met Leu Arg Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Leu Ala Arg Thr Thr Ala Met Asp Tyr Trp Gly Gln Gly
100 105 110

Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125

Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu
130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190

Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro
195 200 205

Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Ser Cys Val Glu
210 215 220

Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu
225 230 235 240

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
245 250 255

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln
260 265 270

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
275 280 285

Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu
290 295 300

Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
305 310 315 320

Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
325 330 335

Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
340 345 350

Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
355 360 365

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
370 375 380

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly
385 390 395 400

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
405 410 415

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
420 425 430

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440 445

<210> 158
<211> 445
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 158

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Ser Ile Thr Ser Asp
20 25 30

His Ala Trp Ser Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp
35 40 45

Ile Gly Tyr Ile Ser Tyr Ser Gly Ile Thr Thr Tyr Asn Pro Ser Leu
50 55 60

Lys Ser Arg Val Thr Met Leu Arg Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Leu Ala Arg Thr Thr Ala Met Asp Tyr Trp Gly Gln Gly
100 105 110

Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125

Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu
130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190

Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro
195 200 205

Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Ser Val Glu
210 215 220

Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu
225 230 235 240

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
245 250 255

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln
260 265 270

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
275 280 285

Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu
290 295 300

Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
305 310 315 320

Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
325 330 335

Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
340 345 350

Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
355 360 365

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
370 375 380

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly
385 390 395 400

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
405 410 415

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
420 425 430

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440 445

<210> 159
<211> 119
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 159

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Ser Gly Tyr Ser Ile Ser Asp Asp
20 25 30

His Ala Trp Ser Trp Val Arg Gln Pro Pro Gly Glu Gly Leu Glu Trp
35 40 45

Ile Gly Tyr Ile Ser Tyr Ser Gly Ile Thr Asn Tyr Asn Pro Ser Leu
50 55 60

Lys Gly Arg Val Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Val Leu Ala Arg Ile Thr Ala Met Asp Tyr Trp Gly Glu Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

<210> 160
<211> 119
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 160

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Ser Gly Tyr Ser Ile Ser Asp Asp
20 25 30

His Ala Trp Ser Trp Val Arg Gln Pro Pro Gly Glu Gly Leu Glu Trp
35 40 45

Ile Gly Tyr Ile Ser Tyr Ser Gly Ile Thr Asn Tyr Asn Pro Ser Leu
50 55 60

Gln Asp Arg Val Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Leu Leu Ala Arg Ala Thr Ala Met Asp Tyr Trp Gly Gln Gly
100 105 110

Ile Leu Val Thr Val Ser Ser
115

<210> 161
<211> 119
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 161

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Ser Gly Tyr Ser Ile Ser Asp Asp
20 25 30

His Ala Val Ser Trp Val Arg Gln Pro Pro Gly Glu Gly Leu Glu Trp
35 40 45

Ile Gly Phe Ile Ser Tyr Ser Gly Ile Thr Asn Tyr Asn Pro Thr Leu
50 55 60

Gln Asp Arg Val Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Leu Leu Ala Arg Ala Thr Ala Met Asp Val Trp Gly Glu Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

<210> 162
<211> 107
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 162

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Ser Val Thr Ile Thr Cys Gln Ala Ser Glu Asp Ile Ser Ser Tyr
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Glu Leu Leu Ile
35 40 45

Tyr Tyr Gly Ser Glu Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Glu Ala
65 70 75 80

Glu Asp Ala Ala Thr Tyr Tyr Cys Gly Gln Gly Asn Arg Leu Pro Tyr
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Glu
100 105

<210> 163
<211> 107

<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 163

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Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                  10                  15

Asp Ser Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Ile Ser Ser Tyr
            20                  25                  30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Glu Leu Leu Ile
        35                  40                  45

Tyr Tyr Gly Ser Glu Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Glu Ala
65                  70                  75                  80

Glu Asp Ala Ala Thr Tyr Tyr Cys Gly Gln Gly Asn Arg Leu Pro Tyr
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Glu
            100                 105
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<210> 164
<211> 328
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 164

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1 5 10 15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65 70 75 80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
100 105 110

Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
115 120 125

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
130 135 140

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145 150 155 160

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
165 170 175

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
180 185 190

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
195 200 205

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
210 215 220

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
225 230 235 240

Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
245 250 255

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
260 265 270

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
275 280 285

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
290 295 300

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Ala His Tyr Thr
305 310 315 320

Gln Lys Ser Leu Ser Leu Ser Pro
325

<210> 165
<211> 6
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 165

Asp Asp His Ala Val Ser
1 5

<210> 166
<211> 16
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 166

Phe Ile Ser Tyr Ser Gly Ile Thr Asn Tyr Asn Pro Thr Leu Gln Asp
1 5 10 15

<210> 167
<211> 10
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 167

Leu Leu Ala Arg Ala Thr Ala Met Asp Val
1 5 10

<210> 168
<211> 7
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 168

Tyr Gly Ser Glu Leu Glu Ser
1 5

<210> 169
<211> 6
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 169

His Asp His Ala Trp Ser
1 5

<210> 170
<211> 16
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 170

Phe Ile Ser Tyr Ser Gly Ile Thr Asn Tyr Asn Pro Ser Leu Gln Gly
1 5 10 15

<210> 171
<211> 10

<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 171

Ser Leu Ala Arg Thr Thr Ala Met Asp Tyr
1 5 10

<210> 172
<211> 11
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 172

Gln Ala Ser Thr Asp Ile Ser Ser His Leu Asn
1 5 10

<210> 173
<211> 7
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 173

Tyr Gly Ser His Leu Leu Ser
1 5

<210> 174
<211> 16
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 174

Phe Ile Ser Tyr Ser Gly Ile Thr Asn Tyr Asn Pro Thr Leu Gln Gly
1 5 10 15

<210> 175
<211> 11
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 175

Gln Ala Ser Arg Asp Ile Ser Ser His Leu Asn
1 5 10

<210> 176
<211> 119
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 176

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Ser Gly His Ser Ile Ser His Asp
20 25 30

His Ala Trp Ser Trp Val Arg Gln Pro Pro Gly Glu Gly Leu Glu Trp
35 40 45

Ile Gly Phe Ile Ser Tyr Ser Gly Ile Thr Asn Tyr Asn Pro Ser Leu
50 55 60

Gln Gly Arg Val Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Leu Ala Arg Thr Thr Ala Met Asp Tyr Trp Gly Glu Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

<210> 177
<211> 107
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 177

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Ser Val Thr Ile Thr Cys Gln Ala Ser Thr Asp Ile Ser Ser His
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Glu Leu Leu Ile
35 40 45

Tyr Tyr Gly Ser His Leu Leu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Glu Ala
65 70 75 80

Glu Asp Ala Ala Thr Tyr Tyr Cys Gly Gln Gly Asn Arg Leu Pro Tyr
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Glu
100 105

<210> 178
<211> 119
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 178

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Ser Gly His Ser Ile Ser His Asp
20 25 30

His Ala Trp Ser Trp Val Arg Gln Pro Pro Gly Glu Gly Leu Glu Trp
35 40 45

Ile Gly Phe Ile Ser Tyr Ser Gly Ile Thr Asn Tyr Asn Pro Thr Leu
50 55 60

Gln Gly Arg Val Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Leu Ala Arg Thr Thr Ala Met Asp Tyr Trp Gly Glu Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

<210> 179
<211> 107
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 179

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Ser Val Thr Ile Thr Cys Gln Ala Ser Arg Asp Ile Ser Ser His
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Glu Leu Leu Ile
35 40 45

Tyr Tyr Gly Ser His Leu Leu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Glu Ala
65 70 75 80

Glu Asp Ala Ala Thr Tyr Tyr Cys Gly Gln Gly Asn Arg Leu Pro Tyr
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Glu
100 105

<210> 180
<211> 443
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 180

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Ser Gly His Ser Ile Ser His Asp
20 25 30

His Ala Trp Ser Trp Val Arg Gln Pro Pro Gly Glu Gly Leu Glu Trp
35 40 45

Ile Gly Phe Ile Ser Tyr Ser Gly Ile Thr Asn Tyr Asn Pro Ser Leu
50 55 60

Gln Gly Arg Val Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Leu Ala Arg Thr Thr Ala Met Asp Tyr Trp Gly Glu Gly
100 105 110

Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190

Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro
195 200 205

Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Ser Cys Val Glu
210 215 220

Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu
225 230 235 240

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
245 250 255

Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln
260 265 270

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
275 280 285

Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu
290 295 300

Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
305 310 315 320

Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
325 330 335

Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
340 345 350

Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
355 360 365

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
370 375 380

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly
385 390 395 400

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
405 410 415

Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Ala
420 425 430

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
435 440

<210> 181
<211> 214
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 181

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Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                  10                  15

Asp Ser Val Thr Ile Thr Cys Gln Ala Ser Thr Asp Ile Ser Ser His
            20                  25                  30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Glu Leu Leu Ile
        35                  40                  45

Tyr Tyr Gly Ser His Leu Leu Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Glu Ala
65                  70                  75                  80

Glu Asp Ala Ala Thr Tyr Tyr Cys Gly Gln Gly Asn Arg Leu Pro Tyr
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Glu Arg Thr Val Ala Ala
            100                 105                 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140
```

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205

Phe Asn Arg Gly Glu Cys
210

<210> 182
<211> 443
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 182

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Ser Gly His Ser Ile Ser His Asp
20 25 30

His Ala Trp Ser Trp Val Arg Gln Pro Pro Gly Glu Gly Leu Glu Trp
35 40 45

Ile Gly Phe Ile Ser Tyr Ser Gly Ile Thr Asn Tyr Asn Pro Thr Leu
50 55 60

Gln Gly Arg Val Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Leu Ala Arg Thr Thr Ala Met Asp Tyr Trp Gly Glu Gly
100 105 110

Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190

Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro
195 200 205

Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Ser Cys Val Glu
210 215 220

Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu
225 230 235 240

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
245 250 255

Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln
260 265 270

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
275 280 285

Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu
290 295 300

Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
305 310 315 320

Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
325 330 335

Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
340 345 350

Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
355 360 365

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
370 375 380

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly
385 390 395 400

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
405 410 415

Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Ala
420 425 430

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
435 440

<210> 183
<211> 214

<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 183

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Ser Val Thr Ile Thr Cys Gln Ala Ser Arg Asp Ile Ser Ser His
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Glu Leu Leu Ile
35 40 45

Tyr Tyr Gly Ser His Leu Leu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Glu Ala
65 70 75 80

Glu Asp Ala Ala Thr Tyr Tyr Cys Gly Gln Gly Asn Arg Leu Pro Tyr
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Glu Arg Thr Val Ala Ala
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205

Phe Asn Arg Gly Glu Cys
210

<210> 184
<211> 32
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 184

Arg Val Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
20 25 30

<210> 185
<211> 447
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 185

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Ser Ile Thr Ser Asp
20 25 30

His Ala Trp Ser Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp
35 40 45

Ile Gly Tyr Ile Ser Tyr Ser Gly Ile Thr Thr Tyr Asn Pro Ser Leu
50 55 60

Lys Ser Arg Val Thr Met Leu Arg Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Leu Ala Arg Thr Thr Ala Met Asp Tyr Trp Gly Gln Gly
100 105 110

Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
195 200 205

Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
210 215 220

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225 230 235 240

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
245 250 255

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
260 265 270

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
275 280 285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
290 295 300

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305 310 315 320

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
325 330 335

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
340 345 350

Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
355 360 365

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
370 375 380

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385 390 395 400

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
405 410 415

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
420 425 430

Ala Leu His Ala His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
435 440 445

<210> 186
<211> 30
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 186

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Ser Gly His Ser Ile Ser
20 25 30

<210> 187
<211> 445
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 187

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Ser Ile Thr Ser Asp
20 25 30

His Ala Trp Ser Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp
35 40 45

Ile Gly Tyr Ile Ser Tyr Ser Gly Ile Thr Thr Tyr Asn Pro Ser Leu
50 55 60

Lys Ser Arg Val Thr Met Leu Arg Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Leu Ala Arg Thr Thr Ala Met Asp Tyr Trp Gly Gln Gly
100 105 110

Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190

Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro
195 200 205

Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Ser Cys Val Glu
210 215 220

Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu
225 230 235 240

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
245 250 255

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln
260 265 270

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
275 280 285

Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu
290 295 300

Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
305 310 315 320

Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys
325 330 335

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
340 345 350

Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
355 360 365

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
370 375 380

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly
385 390 395 400

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
405 410 415

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
420 425 430

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440 445

<210> 188
<211> 443
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 188

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Ser Ile Thr Ser Asp
20 25 30

His Ala Trp Ser Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp
35 40 45

Ile Gly Tyr Ile Ser Tyr Ser Gly Ile Thr Thr Tyr Asn Pro Ser Leu
50 55 60

Lys Ser Arg Val Thr Met Leu Arg Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Leu Ala Arg Thr Thr Ala Met Asp Tyr Trp Gly Gln Gly
100 105 110

Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190

Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro
195 200 205

Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Ser Cys Val Glu
210 215 220

Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu
225 230 235 240

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
245 250 255

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln
260 265 270

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
275 280 285

Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu
290 295 300

Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
305 310 315 320

Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys
325 330 335

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
340 345 350

Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
355 360 365

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
370 375 380

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly
385 390 395 400

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
405 410 415

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
420 425 430

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
435 440

<210> 189
<211> 445

<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 189

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Ser Ile Thr Ser Asp
20 25 30

His Ala Trp Ser Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp
35 40 45

Ile Gly Tyr Ile Ser Tyr Ser Gly Ile Thr Thr Tyr Asn Pro Ser Leu
50 55 60

Lys Ser Arg Val Thr Met Leu Arg Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Leu Ala Arg Thr Thr Ala Met Asp Tyr Trp Gly Gln Gly
100 105 110

Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190

Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro
195 200 205

Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Ser Cys Val Glu
210 215 220

Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu
225 230 235 240

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
245 250 255

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln
260 265 270

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
275 280 285

Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu
290 295 300

Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
305 310 315 320

Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
325 330 335

Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
340 345 350

Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
355 360 365

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
370 375 380

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly
385 390 395 400

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
405 410 415

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
420 425 430

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440 445

<210> 190
<211> 443
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 190

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Ser Ile Thr Ser Asp
20 25 30

His Ala Trp Ser Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp
35 40 45

Ile Gly Tyr Ile Ser Tyr Ser Gly Ile Thr Thr Tyr Asn Pro Ser Leu
50 55 60

Lys Ser Arg Val Thr Met Leu Arg Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Leu Ala Arg Thr Thr Ala Met Asp Tyr Trp Gly Gln Gly
100 105 110

Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180 185 190

Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro
195 200 205

Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Ser Cys Val Glu
210 215 220

Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu
225 230 235 240

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
245 250 255

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln
260 265 270

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
275 280 285

Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu
290 295 300

Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
305 310 315 320

Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
325 330 335

Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
340 345 350

Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
355 360 365

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
370 375 380

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly
385 390 395 400

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
405 410 415

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
420 425 430

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
435 440

<210> 191
<211> 326
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 191

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1 5 10 15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr
65 70 75 80

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Thr Val Glu Arg Lys Ser Cys Val Glu Cys Pro Pro Cys Pro Ala Pro
100 105 110

Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
115 120 125

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
130 135 140

Val Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly
145 150 155 160

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn
165 170 175

Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp
180 185 190

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro
195 200 205

Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
210 215 220

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn
225 230 235 240

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
245 250 255

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
260 265 270

Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
275 280 285

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
290 295 300

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
305 310 315 320

Ser Leu Ser Pro Gly Lys
325

<210> 192
<211> 324
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 192

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1 5 10 15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr
65 70 75 80

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Thr Val Glu Arg Lys Ser Cys Val Glu Cys Pro Pro Cys Pro Ala Pro
100 105 110

Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
115 120 125

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
130 135 140

Val Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly
145 150 155 160

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn
165 170 175

Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp
180 185 190

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro
195 200 205

Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
210 215 220

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn
225 230 235 240

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
245 250 255

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
260 265 270

Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
275 280 285

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
290 295 300

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
305 310 315 320

Ser Leu Ser Pro

<210> 193
<211> 326

<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 193

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1 5 10 15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr
65 70 75 80

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Thr Val Glu Arg Lys Ser Cys Val Glu Cys Pro Pro Cys Pro Ala Pro
100 105 110

Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
115 120 125

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
130 135 140

Val Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly
145 150 155 160

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn
165 170 175

Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp
180 185 190

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro
195 200 205

Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu
210 215 220

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn
225 230 235 240

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
245 250 255

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
260 265 270

Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
275 280 285

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
290 295 300

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
305 310 315 320

Ser Leu Ser Pro Gly Lys
325

<210> 194
<211> 324
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide sequence

<400> 194

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1 5 10 15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr
65 70 75 80

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Thr Val Glu Arg Lys Ser Cys Val Glu Cys Pro Pro Cys Pro Ala Pro
100 105 110

Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
115 120 125

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
130 135 140

Val Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly
145 150 155 160

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn
165 170 175

Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp
180 185 190

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro
195 200 205

Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu
210 215 220

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn
225 230 235 240

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
245 250 255

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
260 265 270

Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
275 280 285

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
290 295 300

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
305 310 315 320

Ser Leu Ser Pro