HU000027533T2

(19) **HU** (11) Lajstromszám: **E 027 533** (13) **T2**

**MAGYARORSZÁG**
**Szellemi Tulajdon Nemzeti Hivatala**

# EURÓPAI SZABADALOM
## SZÖVEGÉNEK FORDÍTÁSA

(21) Magyar ügyszám: **E 09 816184**
(22) A bejelentés napja: **2009. 09. 25.**

(96) Az európai bejelentés bejelentési száma:
**EP 20090816184**
(97) Az európai bejelentés közzétételi adatai:
**EP 2330193 A1** **2011. 05. 08.**
(97) Az európai szabadalom megadásának meghirdetési adatai:
**EP 2330193 B1** **2015. 06. 17.**

(51) Int. Cl.:

| | |
|---|---|
| ***C12N 15/09*** | *(2006.01)* |
| ***A61K 39/00*** | *(2006.01)* |
| ***A61K 393/95*** | *(2006.01)* |
| ***A61P 1/04*** | *(2006.01)* |
| ***A61P 1/16*** | *(2006.01)* |
| ***A61P 3/10*** | *(2006.01)* |
| ***A61P 7/06*** | *(2006.01)* |
| ***A61P 9/00*** | *(2006.01)* |
| ***A61P 9/10*** | *(2006.01)* |
| ***A61P 11/00*** | *(2006.01)* |
| ***A61P 11/06*** | *(2006.01)* |
| ***A61P 13/12*** | *(2006.01)* |
| ***A61P 15/08*** | *(2006.01)* |
| ***A61P 17/00*** | *(2006.01)* |
| ***A61P 17/06*** | *(2006.01)* |
| ***A61P 19/02*** | *(2006.01)* |
| ***A61P 19/10*** | *(2006.01)* |
| ***A61P 21/00*** | *(2006.01)* |
| ***A61P 25/00*** | *(2006.01)* |
| ***C07K 16/28*** | *(2006.01)* |

(86) A nemzetközi (PCT) bejelentési szám:
**PCT/JP 09/066590**
(87) A nemzetközi közzétételi szám:
**WO 10035769**

(30) Elsőbbségi adatok:

| | | |
|---|---|---|
| **2008248213** | **2008. 09. 26.** | **JP** |
| **2009060806** | **2009. 03. 13.** | **JP** |
| **2009067925** | **2009. 03. 19.** | **JP** |

(72) Feltaláló(k):
**IGAWA, Tomoyuki, Gotenba-shi Shizuoka 412-8513 (JP)**

**ISHII, Shinya, Gotenba-shi Shizuoka 412-8513 (JP)**
**MAEDA, Atsuhiko, Gotenba-shi Shizuoka 412-8513 (JP)**
**SAKURAI, Mika, Gotenba-shi Shizuoka 412-8513 (JP)**
**KOJIMA, Tetsuo, Gotenba-shi Shizuoka 412-8513 (JP)**
**TACHIBANA, Tatsuhiko, Gotenba-shi Shizuoka 412-8513 (JP)**

(73) Jogosult(ak):
**Chugai Seiyaku Kabushiki Kaisha,**
**Kita-kuTokyo 115-8543 (JP)**

(74) Képviselő:
**dr. Láng Tivadarné, SBGK Szabadalmi Ügyvivői Iroda, Budapest**

**SHIRAIWA, Hirotake, Gotenba-shi Shizuoka 412-8513 (JP)**
**TSUNODA, Hiroyuki, Gotenba-shi Shizuoka 412-8513 (JP)**
**HIGUCHI, Yoshinobu, Gotenba-shi Shizuoka 412-8513 (JP)**

(54) **IL-6 receptor elleni javított antitest molekula**

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 2 330 193 B1

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
**17.06.2015 Bulletin 2015/25**

(21) Application number: **09816184.7**

(22) Date of filing: **25.09.2009**

(51) Int Cl.:

| | |
|---|---|
| ***C12N 15/09*** *(2006.01)* | ***A61K 39/395*** *(2006.01)* |
| ***A61P 1/04*** *(2006.01)* | ***A61P 1/16*** *(2006.01)* |
| ***A61P 3/10*** *(2006.01)* | ***A61P 7/06*** *(2006.01)* |
| ***A61P 9/00*** *(2006.01)* | ***A61P 9/10*** *(2006.01)* |
| ***A61P 11/00*** *(2006.01)* | ***A61P 11/06*** *(2006.01)* |
| ***A61P 13/12*** *(2006.01)* | ***A61P 15/08*** *(2006.01)* |
| ***A61P 17/00*** *(2006.01)* | ***A61P 17/06*** *(2006.01)* |
| ***A61P 19/02*** *(2006.01)* | ***A61P 19/10*** *(2006.01)* |
| ***A61P 21/00*** *(2006.01)* | ***A61P 25/00*** *(2006.01)* |
| ***A61K 39/00*** *(2006.01)* | ***C07K 16/28*** *(2006.01)* |

(86) International application number:
**PCT/JP2009/066590**

(87) International publication number:
**WO 2010/035769 (01.04.2010 Gazette 2010/13)**

(54) **IMPROVED ANTIBODY MOLECULE AGAINST IL-6 RECEPTOR**

VERBESSERTES ANTIKÖRPERMOLEKÜL GEGEN IL-6 REZEPTOR

MOLÉCULE D'ANTICORPS AMÉLIORÉE CONTRE LE RÉCEPTEUR A IL-6

(84) Designated Contracting States:
**AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO SE SI SK SM TR**
Designated Extension States:
**AL BA RS**

(30) Priority: **26.09.2008 JP 2008248213**
**13.03.2009 JP 2009060806**
**19.03.2009 JP 2009067925**

(43) Date of publication of application:
**08.06.2011 Bulletin 2011/23**

(73) Proprietor: **Chugai Seiyaku Kabushiki Kaisha**
**Kita-ku**
**Tokyo 115-8543 (JP)**

(72) Inventors:
- **IGAWA, Tomoyuki**
  **Gotenba-shi**
  **Shizuoka 412-8513 (JP)**
- **ISHII, Shinya**
  **Gotenba-shi**
  **Shizuoka 412-8513 (JP)**
- **MAEDA, Atsuhiko**
  **Gotenba-shi**
  **Shizuoka 412-8513 (JP)**
- **SAKURAI, Mika**
  **Gotenba-shi**
  **Shizuoka 412-8513 (JP)**
- **KOJIMA, Tetsuo**
  **Gotenba-shi**
  **Shizuoka 412-8513 (JP)**
- **TACHIBANA, Tatsuhiko**
  **Gotenba-shi**
  **Shizuoka 412-8513 (JP)**
- **SHIRAIWA, Hirotake**
  **Gotenba-shi**
  **Shizuoka 412-8513 (JP)**
- **TSUNODA, Hiroyuki**
  **Gotenba-shi**
  **Shizuoka 412-8513 (JP)**
- **HIGUCHI, Yoshinobu**
  **Gotenba-shi**
  **Shizuoka 412-8513 (JP)**

(74) Representative: **Vossius & Partner**
**Patentanwälte Rechtsanwälte mbB**
**Siebertstrasse 3**
**81675 München (DE)**

(56) References cited:
**EP-A1- 2 194 066**
**EP-A1- 2 202 245**
**EP-A1- 2 206 775**
**EP-A1- 2 206 775**
**EP-A1- 2 275 443**
**WO-A2-2007/143168**





Printed by Jouve, 75001 PARIS (FR)

**(Cont. next page)**

JP-A- 2 163 096

- YOSHIYUKI OSUGI: 'Kotai Iyakuhin no Genzai Shinkokei -Kaihatsu.Seisan Gijutsu.Tokkyo Han'i- Seizo Hanbai Shonin o Eta Kaihatsu Hinmoku no Success Story Nipponhatsu Saisho no Kotai Iyaku 'Hito-ka Ko-hito-interleukin-6 Juyotai Kotai (Tocilizumab)' no Kenkyu Kaihatsu Keii -Kiso Kenkyu, Tansaku Kenkyu kara Soyaku Kenkyu,' GEKKAN PHARM STAGE vol. 7, no. 5, 2007, pages 13 - 18, XP008143206
- RAJPAL, A. ET AL.: 'A general method for greatly improving the affinity of antibodies by using combinatorial libraries.' PROC.NATL.ACAD.SCI.USA vol. 102, no. 24, 2005, pages 8466 - 8471, XP002392777
- ONDA, M. ET AL.: 'Lowering the Isoelectric Point of the Fv Portion of Recombinant Immunotoxins Leads to Decreased Nonspecific Animal Toxicity without Affecting Antitumor Activity.' CANCER RES. vol. 61, 2001, pages 5070 - 5077, XP002359466
- ITO, W. ET AL.: 'The His-probe method: effects of histidine residues introduced into the complementary-determining regions of antibodies on antigen-antibody interactions at different pH values.' FEBS LETT. vol. 309, no. 1, 1992, pages 85 - 88, XP008123385
- CHIRINO, A.J. ET AL.: 'Minimizing the immunogenicity of protein therapeutics.' DRUG DISCOVERY TODAY vol. 9, no. 2, 2004, pages 82 - 90, XP002395255

## Description

### Technical Field

**[0001]** The invention relates to pharmaceutical compositions comprising an anti-IL-6 receptor antibody as defined in the claims as an active ingredient, methods for producing the compositions, and such. Furthermore, the disclosure generally relates to pharmaceutical compositions comprising an anti-IL-6 receptor antibody as an active ingredient, methods for producing the compositions, and such.

### Background Art

**[0002]** Antibodies are drawing attention as pharmaceuticals as they are highly stable in plasma and have few adverse effects. Among 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, inflammatory diseases, malignant tumors, and so on (Non-Patent Document 3). TOCILIZUMAB, a humanized anti-IL-6 receptor IgG1 antibody, specifically binds to the IL-6 receptor. It is thought that TOCILIZUMAB can be used as a therapeutic agent for IL-6-associated diseases such as rheumatoid arthritis, since it neutralizes the biological activity of IL-6 (Patent Documents 1 to 3, and Non-Patent Documents 4 and 25). TOCILIZUMAB has been approved as a therapeutic agent for Castleman's disease and rheumatoid arthritis in Japan (Non-Patent Document 5).

**[0003]** Humanized antibodies such as TOCILIZUMAB are first-generation antibody pharmaceuticals. Second-generation antibody pharmaceuticals are currently being developed by improving the efficacy, convenience, and cost of first-generation antibody pharmaceuticals. Various technologies that are applicable to second-generation antibody pharmaceuticals are being developed. Technologies for enhancing effector function, antigen-binding ability, pharmacokinetics, and stability, as well as technologies for reducing the risk of immunogenicity have been reported (Patent Documents 7 to 10). For altering antibody function, especially amino acid modifications proved to be useful (Patent Documents 7, 10 and 11 and Non-Patent Documents 26 and 27).

**[0004]** As methods for enhancing drug efficacy or reducing dosage, technologies that enhance antibody-dependent cell-mediated cytotoxic activity (ADCC activity) or complement-dependent cytotoxic activity (CDC activity) through amino acid substitution in the Fc region of an IgG antibody have been reported (Non-Patent Document 6). Furthermore, affinity maturation has been reported as a technology for enhancing antigen-binding ability or antigen-neutralizing ability (Non-Patent Document 7). This technology enables one to enhance antigen-binding activity by introducing amino acid mutations into the complementarity determining (CDR) region of a variable region or such. The enhancement of antigen-binding ability improves *in vitro* biological activity or reduces dosage, and furthermore improves *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 efficacy than Palivizumab, a first-generation anti-RSV antibody pharmaceutical (Non-Patent Document 9). An anti-IL-6 receptor antibody with an affinity of about 0.05 nM (i.e., greater affinity than that of TOCILIZUMAB) has been reported (Patent Document 4). However, there is no report describing a human, humanized, or chimeric antibody having an affinity greater than 0.05 nM.

**[0005]** A problem encountered with current antibody pharmaceuticals is the high production cost associated with extremely large quantities of protein to be administered. 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 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 limit for IgG-type antibody formulations is generally about 100 mg/ml (Non-Patent Document 10). 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 to be administered, and by conferring the antibody with high stability.

**[0006]** FcRn is closely involved in antibody pharmacokinetics. With regard to differences in the plasma half-life of antibody isotypes, IgG1 and IgG2 are known to have superior plasma half-life than IgG3 and IgG4 (Non-Patent Document 11). As a method for further improving the plasma half-life of IgG1 and IgG2 antibodies which have superior plasma half-lives, substitution of amino acids in the constant region which enhances the binding to FcRn has been reported (Non-Patent Documents 12 and 13). From the viewpoint of immunogenicity, further improvement of the plasma half-life is performed by substituting amino acids preferably in the variable region rather than in the constant region (Patent Document 5). However, there is no report to date on the improvement of the plasma half-life of IL-6 receptor antibodies through alteration of the variable region.

**[0007]** Another important problem encountered in the development of 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 14). 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 15 and 16). T-cell epitopes may be present even in the CDR of human antibodies, and these T-cell epitopes in CDR are a possible cause of immunogenicity. *In silico* and *in vitro* methods for predicting T-cell epitopes have been reported (Non-Patent documents 17 and 18). It is assumed that immunogenicity risk can be reduced by removing T-cell epitopes predicted using such methods (Non-Patent document 19).

**[0008]** TOCILIZUMAB, a humanized anti-IL-6 receptor IgG1 antibody, is an IgG1 antibody obtained by humanizing mouse antibody PM1. CDR grafting is carried out using human NEW and REI sequences as template framework for H and L chains, respectively; however, five mouse sequence amino acids are retained in the framework as essential amino acids for maintaining the activity (Non-Patent Document 20). There is no previous report that fully humanizes the remaining mouse sequence in the framework of the humanized antibody TOCILIZUMAB without reducing the activity. Furthermore, the CDR sequence of TOCILIZUMAB is a mouse sequence, and thus, like Adalimumab, it may have T-cell epitopes in the CDR, which may have a potential immunogenicity risk. In clinical trials of TOCILIZUMAB, anti-TOCILIZUMAB antibodies were not detected at the effective dose of 8 mg/kg, but they were observed at the doses of 2 mg/kg and 4 mg/kg (Patent Document 6). These suggest that there is still room for improvement for the immunogenicity of TOCILIZUMAB. However, there has been no report on reducing the immunogenicity risk of TOCILIZUMAB by amino acid substitution.

**[0009]** The isotype of TOCILIZUMAB is IgG1. The isotype difference refers to difference in the constant region sequence. Since the constant region sequence is assumed to have strong influence on the effector function, pharmacokinetics, physical properties, and so on, selection of the constant region sequence is very important for the development of antibody pharmaceuticals (Non-Patent Document 11). In recent years, the safety of antibody pharmaceuticals has become of great importance. Interaction between the antibody Fc portion and Fcy receptor (effector function) may have caused serious adverse effects in phase-I clinical trials of TGN1412 (Non-Patent Document 21). For antibody pharmaceuticals designed to neutralize the biological activity of an antigen, the binding to Fcy receptor, which is important for effector functions such as ADCC, is unnecessary. The binding to Fcy receptor may even be unfavorable from the viewpoint of adverse effects. A method for reducing the binding to Fey receptor is to alter the isotype of an IgG antibody from IgG1 to IgG2 or IgG4 (Non-Patent Document 22). IgG2 is more favorable than IgG4 from the viewpoint of pharmacokinetics and Fcy receptor I binding (Non-Patent Document 11). TOCILIZUMAB is an IL-6 receptor-neutralizing antibody, and its isotype is IgG1. Thus, in view of the potential adverse effects, IgG2 may be a preferred isotype since effector functions such as ADCC are not needed.

**[0010]** Meanwhile, when developing antibody pharmaceuticals, physicochemical properties of the proteins, in particular, homogeneity and stability are very crucial. It has been reported that for the IgG2 isotype, there is significant heterogeneity derived from the disulfide bonds in the hinge region (Non-Patent Document 23). It is not easy and would be more costly to manufacture them as 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. Furthermore, for heterogeneity of the H-chain C-terminal sequences of an antibody, deletion of C-terminal amino acid lysine residue, and amidation of the C-terminal carboxyl group due to deletion of both of the two C-terminal amino acids, glycine and lysine, have been reported (Non-Patent Document 24). In developing IgG2 isotype antibodies as pharmaceuticals, it is preferable to reduce such heterogeneity and maintain high stability. To produce convenient, stable, high-concentration, subcutaneously administered formulations, it is preferable that not only the stability is high, but also the plasma half-life is superior to that of IgG1 which is the isotype of TOCILIZUMAB, However, there is no previous report on constant region sequences for antibodies with the IgG2-isotype constant region that have reduced heterogeneity, high stability, and superior plasma half-life than antibodies with the IgG1 isotype constant region.

**[0011]** Prior art documents related to the disclosure are shown below:

[Prior Art Documents]

[Patent Documents]

**[0012]**

[Patent Document 1] WO 92/19759
[Patent Document 2] WO 96/11020
[Patent Document 3] WO 96/12503
[Patent Document 4] WO 2007/143168
[Patent Document 5] WO 2007/114319
[Patent Document 6] WO 2004/096273
[Patent Document 7] EP 2206775
[Patent Document 8] EP 2194066

[Patent Document 9] EP 2275443
[Patent Document 10] EP 2202245
[Patent Document 11] JP 2163096

[Non-Patent Documents]

**[0013]**

[Non-Patent Document 1] Janice M Reichert, Clark J Rosensweig, Laura B Faden & Matthew C Dewitz, Monoclonal antibody successes in the clinic, Nature Biotechnology 23, 1073 - -1078 (2005).
[Non-Patent Document 2] Pavlou AK, Belsey MJ., The therapeutic antibodies market to 2008., Eur J Pharm Biopharm. 2005 Apr; 59(3):389-96.
[Non-Patent Document 3] Nishimoto N, Kishimoto T., Interleukin 6: from bench to bedside., Nat Clin Pract Rheumatol. 2006 Nov; 2(11):619-26.
[Non-Patent Document 4] Maini RN, Taylor PC, Szechinski J, Pavelka K, Broll J, Balint G, Emery P, Raemen F, Petersen J, Smolen J, Thomson D, Kishimoto T; CHARISMA Study Group., Double-blind randomized controlled clinical trial of the interleukin-6 receptor antagonist, Tocilizumab, in European patients with rheumatoid arthritis who had an incomplete response to methotrexate., Arthritis Rheum. 2006 Sep; 54(9):2817-29.
[Non-Patent Document 5] Nishimoto N, Kanakura Y, Aozasa K, Johkoh T, Nakamura M, Nakano S, Nakano N, Ikeda Y, Sasaki T, Nishioka K, Hara M, Taguchi H, Kimura Y, Kato Y, Asaoku H, Kumagai S, Kodama F, Nakahara H, Hagihara K, Yoshizaki K, Kishimoto T. Humanized anti-interleukin-6 receptor antibody treatment of multicentric Castleman disease. Blood. 2005 Oct 15; 106(8):2627-32.
[Non-Patent Document 6] Kim SJ, Park Y, Hong HJ., Antibody engineering for the development of therapeutic antibodies., Mol Cells. 2005 Aug 31; 20(1):17-29. Review.
[Non-Patent Document 7] Rothe A, Hosse RJ, Power BE. Ribosome display for improved biotherapeutic molecules. Expert Opin Biol Ther. 2006 Feb; 6(2):177-87.
[Non-Patent Document 8] Rajpal A, Beyaz N, Haber L, Cappuccilli G, Yee H, Bhatt RR, Takeuchi T, Lerner RA, Crea R., A general method for greatly improving the affinity of antibodies by using combinatorial libraries., Proc Natl Acad Sci USA. 2005 Jun 14; 102(24):8466-71. Epub 2005 Jun 6.
[Non-Patent Document 9] Wu H, Pfarr DS, Johnson S, Brewah YA, Woods RM, Patel NK, White WI, Young JF, Kiener PA. Development of Motavizumab, an Ultra-potent Antibody for the Prevention of Respiratory Syncytial Virus Infection in the Upper and Lower Respiratory Tract. J Mol Biol. 2007, 368, 652-665.
[Non-Patent Document 10] Shire SJ, Shahrokh Z, Liu J. Challenges in the development of high protein concentration formulations. J Pharm Sci. 2004 Jun; 93(6):1390-402.
[Non-patent Document 11] Salfeld JG. Isotype selection in antibody engineering.Nat Biotechnol. 2007 Dec; 25(12):1369-72.
[Non-Patent Document 12] Hinton PR, Xiong JM, Johlfs MG, Tang MT, Keller S, Tsurushita N., An engineered human IgG1 antibody with longer serum half-life., J Immunol. 2006 Jan 1; 176(1):346-56.
[Non-Patent Document 13] Ghetie V, Popov S, Borvak J, Radu C, Matesoi D, Medesan C, Ober RJ, Ward ES., Increasing the serum persistence of an IgG fragment by random mutagenesis., Nat Biotechnol. 1997 Jul; 15(7):637-40.
[Non-Patent Document 14] Hwang WY, Almagro JC, Buss TN, Tan P, Foote J. Use of human germline genes in a CDR homology-based approach to antibody humanization.Methods. 2005 May; 36(1):35-42.
[Non-Patent Document 15] Bartelds GM, Wijbrandts CA, Nurmohamed MT, Stapel S, Lems WF, Aarden L, Dijkmans BA, Tak P, Wolbink GJ. Clinical response to adalimumab: The relationship with anti-adalimumab antibodies and serum adalimumab concentrations in rheumatoid arthritis. Ann Rheum Dis. 2007 Mar 9; [Epub ahead of print]
[Non-Patent Document 16] Bender NK, Heilig CE, Droll B, Wohlgemuth J, Armbruster FP, Heilig B. Immunogenicity, efficacy and adverse events of adalimumab in RA patients. Rheumatol Int. 2007 Jan; 27(3):269-74.
[Non-Patent Document 17] Van Walle I, Gansemans Y, Parren PW, Stas P, Lasters I. Immunogenicity screening in protein drug development.Expert Opin Biol Ther. 2007 Mar; 7(3):405-18.
[Non-Patent Document 18] Jones TD, Phillips WJ, Smith BJ, Bamford CA, Nayee PD, Baglin TP, Gaston JS, Baker MP. Identification and removal of a promiscuous CD4+ T cell epitope from the C1 domain of factor VIII. J Thromb Haemost. 2005 May; 3(5):991-1000.
[Non-Patent Document 19] Chirino AJ, Ary ML, Marshall SA. Minimizing the immunogenicity of protein therapeutics. Drug Discov Today. 2004 Jan 15; 9(2):82-90.
[Non-Patent Document 20] Sato K, Tsuchiya M, Saldanha J, Koishihara Y, Ohsugi Y, Kishimoto T, Bendig MM. Reshaping a human antibody to inhibit the interleukin 6-dependent tumor cell growth. Cancer Res. 1993 Feb 15; 53(4):851-6.

[Non-Patent Document 21] Strand V, Kimberly R, Isaacs JD. Biologic therapies in rheumatology: lessons learned future directions. Nat Rev Drug Discov. 2007 Jan; 6(1):75-92.
[Non-Patent Document 22] Gessner JE, Heiken H, Tamm A, Schmidt RE. The IgG Fc receptor family. Ann Hematol. 1998 Jun; 76(6):231-48.
[Non-Patent Document 23] Dilion TM, Ricci MS, Vezina C, Flynn GC, Liu YD, Rehder DS, Plant M, Henkle B, Li Y, Deechongkit S, Varnum B, Wypych J, Balland A, Bondarenko PV. Structural and functional characterization of disulfide isoforms of the human IgG2 subclass. J Biol Chem. 2008 Jun 6; 283(23):16206-15.
[Non-Patent Document 24] Johnson KA, Paisley-Flango K, Tangarone BS, Porter TJ, Rouse JC. Cation exchange-HPLC and mass spectrometry reveal C-terminal amidation of an IgG1 heavy chain. Anal Biochem. 2007 Jan 1; 360(1):75-83.
[Non-Patent Document 25] Yoshiyuki O, Gekkan Pharm Stage 2007; 7(5):13-18.
[Non-Patent Document 26] Onda M, et al., Lowering the isoelectric point of the Fv portion of recombinant immunotoxins leads to decreased nonspecific animal toxicity without affecting antitumor activity., Cancer Res. 2001; 61:5070-77.
[Non-Patent Document 27] Ito W. et al., The His-probe method: effects of histidine residues introduced into the complementary-determining regions of antibodies on antigen-antibody interactions at different pH values., FEBS Letter 1992; 309(1):85-88.

## Disclosure of the Invention

[Problems to be Solved by the Invention]

**[0014]** The invention was achieved in view of the above circumstances. An objective disclosed herein is to provide pharmaceutical compositions that comprise second-generation molecules that are superior than the humanized anti-IL-6 receptor IgG1 antibody TOCILIZUMAB, by altering the amino acid sequences of the variable and constant regions of TOCILIZUMAB to enhance the antigen-neutralizing ability and improve pharmacokinetics, such that prolonged therapeutic effect is exerted with a less frequency of administration, and immunogenicity, safety, and physicochemical properties (stability and homogeneity) are improved (hereinbelow, these pharmaceutical compositions may also be referred to as the "agents" or the "formulations"). Another objective is to provide methods for producing such pharmaceutical compositions.

[Means for Solving the Problems]

**[0015]** Dedicated studies were conducted to generate second-generation molecules as defined in the claims that are superior than the first-generation humanized anti-IL-6 receptor IgG1 antibody TOCILIZUMAB, by altering the amino acid sequences of the variable and constant regions of TOCILIZUMAB to enhance the efficacy and improve the pharmacokinetics, so that prolonged therapeutic effect is exerted with a lower frequency of administration, and immunogenicity, safety, and physicochemical properties (stability and homogeneity) are improved. As a result, multiple CDR mutations in the variable regions of TOCILIZUMAB that improve the binding ability (affinity) to the antigen were discovered. Thus the affinity was successfully significantly improved using a combination of such mutations. Also improving pharmacokinetics by introducing modifications that lower the isoelectric point of the variable region sequence succeeded.

**[0016]** Also improving pharmacokinetics by making the binding to the IL-6 receptor antigen to be pH-dependent, so that a single antibody molecule can neutralize the antigen multiple times succeeded. Furthermore, the risk of immunogenicity has been successfully reduced by fully humanizing the mouse-derived sequences that remain in the framework of TOCILIZUMAB and reducing the number of T-cell epitope peptides in the variable regions predicted *in silico.* Furthermore, also novel constant region sequences for the constant region of TOCILIZUMAB, that reduce the binding to the Fey receptor as compared to IgG1 to improve safety, improve the pharmacokinetics as compared to IgG1, and reduce the heterogeneity due to the disulfide bonds in the hinge region of IgG2 and the heterogeneity due to the H chain C-terminus without decreasing stability. have been successfully discovered. Second-generation molecules that are superior than TOCILIZUMAB have been successfully produced by appropriately combining these amino acid sequence alterations in the CDR, variable regions, and constant regions.

**[0017]** The present invention relates to pharmaceutical compositions comprising a humanized anti-IL-6 receptor IgG antibody having superior antigen (IL-6 receptor)-binding ability, superior pharmacokinetics, superior safety and physical 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 invention provides:

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

(a) an antibody that comprises a heavy chain variable region comprising the sequence of SEQ ID NO: 20 (variable region of VH3-M73) and a light chain variable region comprising the sequence of SEQ ID NO: 23 (variable region of VL3);
(b) an antibody that comprises a heavy chain variable region comprising the sequence of SEQ ID NO: 19 (variable region of VH4-M73) and a light chain variable region comprising the sequence of SEQ ID NO: 22 (variable region of VL1); and
(c) an antibody that comprises a heavy chain variable region comprising the sequence of SEQ ID NO: 21 (variable region of VH5-M83) and a light chain variable region comprising the sequence of SEQ ID NO: 24 (variable region of VL5);

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

(a) an antibody that comprises a heavy chain comprising the sequence of SEQ ID NO: 26 (VH3-M73) and a light chain comprising the sequence of SEQ ID NO: 29 (VL3);
(b) an antibody that comprises a heavy chain comprising the sequence of SEQ ID NO: 25 (VH4-M73) and a light chain comprising the sequence of SEQ ID NO: 28 (VL1); and
(c) an antibody that comprises a heavy chain comprising the sequence of SEQ ID NO: 27 (VH5-M83) and a light chain comprising the sequence of SEQ ID NO: 30 (VL5);

[3] a gene encoding the antibody of [1] or [2];
[4] a vector carrying the gene of [3];
[5] a host cell carrying the vector of [4];
[6] a method for producing the antibody of [1] or [2] by culturing the host cell of [5]; and [6] a pharmaceutical composition comprising the antibody of [1] or [2] or an antibody produced by the method of [6].

[Effects of the Invention]

**[0018]** The humanized anti-IL-6 receptor IgG antibodies obtained according to the disclosure have enhanced efficacy and improved pharmacokinetics; thus, they can exert a prolonged therapeutic effect with a less administration frequency.

Brief Description of the Drawings

**[0019]**

Fig. 1 is a listing of mutation sites that improve the affinity of TOCILIZUMAB for the IL-6 receptor. The HCDR2 sequence of TOCILIZUMAB is shown in SEQ ID NO: 81; the HCDR2 sequence after mutation (upper line) is shown in SEQ ID NO: 82; the HCDR2 sequence after mutation (lower line) is shown in SEQ ID NO: 83; the HCDR3 sequence of TOCILIZUMAB is shown in SEQ ID NO: 84; the HCDR3 sequence after mutation (upper line) is shown in SEQ ID NO: 85; the HCDR3 sequence after mutation (lower line) is shown in SEQ ID NO: 86; the LCDR1 sequence of TOCILIZUMAB is shown in SEQ ID NO: 87; the LCDR1 sequence after mutation (upper line) is shown in SEQ ID NO: 88; the LCDR1 sequence after mutation (lower line) is shown in SEQ ID NO: 89; the LCDR3 sequence of TOCILIZUMAB is shown in SEQ ID NO: 90; the LCDR3 sequence after mutation (upper line) is shown in SEQ ID NO: 91; and the LCDR3 sequence after mutation (lower line) is shown in SEQ ID NO: 92.
Fig. 2 is a graph showing the neutralizing activities of TOCILIZUMAB and RDC-23 in BaF/gp130.
Fig. 3 is a listing of mutation sites that can reduce the isoelectric point of variable region without significantly reducing the binding of TOCILIZUMAB to the IL-6 receptor. Asterisk in the drawing represents a site that has no influence on the isoelectric point but which was mutated for conversion into a human sequence. The HFR1 sequence of TOCILIZUMAB is shown in SEQ ID NO: 93; the HFR1 sequence after mutation is shown in SEQ ID NO: 94; the HCDR1 sequence of TOCILIZUMAB is shown in SEQ ID NO: 95; the HCDR1 sequence after mutation is shown in SEQ ID NO: 96; the HFR2 sequence of TOCILIZUMAB is shown in SEQ ID NO: 97; the HFR2 sequence after mutation is shown in SEQ ID NO: 98; the HCDR2 sequence of TOCILIZUMAB is shown in SEQ ID NO: 81; the HCDR2 sequence after mutation is shown in SEQ ID NO: 99; the HFR4 sequence of TOCILIZUMAB is shown in SEQ ID NO: 100; the HFR4 sequence after mutation is shown in SEQ ID NO: 101; the LFR1 sequence of TOCILIZUMAB is shown in SEQ ID NO: 102; the LFR1 sequence after mutation is shown in SEQ ID NO: 103; the LCDR1 sequence of TOCILIZUMAB is shown in SEQ ID NO: 87; the LCDR1 sequence after mutation is shown in SEQ ID NO: 104; the LFR2 sequence of TOCILIZUMAB is shown in SEQ ID NO: 105; the LFR2 sequence after mutation is shown in SEQ ID NO: 106; the LCDR2 sequence of TOCILIZUMAB is shown in SEQ ID NO: 107; the LCDR2 sequences after mutation are shown in SEQ ID NOs: 108 and 109; the LFR3 sequence of TOCILIZUMAB is shown

in SEQ ID NO: 110; the LFR3 sequence after mutation is shown in SEQ ID NO: 111; the LFR4 sequence of TOCILIZUMAB is shown in SEQ ID NO: 112; and the LFR4 sequence after mutation is shown in SEQ ID NO: 113.
Fig. 4 is a graph showing the neutralizing activities OF TOCILIZUMAB and H53/L28 in BaF/gp130.
Fig. 5 is a graph showing the time courses of plasma concentration for TOCILIZUMAB and H53/L28 in mice after intravenous administration.
Fig. 6 is a graph showing the time courses of plasma concentration for TOCILIZUMAB and H53/L28 in mice after subcutaneous administration.
Fig. 7 is a schematic illustration showing that an IgG molecule can bind again to another antigen by dissociating from a membrane-type antigen in the endosome.
Fig. 8 is a listing of mutation sites that can confer pH dependency to the binding of TOCILIZUMAB to the IL-6 receptor (binding at pH 7.4 and dissociation at pH 5.8). The HFR1 sequence of TOCILIZUMAB is shown in SEQ ID NO: 93; the HFR1 sequence after mutation is shown in SEQ ID NO: 114; the HCDR1 sequence of TOCILIZUMAB is shown in SEQ ID NO: 95; the HCDR1 sequence after mutation is shown in SEQ ID NO: 115; the LCDR1 sequence of TOCILIZUMAB is shown in SEQ ID NO: 87; the LCDR1 sequence after mutation is shown in SEQ ID NO: 116; the LCDR2 sequence of TOCILIZUMAB is shown in SEQ ID NO: 107; and the LCDR2 sequence after mutation is shown in SEQ ID NO: 117.
Fig. 9 is a graph showing the neutralizing activities of TOCILIZUMAB and H3pI/L73 in BaF/gp130.
Fig. 10 is a graph showing the time courses of plasma concentration for TOCILIZUMAB and H3pI/L73 in cynomolgus monkeys after intravenous administration.
Fig. 11 is a graph showing the time courses of plasma concentration for TOCILIZUMAB and H3pI/L73 in human IL-6 receptor transgenic mice after intravenous administration.
Fig. 12 is a diagram showing the result of assessment of the C-terminus-derived heterogeneity of TOCILIZUMAB, TOCILIZUMABΔK, and TOCILIZUMABΔGK by cation exchange chromatography.
Fig. 13 is a diagram showing the result of assessment of the disulfide bond-derived heterogeneity of TOCILIZUMAB-IgG1, TOCILIZUMAB-IgG2, and TOCILIZUMAB-SKSC by cation exchange chromatography.
Fig. 14 is a diagram showing the denaturation curves for TOCILIZUMAB-IgG1, TOCILIZUMAB-IgG2, and TOCILIZUMAB-SKSC obtained by differential scanning calorimetry (DSC), and the Tm value for each Fab domain.
Fig. 15 is a graph showing the time courses of plasma concentration for TOCILIZUMAB-IgG1, TOCILIZUMAB-M44, TOCILIZUMAB-M58, and TOCILIZUMAB-M73 in human FcRn transgenic mice after intravenous administration.
Fig. 16 is a graph showing the neutralizing activities of TOCILIZUMAB, control, and Fv5-M83 in BaF/gp130.
Fig. 17 is a graph showing the neutralizing activities of TOCILIZUMAB, Fv3-M73, and Fv4-M73 in BaF/gp130.
Fig. 18 is a graph showing the time courses of plasma concentrations for TOCILIZUMAB, control, Fv3-M73, Fv4-M73, and Fv5-M83 in cynomolgus monkeys after intravenous administration.
Fig. 19 is a graph showing the time courses of CRP concentration for TOCILIZUMAB, control, Fv3-M73, Fv4-M73, or Fv5-M83 in cynomolgus monkeys after intravenous administration.
Fig. 20 is a graph showing the time courses of percentage of free soluble IL-6 receptor in cynomolgus monkeys after intravenous administration of TOCILIZUMAB, control, Fv3-M73, Fv4-M73, or Fv5-M83.
Fig. 21 is a graph showing the inhibitory effects by TOCILIZUMAB and Fv4-M73 on MCP-1 production from human RA patient-derived synovial cells.
Fig. 22 is a graph showing the inhibitory effects by TOCILIZUMAB and Fv4-M73 on VEGF production from human RA patient-derived synovial cells.

## Mode for Carrying Out the Invention

**[0020]** The invention provides antibodies as claimed. The disclosure also relates to the polypeptides of (a) to (f) below:

(a) a polypeptide that comprises CDR1 comprising the sequence of SEQ ID NO: 1 (CDR1 of VH4-M73), CDR2 comprising the sequence of SEQ ID NO: 2 (CDR2 of VH4-M73), and CDR3 comprising the sequence of SEQ ID NO: 3 (CDR3 of VH4-M73);
(b) a polypeptide that comprises CDR1 comprising the sequence of SEQ ID NO: 4 (CDR1 of VH3-M73), CDR2 comprising the sequence of SEQ ID NO: 5 (CDR2 of VH3-M73), and CDR3 comprising the sequence of SEQ ID NO: 6 (CDR3 of VH3-M73);
(c) a polypeptide that comprises CDR1 comprising the sequence of SEQ ID NO: 7 (CDR1 of VH5-M83), CDR2 comprising the sequence of SEQ ID NO: 8 (CDR2 of VH5-M83), and CDR3 comprising the sequence of SEQ ID NO: 9 (CDR3 of VH5-M83);
(d) a polypeptide that comprises CDR1 comprising the sequence of SEQ ID NO: 10 (CDR1 of VL1), CDR2 comprising the sequence of SEQ ID NO: 11 (CDR2 of VL1), and CDR3 comprising the sequence of SEQ ID NO: 12 (CDR3 of VL1);
(e) a polypeptide that comprises CDR1 comprising the sequence of SEQ ID NO: 13 (CDR1 of VL3), CDR2 comprising

the sequence of SEQ ID NO: 14 (CDR2 of VL3), and CDR3 comprising the sequence of SEQ ID NO: 15 (CDR3 of VL3); and

(f) a polypeptide that comprises CDR1 comprising the sequence of SEQ ID NO: 16 (CDR1 of VL5), CDR2 comprising the sequence of SEQ ID NO: 17 (CDR2 of VL5), and CDR3 comprising the sequence of SEQ ID NO: 18 (CDR3 of VL5).

**[0021]** The polypeptides described herein are not particularly limited; however, they are preferably antigen-binding substances having the activity of binding to human IL-6 receptor. Such antigen-binding substances preferably include, for example, antibody heavy chain variable regions (VH), antibody light chain variable regions (VL), antibody heavy chains, antibody light chains, and antibodies.

**[0022]** Of the polypeptides of (a) to (f) above, the polypeptides of (a) to (c) are preferable examples of antibody heavy chain variable regions, while the polypeptides of (d) to (f) are preferable examples of antibody light chain variable regions.

**[0023]** These variable regions can be used as a portion of an anti-human IL-6 receptor antibody. Anti-human IL-6 receptor antibodies in which such a variable region is used have superior binding activity, excellent pharmacokinetics, excellent safety, reduced immunogenicity, and/or superior physicochemical properties. In the disclosure , excellent pharmacokinetics or improvement of pharmacokinetics refers to any one of: decrease in "clearance (CL)", increase in the "area under the curve (AUC)", increase in "mean residence time", and increase in "plasma half-life (t1/2)", which are pharmacokinetic parameters calculated from the time course of plasma concentration when an antibody is to be administered into the body. Herein, superior physicochemical property or improved physicochemical property refers to, but is not limited to, improved stability, decreased heterogeneity, or the like.

**[0024]** Human antibody framework regions (FRs) to be linked with CDR are selected so that the CDR forms a favorable antigen-binding site. FRs to be used for the variable regions as described herein are not particularly limited and any FR may be used; however; human-derived FRs are preferably used. It is possible to use human-derived FRs having a natural sequence. Alternatively, if needed, substitution, deletion, addition and/or insertion or such of one or more amino acids may be introduced into the framework region having a natural sequence so that the CDR forms an adequate antigen-binding site. Mutant FR sequences having a desired property can be selected, for example, by measuring and evaluating the binding activity to an antigen for an antibody with an FR with amino acid substitutions (Sato, K. et al., Cancer Res. (1993) 53, 851-856).

**[0025]** Moreover, one or more amino acids may be substituted, deleted, added, and/or inserted in the CDR sequence described above. It is preferred that a CDR sequence after substitution, deletion, addition, and/or insertion of one or more amino acids has equivalent activity to the CDR sequence before alteration with regard to binding activity, neutralizing activity, stability, immunogenicity, and/or pharmacokinetics. The number of amino acids to be substituted, deleted, added, and/or inserted is not particularly limited; however, it is preferably three amino acids or less, more preferably two amino acids or less, and still more preferably one amino acid per CDR.

**[0026]** Methods for substituting one or more amino acid residues with other amino acids of interest include, for example, site-directed mutagenesis (Hashimoto-Gotoh, T, Mizuno, T, Ogasahara, Y, and Nakagawa, M. (1995) An oligodeoxyribonucleotide-directed dual amber method for site-directed mutagenesis. Gene 152, 271-275; Zoller, MJ, and Smith, M. (1983) Oligonucleotide-directed mutagenesis of DNA fragments cloned into M13 vectors. Methods Enzymol. 100, 468-500; Kramer, W, Drutsa, V, Jansen, HW, Kramer, B, Pflugfelder, M, and Fritz, HJ (1984) The gapped duplex DNA approach to oligonucleotide-directed mutation construction. Nucleic Acids Res. 12,9441-9456; Kramer W, and Fritz HJ (1987) Oligonucleotide-directed construction of mutations via gapped duplex DNA Methods. Enzymol. 154, 350-367; Kunkel, TA (1985) Rapid and efficient site-specific mutagenesis without phenotypic selection. Proc Natl Acad Sci U. S. A. 82,488-492). This method can be used to substitute desired amino acids in an antibody with other amino acids of interest. Furthermore, amino acids in the frameworks and CDRs can be substituted to other appropriate amino acids using library techniques such as framework shuffling (Mol. Immunol. 2007 Apr; 44(11): 3049-60) and CDR repair (US 2006/0122377).

**[0027]** The disclosure also relates to the antibodies of (a) to (c) below:

(a) an antibody which comprises a heavy chain variable region that comprises CDR1 comprising the sequence of SEQ ID NO: 1 (CDR1 of VH4-M73), CDR2 comprising the sequence of SEQ ID NO: 2 (CDR2 of VH4-M73), and CDR3 comprising the sequence of SEQ ID NO: 3 (CDR3 of VH4-M73), and a light chain variable region that comprises CDR1 comprising the sequence of SEQ ID NO: 10 (CDR1 of VL1), CDR2 comprising the sequence of SEQ ID NO: 11 (CDR2 of VL1), and CDR3 comprising the sequence of SEQ ID NO: 12 (CDR3 of VL1);

(b) an antibody which comprises a heavy chain variable region that comprises CDR1 comprising the sequence of SEQ ID NO: 4 (CDR1 of VH3-M73), CDR2 comprising the sequence of SEQ ID NO: 5 (CDR2 of VH3-M73), and CDR3 comprising the sequence of SEQ ID NO: 6 (CDR3 of VH3-M73), and a light chain variable region that comprises CDR1 comprising the sequence of SEQ ID NO: 13 (CDR1 of VL3), CDR2 comprising the sequence of SEQ ID NO: 14 (CDR2 of VL3), and CDR3 comprising the sequence of SEQ ID NO: 15 (CDR3 of VL3); and

(c) an antibody which comprises a heavy chain variable region that comprises CDR1 comprising the sequence of

SEQ ID NO: 7 (CDR1 of VH5-M83), CDR2 comprising the sequence of SEQ ID NO: 8 (CDR2 of VH5-M83), and CDR3 comprising the sequence of (SEQ ID NO: 9 (CDR3 of VH5-M83), and a light chain variable region that comprises CDR1 comprising the sequence of SEQ ID NO: 16 (CDR1 of VL5), CDR2 comprising the sequence of SEQ ID NO: 17 (CDR2 of VL5), and CDR3 comprising the sequence of SEQ ID NO: 18 (CDR3 of VL5).

**[0028]** The antibodies described above can be used as anti-human IL-6 receptor antibodies having superior binding activity, excellent pharmacokinetics, excellent safety, reduced immunogenicity, and/or superior physicochemical properties.

**[0029]** Human antibody framework regions to be linked with CDR as described herein are selected so that the CDR forms a favorable antigen-binding site. FRs to be used for the variable regions of the disclosure are not particularly limited, and any FR may be used; however, human-derived FR is preferably used. It is possible to use human-derived FRs having a natural sequence. Alternatively, if needed, substitution, deletion, addition and/or insertion or such of one or more amino acids may be introduced into the framework region having a natural sequence so that the CDR forms an adequate antigen-binding site. Mutant FR sequences having a desired property can be selected, for example, by measuring and evaluating the binding activity to an antigen for an antibody having an FR with amino acid substitutions (Sato, K. et al., Cancer Res. (1993) 53, 851-856).

**[0030]** Meanwhile, the constant region to be used for an antibody described herein is not particularly limited, and any constant region may be used. Preferred constant regions to be used for the antibodies described herein include, for example, human-derived constant regions (constant regions derived from IgG1, IgG2, IgG3, IgG4, Cκ, Cλ, and such). One or more amino acids may be substituted, deleted, added, and/or inserted in the human-derived constant regions. The preferred human-derived heavy chain constant regions include, for example, constant regions comprising the amino acid sequence of SEQ ID NO: 31 (constant region of VH4-M73), constant regions comprising the amino acid sequence of SEQ ID NO: 32 (constant region VH3-M73)), and constant regions comprising the amino acid sequence of SEQ ID NO: 33 (constant region of VH5-M83), while the preferred human-derived light chain constant regions include, for example, constant regions comprising the amino acid sequence of SEQ ID NO: 34 (VL1), constant regions comprising the amino acid sequence of SEQ ID NO: 35 (VL3), and constant regions comprising the amino acid sequence of SEQ ID NO: 36 (VL5).

**[0031]** Moreover, one or more amino acids may be substituted, deleted, added, and/or inserted in the CDR sequence described above. It is preferred that a CDR sequence after substitution, deletion, addition, and/or insertion of one or more amino acids has equivalent activity to the CDR sequence before alteration with regard to binding activity, neutralizing activity, stability, immunogenicity, and/or pharmacokinetics. The number of amino acids to be substituted, deleted, added, and/or inserted is not particularly limited; however, it is preferably three amino acids or less, more preferably two amino acids or less, and still more preferably one amino acid per CDR.

**[0032]** Amino acids can also be substituted, deleted, added, and/or inserted by the methods described above.

**[0033]** The disclosure also provides the variable regions of (a) to (f) below:

(a) a heavy chain variable region comprising the sequence of SEQ ID NO: 19 (variable region of VH4-M73);
(b) a heavy chain variable region comprising the sequence of SEQ ID NO: 20 (variable region of VH3-M73);
(c) a heavy chain variable region comprising the sequence of SEQ ID NO: 21 (variable region of VH5-M83);
(d) a light chain variable region comprising the sequence of SEQ ID NO: 22 (variable region of VL1);
(e) a light chain variable region comprising the sequence of SEQ ID NO: 23 (variable region of VL3); and
(f) a light chain variable region comprising the sequence of SEQ ID NO: 24 (variable region of VL5).

**[0034]** The variable regions described above can be used as part of an anti-human IL-6 receptor antibody. Anti-human IL-6 receptor antibodies in which such variable regions are used have superior binding activity, excellent pharmacokinetics, excellent safety, reduced immunogenicity, and/or superior physicochemical properties.

**[0035]** The variable regions described above may also comprise substitutions, deletions, additions, and/or insertions of one or more amino acids (for example, five amino acids or less, preferably three amino acids or less). Methods for substituting one or more amino acid residues with other amino acids of interest include, for example, the methods described above.

**[0036]** The disclosure also relates to polypeptides comprising the variable regions described above.

**[0037]** Furthermore, the disclosure relates to the antibodies of (a) to (c) below:

(a) an antibody that comprises a heavy chain variable region comprising the sequence of SEQ ID NO: 19 (variable region of VH4-M73) and a light chain variable region comprising the sequence of SEQ ID NO: 22 (variable region of VL1);
(b) an antibody that comprises a heavy chain variable region comprising the sequence of SEQ ID NO: 20 (variable region of VH3-M73) and a light chain variable region comprising the sequence of SEQ ID NO: 23 (variable region

of VL3); and
(c) an antibody that comprises a heavy chain variable region comprising the sequence of SEQ ID NO: 21 (variable region of VH5-M83) and a light chain variable region comprising the sequence of SEQ ID NO: 24 (variable region of VL5).

**[0038]** The variable regions described above can be used as part of an anti-human IL-6 receptor antibody. Anti-human IL-6 receptor antibodies in which these variable regions are used have superior binding activity, excellent pharmacokinetics, excellent safety, reduced immunogenicity, and/or superior physical properties.

**[0039]** The variable regions described above may also comprise substitutions, deletions, additions, and/or insertions of one or more amino acids (for example, five amino acids or less, preferably three amino acids or less). Methods for substituting one or more amino acid residues with other amino acids of interest include, for example, the methods described above.

**[0040]** Meanwhile, the constant region to be used for an antibody disclosed herein is not particularly limited, and any constant region may be used. The preferred constant regions to be used for the antibodies of the present invention include, for example, human-derived constant regions (constant regions derived from IgG1, IgG2, IgG3, IgG4, κ chain, λ chain, and such). One or more amino acids may be substituted, deleted, added, and/or inserted in the human-derived constant regions. The preferred human-derived heavy chain constant regions include, for example, constant regions comprising the amino acid sequence of SEQ ID NO: 31 (constant region of VH4-M73), constant regions comprising the amino acid sequence of SEQ ID NO: 32 (constant region VH3-M73), and constant regions comprising the amino acid sequence of SEQ ID NO: 33 (constant region of VH5-M83), while the preferred human-derived light chain constant regions include, for example, constant regions comprising the amino acid sequence of SEQ ID NO: 34 (VL1), constant regions comprising the amino acid sequence of SEQ ID NO: 35 (VL3), and constant regions comprising the amino acid sequence of SEQ ID NO: 36 (VL5).

**[0041]** The disclosure also relates to the heavy or light chains of (a) to (f) below:

(a) a heavy chain comprising the sequence of SEQ ID NO: 25 (VH4-M73);
(b) a heavy chain comprising the sequence of SEQ ID NO: 26 (VH3-M73);
(c) a heavy chain comprising the sequence of SEQ ID NO: 27 (VH5-M83);
(d) a light chain comprising the sequence of SEQ ID NO: 28 (VL1);
(e) a light chain comprising the sequence of SEQ ID NO: 29 (VL3); and
(f) a light chain comprising the sequence of SEQ ID NO: 30 (VL5).

**[0042]** The heavy chains and light chains described above can be used as part of an anti-human IL-6 receptor antibody. Anti-human IL-6 receptor antibodies in which these heavy chains and light chains are used have superior binding activity, excellent pharmacokinetics, excellent safety, reduced immunogenicity, and/or superior physicochemical properties.

**[0043]** The heavy chains and light chains described above may also comprise substitutions, deletions, additions, and/or insertions of one or more amino acids (for example, ten amino acids or less, preferably five amino acids or less, and more preferably three amino acids or less). Methods for substituting one or more amino acid residues with other amino acids of interest include, for example, the methods described above.

**[0044]** Substitutions, deletions, additions, and/or insertions of one or more amino acids may be carried out for the variable regions, constant regions, or both.

**[0045]** The disclosure also relates to the antibodies of (a) to (c) below:

(a) an antibody that comprises a heavy chain comprising the sequence of SEQ ID NO: 25 (VH4-M73) and a light chain comprising the sequence of SEQ ID NO: 28 (VL1);
(b) an antibody that comprises a heavy chain comprising the sequence of SEQ ID NO: 26 (VH3-M73) and a light chain comprising the sequence of SEQ ID NO: 29 (VL3); and
(c) an antibody that comprises a heavy chain comprising the sequence of SEQ ID NO: 27 (VH5-M83) and a light chain comprising the sequence of SEQ ID NO: 30 (VL5).

**[0046]** The antibodies described above are anti-human IL-6 receptor antibodies that have superior binding activity, excellent pharmacokinetics, excellent safety, reduced immunogenicity, and/or superior physicochemical properties.

**[0047]** The antibodies described above may also comprise substitutions, deletions, additions, and/or insertions of one or more amino acids (for example, 20 amino acids or less, preferably ten amino acids or less, and more preferably five amino acids or less). Methods for substituting one or more amino acid residues with other amino acids of interest include, for example, the methods described above.

**[0048]** Substitutions, deletions, additions, and/or insertions of one or more amino acids may be carried out for the variable regions, constant regions, or both.

**[0049]** The antibodies described herein are preferably humanized antibodies.

**[0050]** Humanized antibodies are also referred to as reshaped human antibodies. Such a humanized antibody is obtained by grafting a complementary determining region (CDR) derived from a non-human mammal into the CDR of a human antibody. Conventional genetic recombination techniques for the preparation of such antibodies are also known (see European Patent Application No. EP 125023; and WO 96/02576).

**[0051]** Specifically, for example, a DNA sequence designed such that a CDR of interest and a framework region (FR) of interest are linked is synthesized by PCR, using several oligonucleotides prepared to have overlapping portions with the ends of both CDR and FR as primers (see the method described in WO 98/13388). A humanized antibody is obtained by: ligating the resulting DNA to a DNA that encodes a human antibody constant region or a modified human antibody constant region; inserting this into an expression vector; and introducing this into a host to produce the antibody (see European Patent Application No. EP 239400 and International Patent Application Publication No. WO 96/02576).

**[0052]** Human antibody framework regions to be linked with CDR are selected so that the CDR forms a favorable antigen-binding site. If needed, amino acid substitution, deletion, addition and/or insertion may be introduced into the framework region of an antibody variable region.

**[0053]** A human antibody constant region, or an altered human antibody constant region in which one or more amino acids have been substituted, deleted, added, and/or inserted in a human antibody constant region, can be used as the constant region of a humanized antibody.

**[0054]** For example, Cγ1, Cγ2, Cγ3, Cγ4, Cμ, Cδ, Cα1, Cα2, and Cε can be used for the H chain, and Cκ and Cλ can be used for the L chain. The amino acid sequence of Cκ is shown in SEQ ID NO: 38, and the nucleotide sequence encoding this amino acid sequence is shown in SEQ ID NO: 37. The amino acid sequence of Cγ1 is shown in SEQ ID NO: 40, and the nucleotide sequence encoding this amino acid sequence is shown in SEQ ID NO: 39. The amino acid sequence of Cγ2 is shown in SEQ ID NO: 42, and the nucleotide sequence encoding this amino acid sequence is shown in SEQ ID NO: 41. The amino acid sequence of Cγ4 is shown in SEQ ID NO: 44, and the nucleotide sequence encoding this amino acid sequence is shown in SEQ ID NO: 43.

**[0055]** Furthermore, human antibody C regions may be modified to improve antibody stability or antibody production stability. Human antibodies of any isotype such as IgG, IgM, IgA, IgE, or IgD may be used in antibody humanization; however, IgG is preferably used herein.

**[0056]** IgG1, IgG2, IgG3, IgG4, or the like can be used as the IgG.

**[0057]** Amino acids in the variable region (for example, CDR and FR) and constant region of a humanized antibody may be deleted, added, inserted, and/or substituted with amino acids after preparation. The antibodies described herein also include such humanized antibodies comprising amino acid substitutions and the like.

**[0058]** The antibodies described herein include not only divalent antibodies as represented by IgG, but also monovalent antibodies and multivalent antibodies as represented by IgM, as long as they have IL-6 receptor-binding activity and/or neutralizing activity. The multivalent antibodies described herein include multivalent antibodies in which the antigen-binding sites are all identical, and multivalent antibodies in which all or some of the antigen-binding sites are different The antibodies described herein include not only whole antibody molecules, but also minibodies and modified products thereof, as long as they bind to the IL-6 receptor protein.

**[0059]** Minibodies are antibodies comprising an antibody fragment lacking a portion of a whole antibody (for example, whole IgG or such), and are not particularly limited as long as they have IL-6 receptor-binding activity and/or neutralizing activity and comprise an antibody fragment that lacks a portion of a whole antibody (for example, whole IgG or such). The minibodies described herein are not particularly limited, as long as they comprise a portion of a whole antibody. However, the minibodies preferably comprise VH or VL, and particularly preferably comprise both VH and VL. Other preferable minibodies described herein include, for example, minibodies comprising antibody CDRs. The minibodies may comprise all or some of the six CDRs of an antibody.

**[0060]** The minibodies described herein preferably have a smaller molecular weight than whole antibodies. However, the minibodies may form multimers, for example, dimers, trimers, or tetramers, and thus their molecular weight is sometimes greater than that of whole antibodies.

**[0061]** Specifically, antibody fragments include, for example, Fab, Fab', F(ab')2, and Fv. Meanwhile, minibodies include, for example, Fab, Fab', F(ab')2, Fv, scFv (single chain Fv), diabodies, and sc(Fv)2 (single chain (Fv)2). Multimers (for example, dimers, trimers, tetramers, and polymers) of these antibodies are also included in the minibodies described herein.

**[0062]** Antibody fragments can be obtained, for example, by treating antibodies with enzymes to produce antibody fragments. Enzymes known to generate antibody fragments include, for example, papain, pepsin, and plasmin. Alternatively, a gene encoding such antibody fragment can be constructed, introduced into an expression vector, and expressed in appropriate host cells (see, for example, Co, M.S. et al., J. Immunol. (1994) 152, 2968-2976; Better, M. & Horwitz, A. H. Methods in Enzymology (1989) 178,476-496; Pluckthun, A. & Skerra, A. Methods in Enzymology (1989) 178, 476-496; Lamoyi, E., Methods in Enzymology (1989) 121,652-663; Rousseaux, J. et al., Methods in Enzymology (1989) 121, 663-669; Bird, R. E. et al., TIBTECH (1991) 9,132-137).

**[0063]** Digestive enzymes cleave at specific sites of an antibody fragment, yielding antibody fragments of specific structures shown below. Genetic engineering techniques can be applied to such enzymatically-obtained antibody fragments to delete an arbitrary portion of the antibody.

**[0064]** Antibody fragments obtained by using the above digestive enzymes are as follows.

Papain digestion: F(ab)2 or Fab
Pepsin digestion: F(ab')2 or Fab'
Plasmin digestion: Facb

**[0065]** The minibodies described herein include antibody fragments lacking an arbitrary region, as long as they have IL-6 receptor-binding activity and/ or neutralizing activity.

**[0066]** "Diabody" refers to a bivalent antibody fragment constructed by gene fusion (Holliger P et al., 1993, Proc. Natl. Acad. Sci. USA 90: 6444-6448; EP 404,097; WO 93/11161, etc). Diabodies are dimers composed of two polypeptide chains. In each of the polypeptide chains forming a dimer, a VL and a VH are generally linked by a linker in the same chain. In general, a linker in a diabody is short enough such that the VL and VH cannot bind to each other. Specifically, the number of amino acid residues constituting the linker is, for example, about five residues. Thus, the VL and VH encoded on the same polypeptide cannot form a single-chain variable region fragment, and will form a dimer with another single-chain variable region fragment. As a result, the diabody has two antigen binding sites.

**[0067]** ScFv antibodies are single-chain polypeptides produced by linking VH and VL via a linker or such (Huston, J. S. et al., Proc. Natl. Acad. Sci. U.S.A. (1988) 85, 5879-5883; Pluckthun "The Pharmacology of Monoclonal Antibodies" Vol. 113, eds., Resenburg and Moore, Springer Verlag, New York, pp. 269-315, (1994)). The H-chain V region and L-chain V region of scFv may be derived from any antibody described herein. The peptide linker for linking the V regions is not particularly limited. For example, an arbitrary single-chain peptide containing about three to 25 residues can be used as the linker. Specifically, it is possible to use the peptide linkers described below or such.

**[0068]** The V regions of the two chains can be linked, for example, by PCR as described above. First, a DNA encoding the complete amino acid sequence or a desired partial amino acid sequence of one of the DNAs shown below is used as a template to link the V regions by PCR:

a DNA sequence encoding an H chain or H-chain V region of an antibody, and
a DNA sequence encoding an L chain or L-chain V region of an antibody.

**[0069]** DNAs encoding the V region of an H chain or L chain are amplified by PCR using a pair of primers containing corresponding sequences of the two ends of the DNA to be amplified. Then, a DNA encoding the peptide linker portion is prepared. The peptide linker-encoding DNA can also be synthesized by PCR. A nucleotide sequence that can be used to link the separately synthesized amplification products of V region is added to the 5' end of the primers to be used. Then, PCR is carried out using each of the DNAs in [H chain V region DNA]-[peptide linker DNA]-[L chain V region DNA] and assembly PCR primers.

**[0070]** The assembly PCR primers contain a combination of a primer that anneals with the 5' end of the [H chain V region DNA] and a primer that anneals with the 3' end of the [L chain V region DNA]. In other words, the assembly PCR primers are a set of primers that can be used to amplify DNAs encoding the full-length sequence of the scFv to be synthesized. Meanwhile, nucleic sequences that can be used to link each of the V-region DNAs are added to the [peptide linker DNA]. Then, these DNAs are linked, and then the whole scFv is ultimately generated as an amplification product using the assembly PCR primers. Once the scFv-encoding DNAs are generated, expression vectors containing these DNAs and recombinant cells transformed with these expression vectors can be obtained by conventional methods. Further, the scFv can be obtained through expression of the scFv-encoding DNAs by culturing the resulting recombinant cells.

**[0071]** The order of VH and VL to be linked is not particularly limited, and they may be arranged in any order. Examples of the arrangement are listed below.

[VH] linker [VL]

[VL] linker [VH]

**[0072]** sc(Fv)2 is a single-chain minibody produced by linking two VHs and two VLs using linkers and such (Hudson et al., 1999, J Immunol. Methods 231:177-189). sc(Fv)2 can be produced, for example, by linking scFv using a linker.

**[0073]** Preferably, the two VHs and two VLs of an antibody are arranged in the order of VH, VL, VH, and VL ([VH] linker [VL] linker [VH] linker [VL]) from the N terminus of the single-chain polypeptide; however, the order of the two VHs and two VLs is not limited to the above arrangement, and they may be arranged in any order. Examples of the arrangement

are listed below:

[VL] linker [VH] linker [VH] linker [VL]
[VH] linker [VL] linker [VL] linker [VH]
[VH] linker [VH] linker [VL] linker [VL]
[VL] linker [VL] linker [VH] linker [VH]
[VL] linker [VH] linker [VL] linker [VH]

**[0074]** The amino acid sequence of the minibody VH or VL may contain substitutions, deletions, additions, and/or insertions. Furthermore, as long as VH and VL have antigen-binding activity when assembled, a portion may be deleted or other polypeptides may be added. Moreover, the variable regions may be chimerized or humanized.

**[0075]** In the disclosure, linkers that can be used to link the antibody variable regions include arbitrary peptide linkers that can be introduced by genetic engineering, and synthetic linkers, for example, the linkers disclosed in Protein Engineering, (1996) 9(3), 299-305.

**[0076]** The preferred linkers in the disclosure are peptide linkers. The length of the peptide linkers is not particularly limited and those skilled in the art can appropriately select the length according to the purpose. The typical length is one to 100 amino acids, preferably 3 to 50 amino acids, more preferably 5 to 30 amino acids, and particularly preferably 12 to 18 amino acids (for example; 15 amino acids).

**[0077]** For example, amino acid sequences for peptide linkers include the following sequences:

Ser
Gly·Ser
Gly·Gly·Ser
Ser·Gly·Gly
Gly·Gly·Gly·Ser (SEQ ID NO: 45)
Ser·Gly·Gly·Gly (SEQ ID NO: 46)
Gly·Gly·Gly·Gly·Ser (SEQ ID NO: 47)
Ser·Gly·Gly·Gly·Gly (SEQ ID NO: 48)
Gly·Gly·Gly·Gly·Gly·Ser (SEQ ID NO: 49)
Ser·Gly·Gly·Gly·Gly·Gly (SEQ ID NO: 50)
Gly·Gly·Gly·Gly·Gly·Gly·Ser (SEQ ID NO: 51)
Ser·Gly·Gly·Gly·Gly·Gly·Gly (SEQ ID NO: 52)
(Gly·Gly·Gly·Gly·Ser [SEQ ID NO: 47])n
(Ser·Gly·Gly·Gly·Gly [SEQ ID NO: 48])n

where n is an integer of 1 or more.

**[0078]** The amino acid sequences of peptide linkers can be appropriately selected by those skilled in the art according to the purpose. For example, the above "n" which determines the length of the peptide linker is typically one to five, preferably one to three, and more preferably one or two.

**[0079]** Synthetic linkers (chemical crosslinking agents) include, crosslinking agents routinely used to crosslink peptides, for example, *N*-hydroxysuccinimide (NHS), disuccinimidyl suberate (DSS), bis(sulfosuccinimidyl)suberate (BS3), dithiobis(succinimidyl propionate) (DSP), dithiobis(sulfosuccinimidyl propionate) (DTSSP), ethylene glycol bis(succinimidyl succinate) (EGS), ethylene glycol bis(sulfosuccinimidyl succinate) (sulfo-EGS), disuccinimidyl tartarate (DST), disulfosuccinimidyl tartarate (sulfo-DST), bis[2-(succinimidooxycarbonyloxy)ethyl]sulfone (BSOCOES), and bis[2-(sulfosuccinimidooxycarbonyloxy)ethyl]sulfone (sulfo-BSOCOES). These crosslinking agents are commercially available.

**[0080]** In general, three linkers are required to link four antibody variable regions. These multiple linkers may be the same or different linkers.

**[0081]** The antibodies described herein also include antibodies in which one or more amino acid residues have been added to the amino acid sequence of an antibody described herein.

**[0082]** Furthermore, the antibodies described herein also include fusion proteins in which an above-described antibody is fused with another peptide or protein. The fusion protein can be prepared by ligating a polynucleotide encoding an antibody described herein and a polynucleotide encoding another peptide or polypeptide in frame, introducing this into an expression vector, and expressing this in a host. Techniques known to those skilled in the art can be used. The peptide or polypeptide to be fused with an antibody described herein may be a known peptide, for example, FLAG (Hopp, T. P. et al., BioTechnology 6, 1204-1210 (1988)), 6x His consisting of six His (histidine) residues, 10x His, influenza hemagglutinin (HA), human c-myc fragment, VSV-GP fragment, p18HIV fragment, T7-tag, HSV-tag, E-tag, SV40 T antigen fragment, lck tag, $\alpha$-tubulin fragment, B-tag, and Protein C fragment. Polypeptides to be fused with the antibodies described herein include, for example, GST (glutathione-S-transferase), HA (influenza hemagglutinin), immunoglobulin

constant region, β-galactosidase, and MBP (maltose-binding protein). Commercially available polynucleotides encoding these peptides or polypeptides can be fused with a polynucleotide encoding an antibody described herein. A fusion polypeptide can be prepared by expressing the fusion polynucleotide thus prepared.

**[0083]** Moreover, the antibodies described herein may also be conjugated antibodies linked to various molecules such as polymers, including polyethylene glycol (PEG) and hyaluronic acid; radioactive substances; fluorescent substances; luminescent substances; enzymes; and toxins. Such conjugated antibodies can be obtained by chemically modifying the obtained antibodies. Methods for antibody modification are already established in the art (see, for example, US 5,057,313 and US 5,156,840). The "antibodies" described herein also include such conjugated antibodies.

**[0084]** Furthermore, the antibodies described herein include antibodies with altered sugar chains.

**[0085]** Furthermore, the antibodies used in the disclosure may be bispecific antibodies. Bispecific antibody refers to an antibody that has variable regions that recognize different epitopes in the same antibody molecule. A bispecific antibody described herein may be a bispecific antibody that recognizes different epitopes on the IL-6 receptor molecule, or a bispecific antibody in which one of the antigen-binding sites recognizes the IL-6 receptor and the other antigen-binding site recognizes another substance. Examples of antigens that bind to the other antigen-binding site of a bispecific antibody that comprises an IL-6 receptor-recognizing antibody described herein include IL-6, TNFα, TNFR1, TNFR2, CD80, CD86, CD28, CD20, CD19, IL-1α, IL-β, IL-1R, RANKL, RANK, IL-17, IL-17R, IL-23, IL-23R, IL-15, IL-15R, BlyS, lymphotoxin α, lymphotoxin β, LIGHT ligand, LIGHT, VL-4, CD25, IL-12, IL-12R, CD40, CD40L, BAFF, CD52, CD22, IL-32, IL-21, IL-21R, GM-CSF, GM-CSFR, M-CSF, M-CSFR, IFN-alpha, VEGF, VEGFR, EGF, EGFR, CCR5, APRIL, and APRILR.

**[0086]** Methods for producing bispecific antibodies are known. Bispecific antibodies can be prepared, for example, by linking two types of antibodies recognizing different antigens. Antibodies to be linked may be a half molecule each containing an H chain and an L chain, or a quarter molecule containing only one H chain. Alternatively, fusion cells producing bispecific antibodies can be prepared by fusing hybridomas producing different monoclonal antibodies. Furthermore, bispecific antibodies can be produced by genetic engineering techniques.

**[0087]** As described below, the antibodies described herein may differ in amino acid sequence, molecular weight, isoelectric point, presence/absence of sugar chains, and conformation, depending on the purification method, or the cell or host used to produce the antibodies. However, as long as the antibody obtained is functionally equivalent to an antibody described herein, it is included in the disclosure. For example, when an antibody described herein is expressed in prokaryotic cells, for example, *Escherichia coli,* a methionine residue is added to the N terminus of the original antibody amino acid sequence. Such antibodies are also included in the antibodies described herein.

**[0088]** Polypeptides of anti-IL-6 receptor antibodies and such described herein can be produced by methods known to those skilled in the art.

**[0089]** An anti-IL-6 receptor antibody can be prepared, for example, by genetic recombination techniques known to those skilled in the art based on the sequence of the anti-IL-6 receptor antibody obtained. Specifically, an anti-IL-6 receptor antibody can be prepared by constructing a polynucleotide encoding the antibody based on the sequence of an IL-6 receptor-recognizing antibody, inserting the polynucleotide into an expression vector, and then expressing it in an appropriate host cell (see for example, Co, M. S. et al., J. Immunol. (1994) 152, 2968-2976; Better, M. and Horwitz, A. H., Methods Enzymol. (1989) 178,476-496; Pluckthun, A. and Skerra, A., Methods Enzymol. (1989) 178, 497-515; Lamoyi, E., Methods Enzymol. (1986) 121, 652-663; Rousseaux, J. et al., Methods Enzymol. (1986) 121,663-669; Bird, R. E. and Walker, B. W., Trends Biotechnol. (1991) 9, 132-137).

**[0090]** Thus, the disclosure provides methods of producing (i) a polypeptide described herein, or (ii) a polypeptide encoded by a gene encoding the polypeptide described herein, wherein the methods comprise the step of culturing a host cell comprising a vector into which a polynucleotide encoding the polypeptide described herein is introduced.

**[0091]** More specifically, the disclosure provides methods of producing a polypeptide described herein, which comprise the steps of:

(a) culturing a host cell comprising a vector into which a gene encoding the polypeptide described herein is introduced; and
(b) obtaining the polypeptide encoded by the gene.

**[0092]** Examples of the vector include M13-type vectors, pUC-type vectors, pBR322, pBluescript, and pCR-Script. Alternatively, when the objective is to subclone and excise the cDNA, other examples of the vector in addition to the ones described above include pGEM-T, pDIRECT, and pT7. Expression vectors are particularly useful for producing antibodies described herein. For example, when the expression vector is used for expression in *E. coli,* the vector should have features that allow its amplification in *E. coli.* In addition, when the host is *E. coli* such as JM109, DH5α, HB101, or XL1-Blue, it is essential that the vector carries a promoter that allows its efficient expression in *E. coli,* for example, lacZ promoter (Ward et al., Nature (1989) 341, 544-546; FASEB J. (1992) 6, 2422-2427), araB promoter (Better et al., Science (1988) 240,1041-1043), T7 promoter or such. Such vector includes pGEX-5X-1 (Pharmacia), "QIAexpress

system" (Quiagen), pEGFP, and pET (in this case, the host is preferably BL21 which expresses T7 RNA polymerase), in addition to the ones described above.

**[0093]** Furthermore, the expression plasmid vectors may contain signal sequences for antibody secretion. As a signal sequence for antibody secretion, the pelB signal sequence (Lei, S. P. et al., J. Bacteriol. (1987) 169,4379) may be used for production into the *E. coli* periplasm. The vectors can be introduced into host cells, for example, by calcium chloride methods or electroporation.

**[0094]** In addition to vectors for *E. coli,* the vectors for producing antibodies described herein include, for example, mammal-derived expression vectors (for example, pcDNA3 (Invitrogen), pEF-BOS (Nucleic Acids. Res. (1990) 18(17), p5322), pEF, and pCDM8), insect cell-derived expression vectors (for example, the "Bac-to-BAC baculovirus expression system" (Gibco-BRL) and pBacPAKB), plant-derived expression vectors (for example, pMH1 and pMH2), animal virus-derived expression vectors (for example, pHSV, pMV, and pAdexLcw), retrovirus-derived expression vectors (for example, pZIPneo), yeast-derived expression vectors (for example, "Pichia Expression Kit" (Invitrogen), pNV11, and SP-Q01), and *Bacillus subtilis*-derived expression vectors (for example, pPL608 and pKTH50).

**[0095]** When the expression plasmid vector is used for expression in animal cells such as CHO, COS, and NIH3T3 cells, it must have a promoter necessary for expression in those cells, for example, SV40 promoter (Mulligan et al., Nature (1979) 277, 108), MMLV-LTR promoter, EF1$\alpha$ promoter (Mizushima et al., Nucleic Acids Res. (1990) 18,5322), or CMV promoter. It is even more preferable if the vector has a gene for selection of transformed cells (for example, a drug resistance gene that allows distinction by an agent (neomycin, G418, or such). Vectors with such characteristics include, for example, pMAM, pDR2, pBK-RSV, pHK-CMV, pOPRSV, and pOP 13.

**[0096]** In addition, when the objective is to stably express genes and amplify a gene's copy number in the cells, a method in which CHO cells deficient in a nucleic acid synthesis pathway are introduced with a vector having a DHFR gene which compensates for the deficiency (for example, pSV2-dhfr ("Molecular Cloning 2nd edition" Cold Spring Harbor Laboratory Press, (1989))) and the vector is amplified using methotrexate (MTX) can be used. Further, when the objective is transient gene expression, a method in which COS cells carrying a gene expressing the SV40 T antigen on their chromosome are transformed with a vector carrying an SV40 replication origin (pcD and such) can be used. It is possible to use replication origins derived from polyoma virus, adenovirus, bovine papilloma virus (BPV), and such. Moreover, to amplify the gene copy number in host cell lines, the expression vectors may comprise the aminoglycoside transferase (APH) gene, thymidine kinase (TK) gene, *E. coli* xanthine-guanine phosphoribosyltransferase (Ecogpt) gene, dihydrofolate reductase (dhfr) gene, and such as a selection marker.

**[0097]** The resulting antibodies described herein can be isolated from host cells or from outside the cells (the medium, or such), and purified as substantially pure and homogenous antibodies. The antibodies can be separated and purified using conventional separation and purification methods for antibody purification, without being limited thereto. For example, the antibodies can be separated and purified by appropriately selecting and combining column chromatography, filtration, ultrafiltration, salting out, solvent precipitation, solvent extraction, distillation, immunoprecipitation, SDS-polyacrylamide gel electrophoresis, isoelectrofocusing, dialysis, recrystallization, and such.

**[0098]** Chromatography includes, for example, affinity chromatography, ion exchange chromatography, hydrophobic chromatography, gel filtration, reverse phase chromatography, and adsorption chromatography (Strategies for Protein Purification and Characterization: A Laboratory Course Manual. Ed Daniel R. Marshak et al., Cold Spring Harbor Laboratory Press, 1996). These chromatographies can be carried out using liquid-phase chromatography, for example, HPLC and FPLC. Columns used for affinity chromatography include protein A columns and protein G columns. Examples of columns using Protein A include Hyper D, POROS, and Sepharose FF (GE Amersham Biosciences). The disclosure also includes antibodies highly purified using such purification methods.

**[0099]** The IL-6 receptor binding activity of the obtained antibodies can be measured by methods known to those skilled in the art. Methods for measuring the antigen-binding activity of an antibody include, for example, enzyme-linked immunosorbent assay (ELISA), enzyme immunoassay (EIA), radioimmunoassay (RIA), and fluorescent antibody methods. For example, when enzyme immunoassay is used, antibody-containing samples such as purified antibodies and culture supernatants of antibody-producing cells are added to antigen-coated plates. A secondary antibody labeled with an enzyme such as alkaline phosphatase is added, and the plates are incubated. After washing, an enzyme substrate such as p-nitrophenyl phosphate is added, and the absorbance is measured to evaluate the antigen-binding activity.

Pharmaceutical compositions

**[0100]** The disclosure also describes pharmaceutical compositions that comprise a polypeptide as described herein as an active ingredient. The pharmaceutical compositions described herein can be used in a method for treating IL-6-associated diseases such as rheumatoid arthritis. Thus, the disclosure also describes agents for use in a method for treating diseases such as rheumatoid arthritis, which comprise an antibody described above as an active ingredient. Preferred examples of target diseases in the disclosure include, but are not limited to, rheumatoid arthritis, juvenile idiopathic arthritis, systemic juvenile idiopathic arthritis, Castleman's disease, systemic lupus erythematosus (SLE),

lupus nephritis, Crohn's disease, lymphoma, ulcerative colitis, anemia, vasculitis, Kawasaki disease, Still's disease, amyloidosis, multiple sclerosis, transplantation, age-related macular degeneration, ankylosing spondylitis, psoriasis, psoriatic arthritis, chronic obstructive pulmonary disease (COPD), IgA nephropathy, osteoarthritis, asthma, diabetic nephropathy, GVHD, endometriosis, hepatitis (NASH), myocardial infarction, arteriosclerosis, sepsis, osteoporosis, diabetes, multiple myeloma, prostate cancer, kidney cancer, B-cell non-Hodgkin's lymphoma, pancreatic cancer, lung cancer, esophageal cancer, colon cancer, cancer cachexia, cancer neuroinvasion, myocardial infarction, myopic choroidal neovascularization, idiopathic choroidal neovascularization, uveitis, chronic thyroiditis, delayed hypersensitivity, contact dermatitis, atopic dermatitis, mesothelioma, polymyositis, dermatomyositis, panuveitis, anterior uveitis, intermediate uveitis, scleritis, keratitis, orbital inflammation, optic neuritis, diabetic retinopathy, proliferative vitreoretinopathy, dry eye, and post-operative inflammation.

**[0101]** The phrase "to comprise an anti-IL-6 receptor antibody as an active ingredient" means comprising an anti-IL-6 receptor antibody as at least one of the active ingredients, without particular limitation on its content. Furthermore, the pharmaceutical compositions described herein may contain other active ingredients in combination with the polypeptides described above.

**[0102]** The pharmaceutical compositions described herein may be used not only for therapeutic purposes, but also for preventive purposes.

**[0103]** The polypeptides described herein can be formulated according to conventional methods (see, for example, Remington's Pharmaceutical Science, latest edition, Mark Publishing Company, Easton, USA). If needed, they may contain pharmaceutically acceptable carriers and/or additives. For example, they may include detergents (for example, PEG and Tween), excipients, antioxidants (for example, ascorbic acid), coloring agents, flavoring agents, preservatives, stabilizers, buffering agents (for example, phosphoric acid, citric acid, and other organic acids), chelating agents (for example, EDTA), suspending agents, isotonizing agents, binders, disintegrants, lubricants, fluidity promoters, and corrigents. However, the agents described herein for use in a method for preventing or treating inflammatory diseases are not limited to the above and may appropriately contain other conventional carriers. Specifically, examples include light anhydrous silicic acid, lactose, crystalline cellulose, mannitol, starch, carmellose calcium, carmellose sodium, hydroxypropylcellulose, hydroxypropyl methylcellulose, polyvinyl acetal diethylaminoacetate, polyvinylpyrrolidone, gelatin, medium chain fatty acid triglyceride, polyoxyethylene hydrogenated castor oil 60, saccharose, carboxymethylcellulose, corn starch, and inorganic salts. They may also contain other low-molecular-weight polypeptides; proteins such as serum albumin, gelatin, and immunoglobulin; and amino acids. When preparing aqueous solutions for use in injecting, the anti-IL-6 receptor antibodies are dissolved, for example, in isotonic solutions containing physiological saline, glucose, or other adjuvants. Adjuvants include, for example, D-sorbitol, D-mannose, D-mannitol, and sodium chloride. Furthermore, appropriate solubilizing agents, for example, alcohol (ethanol, and the like), polyalcohol (propylene glycol, PEG, and the like), and non-ionic surfactants (polysorbate 80 and HCO-50) may be combined.

**[0104]** If necessary, the polypeptides may be encapsulated in microcapsules (microcapsules made of hydroxycellulose, gelatin, poly(methyl methacrylate), and the like), or made into a colloidal drug delivery system (liposomes, albumin microspheres, microemulsions, nanoparticles, nanocapsules, etc) (see, for example, "Remington's Pharmaceutical Science 16th edition", Oslo Ed. (1980)). Moreover, methods for preparing agents as sustained-release agents are known, and these can be applied to the polypeptides (Langer et al., J. Biomed. Mater. Res. (1981) 15: 167-277; Langer, Chem. Tech. (1982) 12: 98-105; US Patent No. 3,773,919; European Patent Application (EP) No. 58,481; Sidman et al., Biopolymers (1983) 22:547-56; EP No.133,988). Furthermore, liquid volume for use in subcutaneous administration can be increased by adding or mixing hyaluronidase to an agent (for example, see WO 2004/078140).

**[0105]** The pharmaceutical compositions described herein can be administered both orally and parenterally, but are preferably administered parenterally. Specifically, the compositions are to be administered to patients by injection or transdermally. Injections include, for example, systemic and local administrations by intravenous, intramuscular, or subcutaneous injection, or such. The compositions may be locally injected at the site of treatment or in the periphery of the site by intramuscular injection, in particular. Transdermal dosage forms include, for example, ointments, gel, cream, poultices, and patches, which can be administered locally or systemically. Furthermore, administration methods can be appropriately selected according to the patient's age and symptoms. The administered dose can be selected, for example, from the range of 0.0001 mg to 100 mg active ingredient per kg of body weight for each administration. Alternatively, when the compositions are to be administered to human patients, for example, the active ingredient can be selected from the range of 0.001 to 1000 mg per kg body weight for each patient. A single administration dose preferably contains, for example, an antibody disclosed herein at about 0.01 to 50 mg/kg body weight

**[0106]** Amino acids contained in the amino acid sequences in the disclosure may be post-translationally modified (for example, the modification of an N-terminal glutamine into a pyroglutamic acid by pyroglutamylation is well-known to those skilled in the art). Naturally, such post-translationally modified amino acids are included in the amino acid sequences in the disclosure.

**[0107]** Further, sugar chains that are bound to the antibodies according to the disclosure may be of any structure. A sugar chain at position 297 (EU numbering) may be of any sugar chain structure (preferably a fucosylated sugar chain),

or no sugar chain may be bound (for example, this can be achieved by producing antibodies in Escherichia coli or by introducing alteration so that no sugar chain binds to position 297, EU numbering).

Examples

**[0108]** Hereinbelow, the disclosure will be specifically described with reference to the Examples.

[Example 1] Identification of mutation sites in the variable regions for enhancing the affinity of TOCILIZUMAB for IL-6 receptor

**[0109]** A library of CDR sequences into which mutations have been introduced was constructed and assayed to improve the affinity of TOCILIZUMAB (H chain WT-IgG1/SEQ ID NO: 53; L chain WT-kappa/SEQ ID NO: 54) for IL-6 receptor. Screening of a library of CDR mutations revealed mutations that improve the affinity for IL-6 receptor. The mutations are shown in Fig. 1. A combination of these mutations yielded high-affinity TOCILIZUMAB such as RDC-23 (H chain RDC23H-IgG1/SEQ ID NO: 55; L chain RDC-23L-kappa/SEQ ID NO: 56). The affinity for soluble IL-6 receptor and biological activity determined using BaF/gp130 were compared between RDC-23 and TOCILIZUMAB (see Reference Examples for the method).

**[0110]** The result of affinity measurement is shown in Table 1. The result of biological activity determination using BaF/gp130 (the final concentration of IL-6 was 30 ng/ml) is shown in Fig. 2. The results showed that the affinity of RDC-23 was about 60 times higher, and the activity expressed as concentration for 100% inhibition of BaF/gp130 was about 100 times higher when compared to TOCILIZUMAB.

Table 1

| | $k_a$(1/Ms) | $k_d$(1/s) | KD(M) |
|---|---|---|---|
| TOCILIZUMAB | 4.9E+05 | 2.0E-03 | 4.0E-09 |
| RDC-23 | 6.4E+05 | 4.3E-05 | 6.7E-11 |

[Example 2] Identification of mutations for improving the pharmacokinetics of TOCILIZUMAB via reduction of its isoelectric point

**[0111]** To improve the pharmacokinetics of TOCILIZUMAB, investigation was carried out to identify mutation sites that would decrease the isoelectric point of the variable regions without significantly reducing the binding to the IL-6 receptor. Screening of mutation sites in the variable regions, which were predicted based on a three-dimensional structure model of TOCILIZUMAB, revealed mutation sites that would decrease the isoelectric point of the variable regions without significantly reducing its binding to the IL-6 receptor. These are shown in Fig. 3. A combination of these mutations yielded TOCILIZUMAB with reduced isoelectric point including, for example, H53/L28 (H chain H53-IgG1/SEQ ID NO: 57; L chain L28-kappa/SEQ ID NO:58). The affinity for soluble IL-6 receptor, isoelectric point, pharmacokinetics in mice, and biological activity determined using BaF/gp130 were compared between H53/L28 and TOCILIZUMAB (see Reference Examples for the method).

**[0112]** The result of affinity measurement is shown in Table 2. The measurement result for the biological activity obtained using BaF/gp130 (the final concentration of IL-6 was 30 ng/ml) is shown in Fig. 4. The results showed that the affinity of H53/L28 was about six times higher and the activity expressed as concentration for 100% inhibition of BaF/gp130 was about several times higher when compared to TOCILIZUMAB.

Table 2

| | $k_a$(1/Ms) | $k_d$(1/s) | KD(M) |
|---|---|---|---|
| TOCILIZUMAB | 4.9E+05 | 2.0E-03 | 4.0E-09 |
| H53/L28 | 7.6E+05 | 5.2E-04 | 6.8E-10 |

**[0113]** The result of isoelectric point determination by isoelectric point electrophoresis known to those skilled in the art showed that the isoelectric points of TOCILIZUMAB and H53/L28 were about 9.3 and 6.5 to 6.7, respectively. Thus, the isoelectric point of H53/L28 was reduced by about 2.7 when compared to TOCILIZUMAB. Furthermore, the theoretical isoelectric point of the VH/VL variable regions was calculated using GENETYX (GENETYX CORPORATION). The result showed that the theoretical isoelectric points of TOCILIZUMAB and H53/L28 were 9.20 and 4.52, respectively. Thus, the isoelectric point of H53/L28 was reduced by about 4.7 when compared to TOCILIZUMAB.

**[0114]** To assess the pharmacokinetics of the altered antibody H53/L28 which has a reduced isoelectric point, the pharmacokinetics OF TOCILIZUMAB and H53/L28 in normal mice were compared. A single dose of TOCILIZUMAB or H53/L28 was intravenously (IV) or subcutaneously (SC) administered at 1 mg/kg to mice (C57BL/6J; Charles River Japan, Inc.) to evaluate the time course of plasma concentration. The time courses of plasma concentration for TOCILIZUMAB and H53/L28 after intravenous administration or subcutaneous administration are shown in Figs. 5 and 6, respectively. Pharmacokinetic parameters (clearance (CL) and half-life (T1/2)) obtained using WinNonlin (Pharsight) are shown in Table 3. The plasma half-life (T1/2) of H53/L28 after intravenous administration was prolonged to about 1.3 times that of TOCILIZUMAB, while the clearance was reduced by about 1.7 times. T1/2 of H53/L28 after subcutaneous administration was increased to about twice that of TOCILIZUMAB, while the clearance was reduced by about 2.1 times. Thus, it was found that the pharmacokinetics could be significantly improved by reducing the isoelectric point of TOCILIZUMAB through amino acid substitution.

Table 3

| | IV | | SC | |
|---|---|---|---|---|
| | CL mL/h/kg | T1/2 day | CL/F mL/h/kg | T1/2 day |
| TOCILIZUMAB | 0.177 | 18.5 | 0.18 | 14.7 |
| H53/L28 | 0.102 | 23.5 | 0.086 | 29.7 |

[Example 3] Identification of mutation sites that reduce the immunogenicity of TOCILIZUMAB Identification of mutations that reduce the immunogenicity risk of T-cell epitopes present in the variable regions

**[0115]** T-cell epitopes present in the variable-region sequence of TOCILIZUMAB were analyzed using TEPITOPE (Methods. 2004 Dec; 34(4):468-75). As a result, the L-chain CDR2 was predicted to have many T-cell epitopes that would bind to HLA (i.e. to have a sequence with a high immunogenicity risk). Thus, TEPITOPE analysis was carried out to examine amino acid substitutions that would reduce the immunogenicity risk of the L-chain CDR2 without decreasing the stability, binding activity, or neutralizing activity.

**[0116]** As described below, the screening result demonstrated that the immunogenicity risk can be reduced without decreasing the stability, binding activity, or neutralizing activity by substituting the threonine at L51 (Kabat's numbering; Kabat EA *et al.*, (1991) Sequences of Proteins of Immunological Interest, NIH)) of the L chain CDR2 (SEQ ID NO: 59) of TOCILIZUMAB with glycine, and the arginine at L53 with glutamic acid (SEQ ID NO: 60).

TOCILIZUMAB L-chain CDR2 (SEQ ID NO: 59)

TOCILIZUMAB L-chain CDR2 with T-cell epitopes removed (SEQ ID NO: 60)

[Example 4] Reduction of immunogenicity risk by full humanization of the variable region framework sequences of TOCILIZUMAB

**[0117]** In the process of TOCILIZUMAB humanization, some mouse sequences remain in the framework sequence to maintain binding activity (Cancer Res. 1993 Feb 15; 53(4):851-6). These sequences are H27, H28, H29, and H30 in the H-chain FR1, and H71 in the H-chain FR3 (Kabat's numbering; Kabat EA et al., (1991) Sequences of Proteins of Immunological Interest, NIH)) of the variable region sequence of TOCILIZUMAB. The mouse sequences that remained are a potential cause of increased immunogenicity risk. Thus, it was assessed whether the framework sequence could be fully humanized to further reduce the immunogenicity risk of TOCILIZUMAB.

**[0118]** The result showed that the entire framework of TOCILIZUMAB could be completely humanized without decreasing the stability, binding activity, or neutralizing activity, by substituting the H-chain FR1 (SEQ ID NO: 61) of TOCILIZUMAB with the humanized H-chain FR1-A (SEQ ID NO: 62) shown below, and substituting the-H chain FR3 (SEQ ID NO: 63) with the humanized H chain FR3 (SEQ ID NO: 64) shown below.

TOCILIZUMAB H chain FR1 (SEQ ID NO: 61)

Humanized H chain FR1-A (SEQ ID NO: 62) (derived from germline IMGT hVH_4)

TOCILIZUMAB H chain FR3 (SEQ ID NO: 63)

**[0119]** Humanized H chain FR3 (SEQ ID NO: 64) (derived from Mol. Immunol. 2007, 44(4):412-422)

[Example 5] Identification of mutation sites to improve the pharmacokinetics based on pH-dependent binding of TOCILIZUMAB to the IL-6 receptor

**[0120]** One of the methods for improving the pharmacokinetics of TOCILIZUMAB is to improve the molecule such that a single molecule of TOCILIZUMAB would repeatedly bind and neutralize several molecules of the IL-6 receptor. It is

assumed that after binding to membrane-type IL-6 receptor, TOCILIZUMAB is taken up into intracellular endosomes via internalization while bound to membrane-type IL-6 receptor, then transferred into lysosomes while bound to membrane-type IL-6 receptor, and becomes degraded by lysosomes. Specifically, one molecule of TOCILIZUMAB typically binds to one or two molecules of membrane-type IL-6 receptor (in a monovalent or divalent manner) and is degraded in lysosomes after internalization. Therefore, one molecule of TOCILIZUMAB can only bind and neutralize one or two molecules of membrane-type IL-6 receptor.

**[0121]** Thus, if it was possible to create TOCILIZUMAB that binds in a pH-dependent manner, in which the binding of TOCILIZUMAB is maintained under neutral conditions but significantly reduced under acidic conditions, TOCILIZUMAB which binds in a pH-dependent manner could dissociate from membrane-type IL-6 receptor (antigen) in the endosomes and return to the plasma by binding to FcRn present in the endosomes, as illustrated in Fig. 7. Once returned to the plasma, TOCILIZUMAB which binds in a pH-dependent manner could again bind to membrane-type IL-6 receptor. By repeating this binding in the plasma and dissociation in the endosomes, it is thought that one molecule of TOCILIZUMAB can repeatedly bind/neutralize several molecules of the IL-6 receptor. Thus, TOCILIZUMAB which binds in a pH-dependent manner is assumed to have improved pharmacokinetics as compared to TOCILIZUMAB.

**[0122]** For TOCILIZUMAB to dissociate from the IL-6 receptor under the acidic condition in the endosome, the binding must be significantly weakened under the acidic condition as compared to under the neutral condition. On the cell surface, strong IL-6 receptor binding is required for neutralization; therefore, at pH 7.4 which is the cell surface pH, the antibody must bind to the IL-6 receptor as strongly as or more strongly than TOCILIZUMAB. It has been reported that the endosomal pH is generally 5.5 to 6.0 (Nat Rev Mol Cell Biol. 2004 Feb;5(2):121-32). Thus, if TOCILIZUMAB which binds in a pH-dependent manner is modified to weakly bind to the IL-6 receptor at pH 5.5 to 6.0, it can be predicted to dissociate from the IL-6 receptor under the acidic condition in the endosomes. Specifically, if TOCILIZUMAB which binds in a pH-dependent manner is improved to strongly bind to the IL-6 receptor at pH 7.4, which is the cell surface pH, and to weakly bind to IL-6 receptor at pH 5.5 to 6.0, which is the endosomal pH, one molecule OF TOCILIZUMAB can bind and neutralize several molecules of the IL-6 receptor, and the pharmacokinetics can therefore be improved.

**[0123]** A possible method for conferring pH dependence on the binding of TOCILIZUMAB to the IL-6 receptor is to introduce histidine residues into the variable region of TOCILIZUMAB, since the pKa of a histidine residue is about 6.0 to 6.5, and its state of proton dissociation changes between neutral (pH 7.4) and acidic (pH 5.5 to 6.0) conditions. Thus, screening was carried out to identify sites for histidine introduction in the variable regions based on a three-dimensional structure model of TOCILIZUMAB. Furthermore, selected variable region sequences of TOCILIZUMAB were randomly substituted with histidine to design a library for screening. The screening was carried out using the binding to the IL-6 receptor at pH 7.4 and dissociation from the IL-6 receptor, or the reduction of affinity at pH 5.5 to 5.8 as an index.

**[0124]** As a result, mutation sites were discovered that confer the binding of TOCILIZUMAB to the IL-6 receptor with pH dependency (the property to bind at pH 7.4 and dissociate at pH 5.8). These are shown in Fig. 8. In Fig. 8, the substitution of tyrosine at H27 to histidine is a mutation in the H-chain FR1, not in the CDR. However, as described in Eur. J. Immunol. (1992) 22: 1719-1728, a sequence with histidine at H27 is a human sequence (SEQ ID NO: 65). Thus, the antibody can be completely humanized by using the following framework in combination with Example 4.
Humanized H-chain FR1-B (SEQ ID NO: 65)

**[0125]** A combination of mutations including, for example, H3pI/L73 (H chain, H3pI-IgG1/SEQ ID NO: 66; L chain L73-kappa/SEQ ID NO: 67) can yield TOCILIZUMAB, with pH-dependent binding properties. H3pI/L73 and TOCILIZUMAB were compared for their affinity towards soluble IL-6 receptor at pH 7.4, rate of dissociation from membrane-type IL-6 receptor at pH 7.4 and pH 5.8, biological activity using BaF/gp130, and pharmacokinetics in cynomolgus monkey and human IL-6 receptor transgenic mice (see Reference Examples for the method).

**[0126]** The result of affinity assay for soluble IL-6 receptor at pH 7.4 is shown in Table 4. The assay result for the biological activity obtained using BaF/gp130 (final IL-6 concentration of 30 ng/ml) is shown in Fig. 9. These results showed that H3pI/L73 is comparable to TOCILIZUMAB in terms of affinity for soluble IL-6 receptor at pH 7.4 and activity on BaF/gp130.

Table 4

| | $k_a$(1/Ms) | $k_d$(1/s) | KD(M) |
|---|---|---|---|
| TOCILIZUMAB | 5.1E+05 | 1.0E-03 | 2.1E-09 |
| H3pI/L73 | 5.4E+05 | 7.4E-04 | 1.4E-09 |

**[0127]** The measurement result for the rate of dissociation of TOCILIZUMAB or H3pI/L73 from membrane-type IL-6 receptor at pH 7.4 and pH 5.8 is shown in Table 5. As compared to TOCILIZUMAB, the dissociation rate of H3pI/L73 at pH 5.8 was faster and the pH dependence of the rate of dissociation from membrane-type IL-6 receptor was increased by about 2.6 times.

Table 5

| | pH7.4 $k_d$(1/s) | pH5.8 $k_d$(1/s) | $k_d$(pH5.8)/$k_{d(pH7.4)}$ pH DEPENDENCY |
|---|---|---|---|
| TOCILIZUMAB | 2.5E-04 | 2.5E-04 | 1.00 |
| H3pI/L73 | 2.6E-04 | 6.7E-04 | 2.59 |

**[0128]** A single dose of TOCILIZUMAB or H3pI/L73 was intravenously administered at 1 mg/kg to cynomolgus monkeys to assess the time course of plasma concentration. The plasma concentration time courses of TOCILIZUMAB or H3pI/L73 after intravenous administration are shown in Fig. 10. The result showed that the pharmacokinetics of H3pI/L73 in cynomolgus monkeys was significantly improved as compared to TOCILIZUMAB.

**[0129]** A single dose of TOCILIZUMAB or H3pI/L73 was intravenously administered at 25 mg/kg to human IL-6 receptor transgenic mice (hIL-6R tg mice; Proc Natl Acad Sci U S A. 1995 May 23; 92(11):4862-6) to assess the time course of plasma concentration. The plasma concentration time courses of TOCILIZUMAB or H3pI/L73 after intravenous administration are shown in Fig. 11. The result showed that the pharmacokinetics of H3pI/L73 in human IL-6 receptor transgenic mice was significantly improved as compared to TOCILIZUMAB.

**[0130]** H3pI/L73, which is a TOCILIZUMAB with pH-dependent binding properties, showed significantly improved pharmacokinetics in cynomolgus monkeys and human IL-6 receptor transgenic mice when compared to TOCILIZUMAB. This suggests that it is possible to bind to and neutralize several molecules of the IL-6 receptor with one single molecule, by conferring the property of binding an antigen at pH 7.4 and dissociating from the antigen at pH 5.8. It was also considered that the pharmacokinetics could be further improved by conferring IL-6 receptor binding with a more pronounced pH dependence than that of H3pI/L73.

[Example 6] Optimization of the TOCILIZUMAB constant region Reduction of the heterogeneity of TOCILIZUMAB H-chain C terminus

**[0131]** For heterogeneity of the H-chain C-terminal sequences of an IgG antibody, deletion of C-terminal amino acid lysine residue, and amidation of the C-terminal carboxyl group due to deletion of both of the two C-terminal amino acids, glycine and lysine, have been reported (Anal Biochem. 2007 Jan 1; 360(1):75-83). Also in TOCILIZUMAB, the major component is a sequence in which the C-terminal amino acid lysine in the nucleotide sequence is deleted by post-translational modification; however, sub-components in which the lysine remains and sub-components in which the C-terminal carboxyl group is amidated due to deletion of both glycine and lysine also exist as heterogeneity. It is not easy and would be more costly to manufacture them as a pharmaceutical in large-scale while maintaining the objective substances/related substances related heterogeneity between productions. If possible, it is desirable to be single substances, and to have reduced heterogeneity when developing antibodies as pharmaceuticals. Thus, it is preferable that the H-chain C-terminal heterogeneity is absent when developing antibodies as pharmaceuticals.

**[0132]** The C-terminal amino acid was altered to reduce the C-terminal amino acid heterogeneity. The result showed that the C-terminus-derived heterogeneity can be prevented by pre-deleting from the nucleotide sequence, the lysine and glycine residues at the C terminus of the H-chain constant region of TOCILIZUMAB. TOCILIZUMAB, TOCILIZUMAB that lacks the C-terminal lysine residue (TOCILIZUMABΔK: H chain WT-IgG1ΔK/SEQ ID NO: 68; L chain WT-kappa/SEQ ID NO: 54), and TOCILIZUMAB that lacks the C-terminal lysine and glycine residues (TOCILIZUMABΔGK: H chain WT-IgGIΔGK/SEQ ID NO: 69; L chain WT-kappa/SEQ ID NO: 54) were assessed for heterogeneity by cation exchange chromatography. The ProPac WCX-10, 4x250 mm (Dionex) column was used; and mobile phase A was 25 mmol/L MES/NaOH (pH 6.1) and mobile phase B was 25 mmol/L MES/NaOH, 250 mmol/L NaCl (pH 6.1). Appropriate flow rate and gradient were used. The assessment result obtained by cation exchange chromatography is shown in Fig. 12. The result showed that the C-terminal amino acid heterogeneity can be reduced by pre-deleting from the nucleotide sequence both the lysine and glycine residues at the C terminus of the H-chain constant region, but not by pre-deleting only the lysine residue at the C terminus of the H-chain constant region. All of the C-terminal sequences of the constant region of human antibodies IgG1, IgG2, and IgG4 contain lysine and glycine at positions 447 and 446, respectively, according to EU numbering (see Sequences of proteins of immunological interest, NIH Publication No.91-3242). Therefore, the method for reducing the C-terminal amino acid heterogeneity found in the study is expected to be also applicable to IgG2 and IgG4 constant regions and variants thereof.

Reduction of disulfide bond-derived heterogeneity in IgG2 isotype TOCILIZUMAB

**[0133]** The isotype of TOCILIZUMAB is IgG1. Since TOCILIZUMAB is a neutralizing antibody, binding to the Fcγ receptor can be unfavorable in view of immunogenicity and adverse effects. A possible method for lowering the Fcγ

receptor binding is to convert the isotype of the IgG antibody from IgG1 to IgG2 or IgG4 (Ann Hematol. 1998 Jun; 76(6):231-48). From the viewpoint of Fcγ receptor I binding and pharmacokinetics, IgG2 was considered to be more desirable than IgG4 (Nat Biotechnol. 2007 Dec; 25(12):1369-72). Meanwhile, physicochemical properties of proteins, in particular, homogeneity and stability are very important when developing antibodies as pharmaceuticals. The IgG2 isotype has been reported to have very high heterogeneity due to the disulfide bonds in the hinge region (J Biol Chem. 2008 Jun 6; 283(23):16206-15). It is not easy and would be more costly to manufacture them as 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. Thus, when developing IgG2 isotype antibodies into pharmaceuticals, it is preferable to reduce the heterogeneity derived from disulfide bonds without lowering the stability.

**[0134]** For the purpose of reducing the heterogeneity of the IgG2 isotype, various variants were assessed. As a result, it was found that heterogeneity could be reduced without decreasing the stability using the WT-SKSC constant region (SEQ ID NO: 70), in which of the IgG2 constant region sequences, the cysteine residue at position 131 and the arginine residue at position 133 (EU numbering) in the H-chain CH1 domain were substituted to serine and lysine, respectively, and the cysteine residue at position 219 (EU numbering) in the H-chain upper hinge was substituted to serine. TOCILIZUMAB-IgG1 (H chain WT-IgG1/SEQ ID NO: 53; L chain WT-kappa/SEQ ID NO: 54), TOCILIZUMAB-IgG2 (H chain WT-IgG2/SEQ ID NO: 71; L chain WT-kappa/SEQ ID NO: 54), and TOCILIZUMAB-SKSC (H chain WT-SKSC/SEQ ID NO: 70; L chain WT-kappa/SEQ ID NO: 54) were prepared and assessed for heterogeneity and stability. The heterogeneity was assessed by cation exchange chromatography. The ProPac WCX-10 (Dionex) column was used; and mobile phase A was 20 mM Sodium Acetate (pH 5.0) and mobile phase B was 20 mM Sodium Acetate, 1 M NaCl (pH 5.0). Appropriate flow rate and gradient were used. The assessment result obtained by cation exchange chromatography is shown in Fig. 13. The stability was assessed based on the intermediate temperature in thermal denaturation (Tm value) determined by differential scanning calorimetry (DSC) (VP-DSC; Microcal). The result of DSC measurement in 20 mM sodium acetate, 150 mM NaCl, pH 6.0 and the Tm value of the Fab domain are shown in Fig. 14.

**[0135]** The result showed that the heterogeneity was markedly increased in TOCILIZUMAB-IgG2 as compared to TOCILIZUMAB-IgG1; however, the heterogeneity could be significantly reduced by conversion to TOCILIZUMAB-SKSC. Furthermore, when compared to TOCILIZUMAB-IgG1, the DSC of TOCILIZUMAB-IgG2 gave a shoulder peak (Fab*) component with low stability, i.e., low Tm, in the thermal denaturation peaks of the Fab domain, which is assumed to be due to a heterogeneous component. However, when converted to TOCILIZUMAB-SKSC, the shoulder peak (low Tm), which is thought to be due to a heterogeneous component, disappeared, and the Tm value was about 94°C, which was equivalent to that of the Fab domain of TOCILIZUMAB-IgG1 and TOCILIZUMAB-IgG2. Thus, TOCILIZUMAB-SKSC was revealed to have high stability.

Identification of pharmacokinetics-improving mutation sites in the constant region of TOCILIZUMAB

**[0136]** As described above, starting from IgG1, which is the isotype of TOCILIZUMAB, reduction of the C-terminal heterogeneity and reduction of heterogeneity of antibodies with IgG2 isotype constant regions while reducing the binding to the Fcγ receptor and maintaining the high stability can be achieved. Moreover, it is preferred that the constant region also has superior pharmacokinetics than IgG1, which is the isotype of TOCILIZUMAB.

**[0137]** In order to find constant regions having a superior plasma half-life than antibodies with IgG1-isotype constant regions, screening was carried out to identify mutation sites for improving the pharmacokinetics of TOCILIZUMAB-SKSC which has high stability and reduced heterogeneity related to antibodies with IgG2-isotype constant regions as mentioned above. As a result, WT-M58 (SEQ ID NO: 72 (amino acid sequence)) was discovered, in which, as compared to WT-SKSC, the glutamic acid at position 137 (EU numbering) is substituted to glycine, the serine at position 138 is substituted to glycine, the histidine at position 268 is substituted to glutamine, the arginine at position 355 is substituted to glutamine, the glutamine at position 419 is substituted to glutamic acid, and in which the glycine at position 446 and the lysine at position 447 is deleted to reduce the heterogeneity of the H-chain C terminus. In addition, WT-M44 (SEQ ID NO: 73 (amino acid sequence)) was prepared to have substitution of asparagine at position 434 to alanine, relative to IgG1. Furthermore, WT-M83 (SEQ ID NO: 74 (amino acid sequence)) was produced by deleting glycine at position 446 and lysine at position 447 from M44 to reduce the heterogeneity of the H-chain C-terminus. In addition, WT-M73 (SEQ ID NO: 75 (amino acid sequence)) was produced by substituting asparagine at position 434 with alanine in WT-M58.

**[0138]** TOCILIZUMAB-M44 (H chain WT-M44/SEQ ID NO: 73; L chain WT-kappa/SEQ ID NO: 54), TOCILIZUMAB-M58 (H chain WT-M58/SEQ ID NO: 72; L chain WT-kappa/SEQ ID NO: 54), and TOCILIZUMAB-M73 (H chain WT-M73/SEQ ID NO: 75; L chain WT-kappa/SEQ ID NO: 54) were prepared and assessed for their affinity towards human FcRn and pharmacokinetics using human FcRn transgenic mice (see Reference Examples for the method).

**[0139]** The binding of TOCILIZUMAB-IgG1, TOCILIZUMAB-M44, TOCILIZUMAB-M58, and TOCILIZUMAB-M73 to human FcRn was assessed using Biacore. As shown in Table 6, the binding of TOCILIZUMAB-M44, TOCILIZUMAB-M58, and TOCILIZUMAB-M73 was about 2.7 times, 1.4 times, and 3.8 times superior than that of TOCILIZUMAB-IgG1,

respectively.

Table 6

| | KD(μM) |
|---|---|
| TOCILIZUMAB-IgG1 | 1.62 |
| TOCILIZUMAB-M44 | 0.59 |
| TOCILIZUMAB-M58 | 1.17 |
| TOCILIZUMAB-M73 | 0.42 |

**[0140]** TOCILIZUMAB-IgG1, TOCILIZUMAB-M44, TOCILIZUMAB-M58, and TOCILIZUMAB-M73 were assessed for their pharmacokinetics in human FcRn transgenic mice. The result is shown in Fig. 15. When compared to TOCILIZUMAB-IgG1, all of TOCILIZUMAB-M44, TOCILIZUMAB-M58, and TOCILIZUMAB-M73 were found to exhibit improved pharmacokinetics, as shown in Fig. 15. The effect of improving the pharmacokinetics correlated with the ability to bind to human FcRn. In particular, the concentration of TOCILIZUMAB-M73 in plasma after 28 days was improved by about 16 times as compared to TOCILIZUMAB-IgG1. Thus, antibodies having the constant region of M73 were also assumed to have significantly improved pharmacokinetics in humans as compared to antibodies having the IgG1 constant region.

[Example 7] Preparation of fully humanized IL-6 receptor antibodies with improved PK/PD

**[0141]** TOCILIZUMAB variants were prepared by combining multiple mutations in the variable and constant regions of TOCILIZUMAB found in the examples above. Fully humanized IL-6 receptor antibodies discovered from various screenings were: Fv3-M73 (H chain VH4-M73/SEQ ID NO: 25; L chain VL1-kappa/SEQ ID NO: 28), Fv4-M73 (H chain VH3-M73/SEQ ID NO: 26; L chain VL3-kappa/SEQ ID NO: 29), and Fv5-M83 (H chain VH5-M83/SEQ ID NO: 27; L chain VL5-kappa/SEQ ID NO: 30).

**[0142]** 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 antibodies for the soluble IL-6 receptor determined at pH 7.4 are shown in Table 7. 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. 16 (TOCILIZUMAB, the control, and Fv5-M83 with a final IL-6 concentration of 300 ng/ml) and Fig. 17 (TOCILIZUMAB, Fv3-M73, and Fv4-M73 with a final IL-6 concentration of 30 ng/ml). As shown in Table 7, 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 (H chain VH5-IgG1 /SEQ ID NO: 76; L chain VL5-kappa /SEQ ID NO: 30), which has an IgG1-type constant region; the constant region is generally thought to have no effect on affinity). As shown in Fig. 17, Fv3-M73 and Fv4-M73 exhibit slightly stronger activities than TOCILIZUMAB. As shown in Fig. 16, 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 7

| | $k_a$(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 |

**[0143]** The rates of dissociation of TOCILIZUMAB, Fv3-M73, and Fv4-M73 from membrane-type IL-6 receptor at pH 7.4 and 5.8 were determined. As demonstrated by the result shown in Table 8 (see Reference Example for method), the pH dependency of the dissociation rate of Fv3-M73 and Fv4-M73 from membrane-type IL-6 receptor was about 11 times and 10 times improved, respectively, as compared to TOCILIZUMAB. The considerable improvement of the pH dependency of the dissociation rate relative to H3pI/L73 described in Example 5 suggested that when compared to H3pI/L73, pharmacokinetics of Fv3-M73 and Fv4-M73 would be significantly improved.

Table 8

| | pH7.4 $k_d$(1/s) | pH5.8 $k_d$(1/s) | $k_{d(pH5.8)}/k_{d(pH7.4)}$ pH DEPENDENCY |
|---|---|---|---|
| TOCILIZUMAB | 2.5E-04 | 2.5E-04 | 1.00 |
| Fv3-M73 | 4.9E-04 | 5.3E-03 | 10.88 |
| Fv4-M73 | 5.1E-04 | 5.1E-03 | 10.06 |

**[0144]** The isoelectric points of TOCILIZUMAB, the control, Fv3-M73, Fv4-M73, and Fv5-M83 were determined by isoelectric focusing electrophoresis 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. Since it was shown in Example 2 that pharmacokinetics is improved by reducing the isoelectric point, the pharmacokinetics of Fv3-M73, Fv4-M73, and Fv5-M83 was thought to be improved when compared to TOCILIZUMAB and the control.

**[0145]** 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, as shown in Example 3. 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 8] PK/PD test of fully humanized IL-6 receptor antibodies in monkeys

**[0146]** 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 their time course of plasma concentration (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. 18. The result showed that each of Fv3-M73, Fv4-M73, and Fv5-M83 exhibited significantly improved pharmacokinetics in cynomolgus monkeys when compared to TOCILIZUMAB and the control. Of them, Fv3-M73 and Fv4-M73 exhibited highly improved pharmacokinetics when compared to TOCILIZUMAB.

**[0147]** The efficacy of each antibody to neutralize membrane-type 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. 19. 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 the percentages of free soluble IL-6 receptor were calculated (see Reference Example for method). The time course of percentage of free soluble IL-6 receptor after administration of each antibody is shown in Fig. 20.

**[0148]** Each of Fv3-M73, Fv4-M73, and Fv5-M83 neutralized membrane-type 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-type 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-type 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.

**[0149]** 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.

[Example 9]

**[0150]** Monocyte chemoattractant protein (MCP)-1 is known to be involved in cellular invasion of monocytes, T cells, NK cells, and basophils. MCP-1 has been reported to be highly expressed in synovial tissues/synovial fluid of RA patients (J. Clin. Invest., Sep 1992, 90(3):772-779) and is thought to be involved in the pathological condition of RA (Inflamm. Allergy Drug Targets, Mar 2008, 7(1):53-66).

**[0151]** VEGF is a potent angiogenic factor and is known to be produced, for example, by macrophages, fibroblasts, and synovial cells in the synovial membrane of RA patients (J. Rheumatol., Sep 1995, 22(9):1624-1630). Moreover, the VEGF level in the serum of RA patients correlates with disease activity and radiographic progression (Arthritis Rheum., Jun 2003, 48(6):1521-1529; and Arthritis Rheum., Sep 2001, 44(9):2055-2064) and the VEGF level in the serum decreases by treating RA patients with the anti-IL-6R antibody TOCILIZUMAB; therefore, VEGF is also considered to play an important role in the pathological condition of RA (Mod. Rheumatol. 2009, 19(1):12-19; and Mediators Inflamm. 2008, 2008:129873).

**[0152]** Thus, whether TOCILIZUMAB and Fv4-M73 can inhibit MCP-1 and VEGF productions from human RA patient-derived synovial cells which occur from sIL-6R and IL-6 stimulation was examined.

**[0153]** Human RA patient-derived synovial cells (TOYOBO) were plated onto 96 well plates in 5% FCS-containing IMDM medium at 2 x $10^4$ cells/0.05 mL/well, and placed for 90 minutes in a $CO_2$ incubator (37°C, 5% $CO_2$). 0.05 mL of TOCILIZUMAB and Fv4-M73 diluted to appropriate concentrations were added, the plates were left still for 15 minutes, then 0.05 mL of soluble IL-6 receptor (SR344: prepared according to the method described in Reference Examples) were added. The plates were further left still for 30 minutes, and 0.05 mL of IL-6 (TORAY) were further added (the final concentrations of soluble IL-6 receptor and IL-6 were 50 ng/mL for each). After two days of culture, the culture supernatants were collected, and the MCP-1 and VEGF concentrations in the culture supernatants were measured using ELISA kit (Biosource and Pierce Biotechnology). The results are shown in Figs. 21 and 22. TOCILIZUMAB and Fv4-M73 inhibited MCP-1 and VEGF production from human RA patient-derived synovial cells following soluble IL-6 receptor and IL-6 stimulation in a concentration-dependent manner.

**[0154]** Accordingly, the persistence of the effect of Fv4-M73 as an anti-IL-6 receptor neutralizing antibody (the effect of binding to the IL-6 receptor and blocking the signals of the membrane-type IL6 receptor and soluble IL-6 receptor) is significantly superior as compared to TOCILIZUMAB, the administration frequency and dose can be greatly reduced as compared to TOCILIZUMAB, and furthermore, Fv4-M73 inhibits MCP-1 and VEGF production from human RA patient-derived synovial cells. Therefore, Fv4-M73 was shown to be a very effective therapeutic agent against RA.

Reference Examples

Preparation of soluble recombinant human IL-6 receptor

**[0155]** Soluble recombinant human IL-6 receptor of the human IL-6 receptor, which is the antigen, was produced as described below. A CHO cell line constitutively expressing a soluble human IL-6 receptor containing a sequence from the N-terminal 1st to 344th amino acids reported in J. Biochem. (1990) 108, 673-676 (Yamasaki et al., Science (1988) 241, 825-828 (GenBank #X12830)) was generated. Soluble human IL-6 receptor was purified from culture supernatant of CHO cells expressing SR344 by three column chromatographies: Blue Sepharose 6 FF column chromatography, affinity chromatography using a column immobilized with an antibody specific to SR344, and gel filtration column chromatography. The fraction eluted as the main peak was used as the final purified sample.

Preparation of soluble recombinant Cynomolgus monkey IL-6 receptor (cIL-6R)

**[0156]** 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 a mammalian 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)

**[0157]** 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 a mammalian 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

**[0158]** A mammalian 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: 77 and 78, respectively). The antibody variable region was constructed by PCR using a combination of synthetic oligo DNAs (assembly PCR) and IgG1 was used for the constant region. The antibody variable and constant regions were combined together by assembly PCR, and then inserted into a mammalian 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.

Preparation, expression, and purification of TOCILIZUMAB variants

**[0159]** TOCILIZUMAB variants were prepared using the QuikChange Site-Directed Mutagenesis Kit (Stratagene) according to the method described in the appended instruction manual. The resulting plasmid fragments were inserted into mammalian cell expression vectors to construct expression vectors for the H chains and L chains of interest. The nucleotide sequences of the obtained expression vectors were determined by a method known to skilled artisans. The antibodies were expressed by the method described below. Human embryonic kidney cancer-derived HEK293H cell line (Invitrogen) was suspended in DMEM (Invitrogen) supplemented with 10% Fetal Bovine Serum (Invitrogen). The cells were plated at 10 ml per dish in dishes for adherent cells (10 cm in diameter; CORNING) at a cell density of 5 to 6 x $10^5$ cells/ml 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 was introduced into the cells by the lipofection method. The resulting culture supernatants were collected, centrifuged (approximately 2000 g, 5 min, room temperature) to remove cells, and sterilized by filtering through 0.22-μm filter MILLEX(R)-GV (Millipore) to obtain the supernatants. Antibodies were purified from the obtained culture supernatants by a method known to those skilled in the art using rProtein A Sepharose™ Fast Flow (Amersham Biosciences). To determine the concentration of the purified antibody, absorbance was measured at 280 nm using a spectrophotometer. Antibody concentrations were calculated from the determined values using an absorbance coefficient calculated by the PACE method (Protein Science 1995; 4:2411-2423).

Establishment of a human gp130-expressing BaF3 cell line

**[0160]** 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.

**[0161]** 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(+).

**[0162]** 10 μg of pCOS2Zeo/gp130 was mixed with BaF3 cells (0.8 x $10^7$ 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 soluble human IL-6 receptor, and thus can be used to assess the growth inhibition activity (or IL-6 receptor neutralizing activity) of an anti-IL-6 receptor

antibody.

Assessment for the biological activity by human gp130-expressing BaF3 cells (BaF/gp130)

**[0163]** 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 600 ng/ml or 60 ng/ml human interleukin-6 (TORAY) (final concentration of 300 ng/ml or 30 ng/ml), appropriate amount of soluble human IL-6 receptor, 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.

Biacore-based analysis of binding to soluble human IL-6 receptor

**[0164]** Antigen-antibody reaction kinetics was analyzed using Biacore T100 (GE Healthcare). The soluble human IL-6 receptor-antibody interaction was measured by immobilizing appropriate amounts of protein A or protein A/G or 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 soluble IL-6 receptor adjusted to 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).

Assessment for the pH-dependent dissociation from membrane-type IL-6 receptor using Biacore

**[0165]** The antigen-antibody reaction with membrane-type IL-6 receptor at pH 5.8 and pH 7.4 was observed using Biacore T100 (GE Healthcare). The binding to membrane-type IL-6 receptor was assessed by evaluating the binding to soluble human IL-6 receptor immobilized onto the sensor chip. SR344 was biotinylated by a method known to those skilled in the art. Based on the affinity between biotin and streptavidin, biotinylated soluble human IL-6 receptor was immobilized onto the sensor chip via streptavidin. All measurements were conducted at 37°C. The mobile phase buffer was 10 mM MES (pH 5.8), 150 mM NaCl, and 0.05% Tween 20. A clone exhibiting pH-dependent binding was injected under the condition of pH 7.4 to bind to soluble human IL-6 receptor (injection sample buffer was 10 mM MES (pH 7.4), 150 mM NaCl, and 0.05% Tween 20). Then, the pH-dependent dissociation of each clone was observed at pH 5.8, which is the pH of the mobile phase. The dissociation rate constant (kd (1/s)) at pH 5.8 was calculated using Biacore T100 Evaluation Software (GE Healthcare) by fitting only the dissociation phase at pH 5.8. The sample concentration was 0.25 μg/ml. Binding was carried out in 10 mM MES (pH 7.4), 150 mM NaCl, and 0.05% Tween 20, and dissociation was carried out in 10 mM MES (pH 5.8), 150 mM NaCl, and 0.05% Tween 20. Likewise, the dissociation rate constant (kd (1/s)) at pH 7.4 was calculated using Biacore T100 Evaluation Software (GE Healthcare) by fitting only the dissociation phase at pH 7.4. The sample concentration was 0.5 μg/ml. Binding was carried out in 10 mM MES (pH 7.4), 150 mM NaCl, and 0.05% Tween 20, and dissociation was carried out in 10 mM MES (pH 7.4), 150 mM NaCl, and 0.05% Tween 20.

Assessment of the binding to human FcRn

**[0166]** 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 a mammalian cell expression vector (the amino acid sequence of human FcRn as set forth in SEQ ID NO: 79). 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 a mammalian cell expression vector (the amino acid sequence of human β2-microglobulin as

set forth in SEQ ID NO: 80).

**[0167]** 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% FBS (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).

Determination of antibody concentration in mouse plasma

**[0168]** Antibody concentrations in mouse plasma were determined by ELISA according to a method known to those skilled in the art.

PK/PD test to determine the antibody concentration in the plasma, CRP concentration, and free soluble IL-6 receptor in monkeys

**[0169]** The plasma concentrations in cynomolgus monkeys were determined by ELISA using a method known to those skilled in the art.

**[0170]** The concentration of CRP was determined with an automated analyzer (TBA-120FR; Toshiba Medical Systems Co.) using Cias R CRP (KANTO CHEMICAL CO., INC.).

**[0171]** The plasma concentration of free soluble cynomolgus monkey IL-6 receptor in cynomolgus monkeys was determined by the procedure described below. All IgG-type 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. The percentage of free soluble IL-6 receptor was calculated by the following formula.

$$\frac{\text{Free soluble IL-6 receptor concentration after antibody administration}}{\text{Soluble IL-6 receptor concentration before antibody administration}} \times 100$$

SEQUENCE LISTING

**[0172]**

<110> CHUGAI SEIYAKU KABUSHIKI KAISHA

<120> Improved antibody molecules

<130> C1-A0805Y2P

<150> JP 2008-248213
<151> 2008-09-26

<150> JP 2009-60806
<151> 2009-03-13

<150> JP 2009-67925
<151> 2009-03-19

<160> 117

<170> PatentIn version 3.4

<210> 1
<211> 6
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 1

**His Asp His Ala Trp Ser**
**1 5**

<210> 2
<211> 16
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 2

**Phe Ile Ser Tyr Ser Gly Ile Thr Asn Tyr Asn Pro Thr Leu Gln Gly**
**1 5 10 15**

<210> 3
<211> 10
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 3

**Ser Leu Ala Arg Thr Thr Ala Met Asp Tyr**
**1 5 10**

<210> 4
<211> 6
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 4

**His Asp His Ala Trp Ser**
**1 5**

<210> 5
<211> 16
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 5

Phe Ile Ser Tyr Ser Gly Ile Thr Asn Tyr Asn Pro Ser Leu Gln Gly
1 5 10 15

<210> 6
<211> 10
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 6

Ser Leu Ala Arg Thr Thr Ala Met Asp Tyr
1 5 10

<210> 7
<211> 6
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 7

Asp Asp His Ala Val Ser
1 5

<210> 8
<211> 16
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 8

Phe Ile Ser Tyr Ser Gly Ile Thr Asn Tyr Asn Pro Thr Leu Gln Asp
1 5 10 15

<210> 9
<211> 10
<212> PRT
<213> Artificial

<220>

<223> An artificially synthesized polypeptide sequence

<400> 9

**Leu Leu Ala Arg Ala Thr Ala Met Asp Val**
**1 5 10**

<210> 10
<211> 11
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 10

**Gln Ala Ser Arg Asp Ile Ser Ser His Leu Asn**
**1 5 10**

<210> 11
<211> 7
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 11

**Tyr Gly Ser His Leu Leu Ser**
**1 5**

<210> 12
<211> 9
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 12

**Gly Gln Gly Asn Arg Leu Pro Tyr Thr**
**1 5**

<210> 13
<211> 11
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 13

**Gln Ala Ser Thr Asp Ile Ser Ser His Leu Asn**
**1 5 10**

<210> 14
<211> 7
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 14

**Tyr Gly Ser His Leu Leu Ser**
**1 5**

<210> 15
<211> 9
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 15

**Gly Gln Gly Asn Arg Leu Pro Tyr Thr**
**1 5**

<210> 16
<211> 11
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 16

**Gln Ala Ser Gln Asp Ile Ser Ser Tyr Leu Asn**
**1 5 10**

<210> 17
<211> 7
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 17

**Tyr Gly Ser Glu Leu Glu Ser**
**1 5**

<210> 18
<211> 9
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 18

```
Gly Gln Gly Asn Arg Leu Pro Tyr Thr
1               5
```

<210> 19
<211> 119
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 19

```
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> 20
<211> 119
<212> PRT

<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 20

```
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> 21
<211> 119
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 21

```
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> 22
<211> 107
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 22

```
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> 23
<211> 107
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 23

```
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> 24
<211> 107
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 24

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

<210> 25
<211> 443
<212> PRT

<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 25

```
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> 26
<211> 443
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 26

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> 27
<211> 447
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 27

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> 28
<211> 214
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 28

```
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> 29
<211> 214
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 29

```
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> 30
<211> 214

<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 30

```
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> 31
<211> 324
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 31

```
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> 32
<211> 324
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 32

```
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> 33
<211> 328
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 33

```
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> 34
<211> 107
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 34

```
Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
1               5                  10                  15

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
            20                  25                  30

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
        35                  40                  45

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
    50                  55                  60

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
65                  70                  75                  80

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
                85                  90                  95

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
            100                 105
```

<210> 35
<211> 107
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 35

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
1 5 10 15

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
20 25 30

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
35 40 45

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
50 55 60

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
65 70 75 80

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
85 90 95

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
100 105

<210> 36
<211> 107
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 36

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
1 5 10 15

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
20 25 30

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
35 40 45

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
50 55 60

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
65 70 75 80

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
85 90 95

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
100 105

<210> 37
<211> 327
<212> DNA
<213> Homo sapiens

<400> 37

cgtacggtgg ctgcaccatc tgtcttcatc ttcccgccat ctgatgagca gttgaaatct 60

ggaactgcct ctgttgtgtg cctgctgaat aacttctatc ccagagaggc caaagtacag 120

tggaaggtgg ataacgccct ccaatcgggt aactcccagg agagtgtcac agagcaggac 180

agcaaggaca gcacctacag cctcagcagc accctgacgc tgagcaaagc agactacgag 240

aaacacaaag tctacgcctg cgaagtcacc catcagggcc tgagctcgcc cgtcacaaag 300

agcttcaaca ggggagagtg ttgataa 327

<210> 38
<211> 107
<212> PRT
<213> Homo sapiens

<400> 38

```
Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
1               5                  10                  15

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
            20                  25                  30

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
        35                  40                  45

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
    50                  55                  60

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
65                  70                  75                  80

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
                85                  90                  95

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
            100                 105
```

<210> 39
<211> 990
<212> DNA
<213> Homo sapiens

<400> 39

gctagcacca agggcccatc ggtcttcccc ctggcaccct cctccaagag cacctctggg 60

ggcacagcgg ccctgggctg cctggtcaag gactacttcc ccgaaccggt gacggtgtcg 120

tggaactcag gcgccctgac cagcggcgtg cacaccttcc cggctgtcct acagtcctca 180

ggactctact ccctcagcag cgtggtgacc gtgccctcca gcagcttggg cacccagacc 240

tacatctgca acgtgaatca caagcccagc aacaccaagg tggacaagaa agttgagccc 300

aaatcttgtg acaaaactca cacatgccca ccgtgcccag cacctgaact cctgggggga 360

ccgtcagtct tcctcttccc cccaaaaccc aaggacaccc tcatgatctc ccggacccct 420

gaggtcacat gcgtggtggt ggacgtgagc cacgaagacc ctgaggtcaa gttcaactgg 480

tacgtggacg gcgtggaggt gcataatgcc aagacaaagc cgcgggagga gcagtacaac 540

agcacgtacc gtgtggtcag cgtcctcacc gtcctgcacc aggactggct gaatggcaag 600

gagtacaagt gcaaggtctc caacaaagcc ctcccagccc ccatcgagaa aaccatctcc 660

aaagccaaag ggcagccccg agaaccacag gtgtacaccc tgccccatc ccgggatgag 720

ctgaccaaga accaggtcag cctgacctgc ctggtcaaag gcttctatcc cagcgacatc 780

gccgtggagt gggagagcaa tgggcagccg gagaacaact acaagaccac gcctcccgtg 840

ctggactccg acggctcctt cttcctctac agcaagctca ccgtggacaa gagcaggtgg 900

cagcagggga acgtcttctc atgctccgtg atgcatgagg ctctgcacaa ccactacacg 960

cagaagagcc tctccctgtc tccgggtaaa 990

<210> 40
<211> 330
<212> PRT
<213> Homo sapiens

<400> 40

```
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 Asn His Tyr Thr
305 310 315 320

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
325 330

<210> 41
<211> 984
<212> DNA
<213> Homo sapiens

<400> 41

gctagcacca agggcccatc ggtcttcccc ctggcgccct cctccaagag cacctccgag 60

agcacagcgg ccctgggctg cctggtcaag gactacttcc ccgaaccggt gacggtgtcg 120

tggaactcag gcgctctgac cagcggcgtg cacaccttcc cggctgtcct acagtcctca 180

ggactctact ccctcagcag cgtggtgacc gtgccctcca gcaacttcgg cacccagacc 240

tacacctgca acgtagatca caagcccagc aacaccaagg tggacaagac agttgagcgc 300

aaatcttgtg tcgagtgccc accgtgccca gcaccacctg tggcaggacc gtcagtcttc 360

ctcttccccc caaaacccaa ggacaccctc atgatctccc ggacccctga ggtcacgtgc 420

gtggtggtgg acgtgagcca cgaagacccc gaggtccagt tcaactggta cgtggacggc 480

gtggaggtgc ataatgccaa gacaaagcca cgggaggagc agttcaacag cacgttccgt 540

gtggtcagcg tcctcaccgt cgtgcaccag gactggctga acggcaagga gtacaagtgc 600

aaggtctcca acaaaggcct cccagccccc atcgagaaaa ccatctccaa aaccaaaggg 660

cagccccgag aaccacaggt gtacaccctg cccccatccc gggaggagat gaccaagaac 720

caggtcagcc tgacctgcct ggtcaaaggc ttctacccca gcgacatcgc cgtggagtgg 780

gagagcaatg ggcagccgga gaacaactac aagaccacac ctcccatgct ggactccgac 840

ggctccttct tcctctacag caagctcacc gtggacaaga gcaggtggca gcaggggaac 900

gtcttctcat gctccgtgat gcatgaggct ctgcacaacc actacacaca gaagagcctc 960

tccctgtctc cgggtaaatg ataa 984

<210> 42
<211> 326
<212> PRT
<213> Homo sapiens

<400> 42

```
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> 43
<211> 995
<212> DNA
<213> Homo sapiens

<400> 43

gctagcacca agggcccatc cgtcttcccc ctggcgccct gctccaggag cacctccgag 60

agcacagccg ccctgggctg cctggtcaag gactacttcc ccgaaccggt gacggtgtcg 120

tggaactcag gcgccctgac cagcggcgtg cacaccttcc cggctgtcct acagtcctca 180

ggactctact ccctcagcag cgtggtgacc gtgccctcca gcagcttggg cacgaagacc 240

tacacctgca acgtagatca caagcccagc aacaccaagg tggacaagag agttgagtcc 300

aaatatggtc ccccatgccc accatgccca gcacctgagt tcctgggggg accatcagtc 360

ttcctgttcc ccccaaaacc caaggacact ctcatgatct cccggacccc tgaggtcacg 420

tgcgtggtgg tggacgtgag ccaggaagac cccgaggtcc agttcaactg gtacgtggat 480

ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagttcaa cagcacgtac 540

cgtgtggtca gcgtcctcac cgtcctgcac caggactggc tgaacggcaa ggagtacaag 600

tgcaaggtct ccaacaaagg cctcccgtcc tccatcgaga aaaccatctc caaagccaaa 660

gggcagcccc gagagccaca ggtgtacacc ctgcccccat cccaggagga gatgaccaag 720

aaccaggtca gcctgacctg cctggtcaaa ggcttctacc ccagcgacat cgccgtggag 780

tgggagagca atgggcagcc ggagaacaac tacaagacca cgcctcccgt gctggactcc 840

gacggctcct tcttcctcta cagcaggcta accgtggaca agagcaggtg gcaggagggg 900

aatgtcttct catgctccgt gatgcatgag gctctgcaca accactacac acagaagagc 960

ctctccctgt ctctgggtta atgataagcg gccgc 995

<210> 44
<211> 326
<212> PRT
<213> Homo sapiens

<400> 44

```
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 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

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
    130                 135                 140

Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp
145                 150                 155                 160

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe
                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
290 295 300

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
305 310 315 320

Leu Ser Leu Ser Leu Gly
325

<210> 45
<211> 4
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 45

Gly Gly Gly Ser
1

<210> 46
<211> 4
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 46

Ser Gly Gly Gly
1

<210> 47
<211> 5
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 47

Gly Gly Gly Gly Ser
1 5

<210> 48
<211> 5
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 48

Ser Gly Gly Gly Gly
1 5

<210> 49
<211> 6
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 49

Gly Gly Gly Gly Gly Ser
1 5

<210> 50
<211> 6
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 50

Ser Gly Gly Gly Gly Gly
1 5

<210> 51
<211> 7
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 51

Gly Gly Gly Gly Gly Gly Ser
1 5

<210> 52
<211> 7
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 52

Ser Gly Gly Gly Gly Gly Gly
1 5

<210> 53
<211> 449
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 53

```
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 Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly**
**435 440 445**

**Lys**

<210> 54
<211> 214
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 54

```
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 Ala Ser Gln Asp Ile Ser Ser Tyr
            20                  25                  30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

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
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

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> 55
<211> 449
<212> PRT
<213> Artificial

<220>

<223> An artificially synthesized polypeptide sequence

<400> 55

```
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 Val Leu Ala Arg Ile 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 Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly**
**435 440 445**

**Lys**

<210> 56
<211> 214
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 56

```
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 Ala Ser Gln Asp Ile Ser Ser Tyr
            20                  25                  30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

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
65                  70                  75                  80

Glu Asp Ile 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 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

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> 57
<211> 449
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 57

```
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 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> 58
<211> 214
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 58

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 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> 59
<211> 7
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 59

Tyr Thr Ser Arg Leu His Ser
1 5

<210> 60
<211> 7
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 60

Tyr Gly Ser Glu Leu His Ser
1 5

<210> 61
<211> 30
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 61

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
20 25 30

<210> 62
<211> 30
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 62

**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**

<210> 63
<211> 32
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 63

**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 Val Tyr Tyr Cys Ala Arg**
**20 25 30**

<210> 64
<211> 32
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 64

**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> 65
<211> 30
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 65

**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> 66
<211> 449
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 66

```
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 His 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 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> 67
<211> 214
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 67

```
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 His 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 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 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> 68
<211> 448

<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 68

```
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 Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
        435                 440                 445
```

<210> 69
<211> 447
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 69

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 Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
        435                 440                 445
```

<210> 70
<211> 445
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 70

```
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> 71
<211> 445
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 71

```
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 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> 72
<211> 443
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 72

```
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> 73
<211> 449
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 73

```
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> 74
<211> 447
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 74

```
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> 75
<211> 443
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 75

```
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> 76
<211> 449
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 76

```
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> 77
<211> 446
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 77

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Arg
1               5                  10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Arg Phe Thr Phe Asp Asp Tyr
            20                  25                  30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ser Gly Ile Ser Trp Asn Ser Gly Arg Ile Gly Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Glu Asn Ser Leu Phe
65                  70                  75                  80

Leu Gln Met Asn Gly Leu Arg Ala Glu Asp Thr Ala Leu Tyr Tyr Cys
                85                  90                  95

Ala Lys Gly Arg Asp Ser Phe Asp Ile Trp Gly Gln Gly Thr Met Val
            100                 105                 110

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
        115                 120                 125

Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu
    130                 135                 140

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
145                 150                 155                 160

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
                165                 170                 175

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu
            180                 185                 190

Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr
        195                 200                 205

Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr
    210                 215                 220

Cys Pro Pro Cys Pro Ala Pro Glu Leu 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 His Glu Asp Pro Glu Val
            260                 265                 270

Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
        275                 280                 285

Lys Pro Arg Glu Glu Gln Tyr 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 Ala Leu Pro Ala Pro 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 Arg Asp Glu Leu 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 Gln 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 Pro Gly Lys
        435                 440                 445
```

<210> 78
<211> 214
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 78

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Val Ser Ala Ser Val Gly

1               5                  10                  15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Trp
            20                  25                  30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Gly Ala Ser Ser Leu Glu Ser 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 Ser Tyr Tyr Cys Gln Gln Ala Asn Ser Phe Pro Tyr
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Leu 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

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> 79
<211> 267
<212> PRT

<213> Homo sapiens

<400> 79

**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> 80
<211> 99
<212> PRT
<213> Homo sapiens

<400> 80

```
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> 81
<211> 16
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 81

```
Tyr Ile Ser Tyr Ser Gly Ile Thr Thr Tyr Asn Pro Ser Leu Lys Ser
1               5                  10                  15
```

<210> 82
<211> 16
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 82

```
Phe Ile Ser Tyr Ser Gly Ile Thr Thr Tyr Asn Pro Ser Leu Lys Ser
1               5                  10                  15
```

<210> 83
<211> 16
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 83

**Tyr Ile Ser Tyr Ser Gly Ile Thr Asn Tyr Asn Pro Ser Leu Lys Ser**
**1 5 10 15**

<210> 84
<211> 10
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 84

**Ser Leu Ala Arg Thr Thr Ala Met Asp Tyr**
**1 5 10**

<210> 85
<211> 10
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 85

**Leu Leu Ala Arg Thr Thr Ala Met Asp Tyr**
**1 5 10**

<210> 86
<211> 10
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 86

**Ser Leu Ala Arg Ala Thr Ala Met Asp Tyr**
**1 5 10**

<210> 87
<211> 11
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 87

**Arg Ala Ser Gln Asp Ile Ser Ser Tyr Leu Asn**

**1 5 10**

<210> 88
<211> 11
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 88

**Arg Ala Ser Thr Asp Ile Ser Ser Tyr Leu Asn**
**1 5 10**

<210> 89
<211> 11
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 89

**Arg Ala Ser Arg Asp Ile Ser Ser Tyr Leu Asn**
**1 5 10**

<210> 90
<211> 9
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 90

**Gln Gln Gly Asn Thr Leu Pro Tyr Thr**
**1 5**

<210> 91
<211> 9
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 91

Gly Gln Gly Asn Thr Leu Pro Tyr Thr
1 5

<210> 92
<211> 9
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 92

Gln Gln Gly Asn Arg Leu Pro Tyr Thr
1 5

<210> 93
<211> 30
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 93

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
20 25 30

<210> 94
<211> 30
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 94

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

<210> 95
<211> 6
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 95

**Ser Asp His Ala Trp Ser**
**1 5**

<210> 96
<211> 6
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 96

**Asp Asp His Ala Trp Ser**
**1 5**

<210> 97
<211> 14
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 97

**Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile Gly**
**1 5 10**

<210> 98
<211> 14
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 98

**Trp Val Arg Gln Pro Pro Gly Glu Gly Leu Glu Trp Ile Gly**
**1 5 10**

<210> 99
<211> 16
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 99

**Tyr Ile Ser Tyr Ser Gly Ile Thr Thr Tyr Asn Pro Ser Leu Gln Asp**
**1 5 10 15**

<210> 100
<211> 11
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 100

**Trp Gly Gln Gly Ser Leu Val Thr Val Ser Ser**
**1 5 10**

<210> 101
<211> 11
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 101

**Trp Gly Glu Gly Thr Leu Val Thr Val Ser Ser**
**1 5 10**

<210> 102
<211> 23
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 102

**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**
**20**

<210> 103
<211> 23
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 103

**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**
**20**

<210> 104
<211> 11
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 104

**Gln Ala Ser Gln Asp Ile Ser Ser Tyr Leu Asn**
**1 5 10**

<210> 105
<211> 15
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 105

**Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr**
**1 5 10 15**

<210> 106
<211> 15
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 106

**Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Glu Leu Leu Ile Tyr**
**1 5 10 15**

<210> 107
<211> 7
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 107

**Tyr Thr Ser Arg Leu His Ser**
**1 5**

<210> 108
<211> 7
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 108

**Tyr Thr Ser Glu Leu Glu Ser**
**1 5**

<210> 109
<211> 7
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 109

**Tyr Thr Ser Arg Leu Leu Ser**
**1 5**

<210> 110
<211> 32
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 110

**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 Ile Ala Thr Tyr Tyr Cys**
**20 25 30**

<210> 111
<211> 32
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 111

**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> 112
<211> 10
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 112

**Phe Gly Gln Gly Thr Lys Val Glu Ile Lys**
**1 5 10**

<210> 113
<211> 10
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 113

**Phe Gly Gln Gly Thr Lys Val Glu Ile Glu**
**1 5 10**

<210> 114
<211> 30
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide 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 His Ser Ile Thr**
**20 25 30**

<210> 115
<211> 6
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 115

**His Asp His Ala Trp Ser**
**1 5**

<210> 116
<211> 11
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 116

**Arg Ala Ser Gln Asp Ile Ser Ser His Leu Asn**
**1 5 10**

<210> 117
<211> 7
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized polypeptide sequence

<400> 117

**Tyr Thr Ser His Leu His Ser**
**1 5**

## Claims

1. An anti-IL-6 receptor antibody of any one of:

   (a) an antibody that comprises a heavy chain variable region comprising the sequence of SEQ ID NO: 20 (variable region of VH3-M73) and a light chain variable region comprising the sequence of SEQ ID NO: 23 (variable region of VL3);
   (b) an antibody that comprises a heavy chain variable region comprising the sequence of SEQ ID NO: 19 (variable region of VH4-M73) and a light chain variable region comprising the sequence of SEQ ID NO: 22 (variable region of VL1); and
   (c) an antibody that comprises a heavy chain variable region comprising the sequence of SEQ ID NO: 21 (variable region of VH5-M83) and a light chain variable region comprising the sequence of SEQ ID NO: 24 (variable region of VL5).

2. An anti-IL-6 receptor antibody of any one of:

   (a) an antibody that comprises a heavy chain comprising the sequence of SEQ ID NO: 26 (VH3-M73) and a light chain comprising the sequence of SEQ ID NO: 29 (VL3);
   (b) an antibody that comprises a heavy chain comprising the sequence of SEQ ID NO: 25 (VH4-M73) and a light chain comprising the sequence of SEQ ID NO: 28 (VL1); and
   (c) an antibody that comprises a heavy chain comprising the sequence of SEQ ID NO: 27 (VH5-M83) and a light chain comprising the sequence of SEQ ID NO: 30 (VL5).

3. A gene encoding the antibody of claim 1 or 2.

4. A vector carrying the gene of claim 3.

5. A host cell carrying the vector of claim 4.

6. A method for producing the antibody of claim 1 or 2 by culturing the host cell of claim 5.

7. A pharmaceutical composition comprising the antibody of claim 1 or 2 or an antibody produced by the method of claim 6.

**Patentansprüche**

1. Anti-IL6-Rezeptor-Antikörper nach einem der folgenden:

(a) ein Antikörper, umfassend eine variable Region der schweren Kette, die die Sequenz von SEQ ID NO:20 (variable Region von VH3-M73) umfasst und eine variable Region der leichten Kette, die die Sequenz von SEQ ID NO:23 (variable Region von VL3) umfasst;
(b) ein Antikörper, umfassend eine variable Region der schweren Kette, die die Sequenz von SEQ ID NO:19 (variable Region von VH4-M73) umfasst und eine variable Region der leichten Kette, die die Sequenz von SEQ ID NO:22 (variable Region von VL1) umfasst; und
(c) ein Antikörper, umfassend eine variable Region der schweren Kette, die die Sequenz von SEQ ID NO:21 (variable Region von VH5-M83) umfasst und eine variable Region der leichten Kette, die die Sequenz von SEQ ID NO:24 (variable Region von VL5) umfasst.

2. Anti-IL6-Rezeptor-Antikörper nach einem der folgenden:

(a) ein Antikörper, umfassend eine schwere Kette, die die Sequenz von SEQ ID NO:26 (VH3-M73) umfasst und eine leichte Kette, die die Sequenz von SEQ ID NO:29 (VL3) umfasst;
(b) ein Antikörper, umfassend eine schwere Kette, die die Sequenz von SEQ ID NO:25 (VH4-M73) umfasst und eine leichte Kette, die die Sequenz von SEQ ID NO:28 (VL1) umfasst; und
(c) ein Antikörper, umfassend eine schwere Kette, die die Sequenz von SEQ ID NO:27 (VH5-M83) umfasst und eine leichte Kette, die die Sequenz von SEQ ID NO:30 (VL5) umfasst.

3. Gen, das den Antikörper nach Anspruch 1 oder 2 codiert.

4. Vektor, der das Gen nach Anspruch 3 trägt.

5. Wirtszelle, die den Vektor nach Anspruch 4 trägt.

6. Verfahren zur Herstellung des Antikörpers nach Anspruch 1 oder 2 durch Züchten der Wirtszelle nach Anspruch 5.

7. Pharmazeutische Zusammensetzung, die den Antikörper nach Anspruch 1 oder 2 oder einen durch das Verfahren nach Anspruch 6 hergestellten Antikörper umfasst.

**Revendications**

1. Anticorps anti-récepteur de l'IL-6 parmi l'un quelconque de :

(a) un anticorps qui comprend une région variable de chaîne lourde comprenant la séquence de SEQ ID NO : 20 (région variable de VH3-M73) et une région variable de chaîne légère comprenant la séquence de SEQ ID NO : 23 (région variable de VL3) ;
(b) un anticorps qui comprend une région variable de chaîne lourde comprenant la séquence de SEQ ID NO : 19 (région variable de VH4-M73) et une région variable de chaîne légère comprenant la séquence de SEQ ID NO : 22 (région variable de VL1) ; et
(c) un anticorps qui comprend une région variable de chaîne lourde comprenant la séquence de SEQ ID NO : 21 (région variable de VH5-M83) et une région variable de chaîne légère comprenant la séquence de SEQ ID NO : 24 (région variable de VL5).

2. Anticorps anti-récepteur de l'IL-6 parmi l'un quelconque de :

(a) un anticorps qui comprend une chaîne lourde comprenant la séquence de SEQ ID NO : 26 (VH3-M73) et une chaîne légère comprenant la séquence de SEQ ID NO : 29 (VL3) ;
(b) un anticorps qui comprend une chaîne lourde comprenant la séquence de SEQ ID NO : 25 (VH4-M73) et une chaîne légère comprenant la séquence de SEQ ID NO : 28 (VL1) ; et
(c) un anticorps qui comprend une chaîne lourde comprenant la séquence de SEQ ID NO : 27 (VH5-M83) et une chaîne légère comprenant la séquence de SEQ ID NO : 30 (VL5).

3. Gène codant pour l'anticorps selon la revendication 1 ou 2.

4. Vecteur portant le gène selon la revendication 3.

5. Cellule hôte portant le vecteur selon la revendication 4.

6. Méthode de production de l'anticorps selon la revendication 1 ou 2 par culture de la cellule hôte selon la revendication 5.

7. Composition pharmaceutique comprenant l'anticorps selon la revendication 1 ou 2 ou anticorps produit par la méthode selon la revendication 6.

| CDR CLASSI-FICATION | TOCILIZUMAB CDR SEQUENCE | MUTATION SITE (Kabat No.) | AMINO ACID OF TOCILIZUMAB | AMINO ACID AFTER MUTATION | CDR SEQUENCE AFTER MUTATION |
|---|---|---|---|---|---|
| HCDR2 | YISYSGITTYNPSLKS | 50 | Y | F | FISYSGITTYNPSLKS (SEQ ID NO: 82) |
| HCDR2 | YISYSGITTYNPSLKS (SEQ ID NO: 81) | 58 | T | N | YISYSGITNYNPSLKS (SEQ ID NO: 83) |
| HCDR3 | SLARTTAMDY | 95 | S | L | LLARTTAMDY (SEQ ID NO: 85) |
| HCDR3 | SLARTTAMDY (SEQ ID NO: 84) | 99 | T | A | SLARATAMDY (SEQ ID NO: 86) |
| LCDR1 | RASQDISSYLN | 27 | Q | T | RASTDISSYLN (SEQ ID NO: 88) |
| LCDR1 | RASQDISSYLN (SEQ ID NO: 87) | 27 | Q | R | RASRDISSYLN (SEQ ID NO: 89) |
| LCDR3 | QQGNTLPYT | 89 | Q | G | GQGNTLPYT (SEQ ID NO: 91) |
| LCDR3 | QQGNTLPYT (SEQ ID NO: 90) | 93 | T | R | QQGNRLPYT (SEQ ID NO: 92) |

FIG. 1

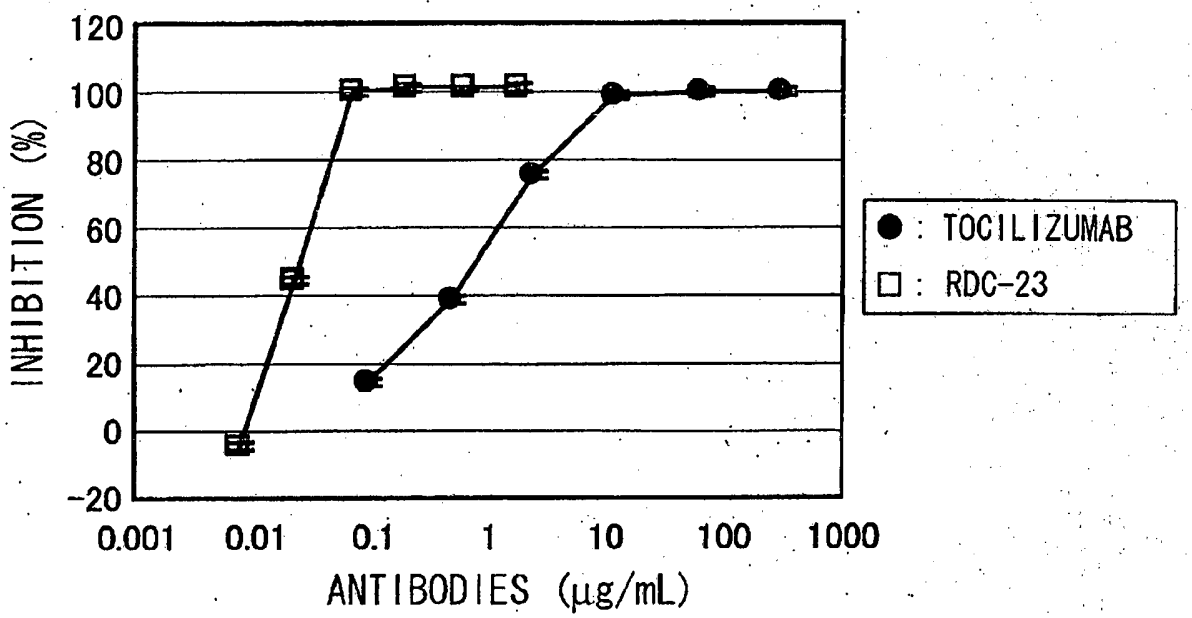
120
100
80
60
40
20
0
-20
INHIBITION (%)
0.001
0.01
0.1
1
10
100
1000
ANTIBODIES (μg/mL)
● : TOCILIZUMAB
□ : RDC-23

FIG. 2

| CLASSIFICATION | TOCILIZUMAB SEQUENCE | MUTATION SITE (Kabat No.) | AMINO ACID OF TOCILIZUMAB | AMINO ACID AFTER MUTATION | SEQUENCE AFTER MUTATION |
|---|---|---|---|---|---|
| HFR1 | QVQLQESGPGLVRPSQTLSLTC TVSGYSIT (SEQ ID NO: 93) | 13 | R | K | QVQLQESGPGLVKPSETLSLTC AVSGYSIS (SEQ ID NO: 94) |
| | | 16 | Q | E | |
| | | 23* | T | A | |
| | | 30* | T | S | |
| HCDR1 | SDHAWS (SEQ ID NO: 95) | 31 | S | D | DDHAWS (SEQ ID NO: 96) |
| HFR2 | WVRQPPGRGLEWIG (SEQ ID NO: 97) | 43 | R | E | WVRQPPGEGLEWIG (SEQ ID NO: 98) |
| HCDR2 | YISYSGITTYNPSLKS (SEQ ID NO: 81) | 64 | K | Q | YISYSGITTYNPSLQD (SEQ ID NO: 99) |
| | | 65 | S | D | |
| HFR4 | WGQGSLVTVSS (SEQ ID NO: 100) | 105 | Q | E | WGEGTLVTVSS (SEQ ID NO: 101) |
| | | 107* | S | T | |
| LFR1 | DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO: 102) | 18 | R | S | DIQMTQSPSSLSASVGDSVTITC (SEQ ID NO: 103) |
| LCDR1 | RASQDISSYLN (SEQ ID NO: 87) | 24 | R | Q | QASQDISSYLN (SEQ ID NO: 104) |
| LFR2 | WYQQKPGKAPKLLIY (SEQ ID NO: 105) | 45 | K | E | WYQQKPGKAPELLIY (SEQ ID NO: 106) |
| LCDR2 | YTSRLHS (SEQ ID NO: 107) | 53 | R | E | YTSELES (SEQ ID NO: 108) |
| | | 55 | H | E | |
| | | 55 | H | L | YTSRLLS (SEQ ID NO: 109) |
| LFR3 | GVPSRFSGSGSGTDFTFTISSLQPE DIATYYC (SEQ ID NO: 110) | 80 | Q | E | GVPSRFSGSGSGTDFTFTISSLEAE DAATYYC (SEQ ID NO: 111) |
| | | 81* | P | A | |
| | | 83* | I | A | |
| LFR4 | FGQGTKVEIK (SEQ ID NO: 112) | 107 | K | E | FGQGTKVEIE (SEQ ID NO: 113) |

FIG. 3

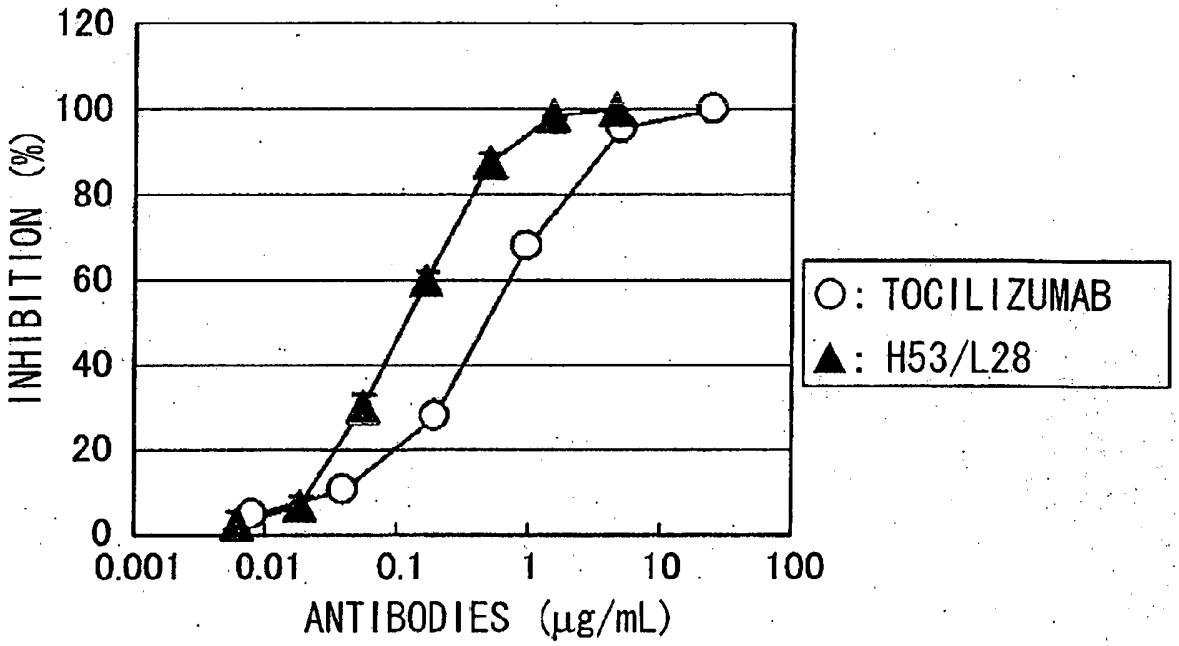
120
100
80
60
40
20
0
INHIBITION (%)
0.001
0.01
0.1
1
10
100
ANTIBODIES (μg/mL)
○: TOCILIZUMAB
▲: H53/L28

FIG. 4

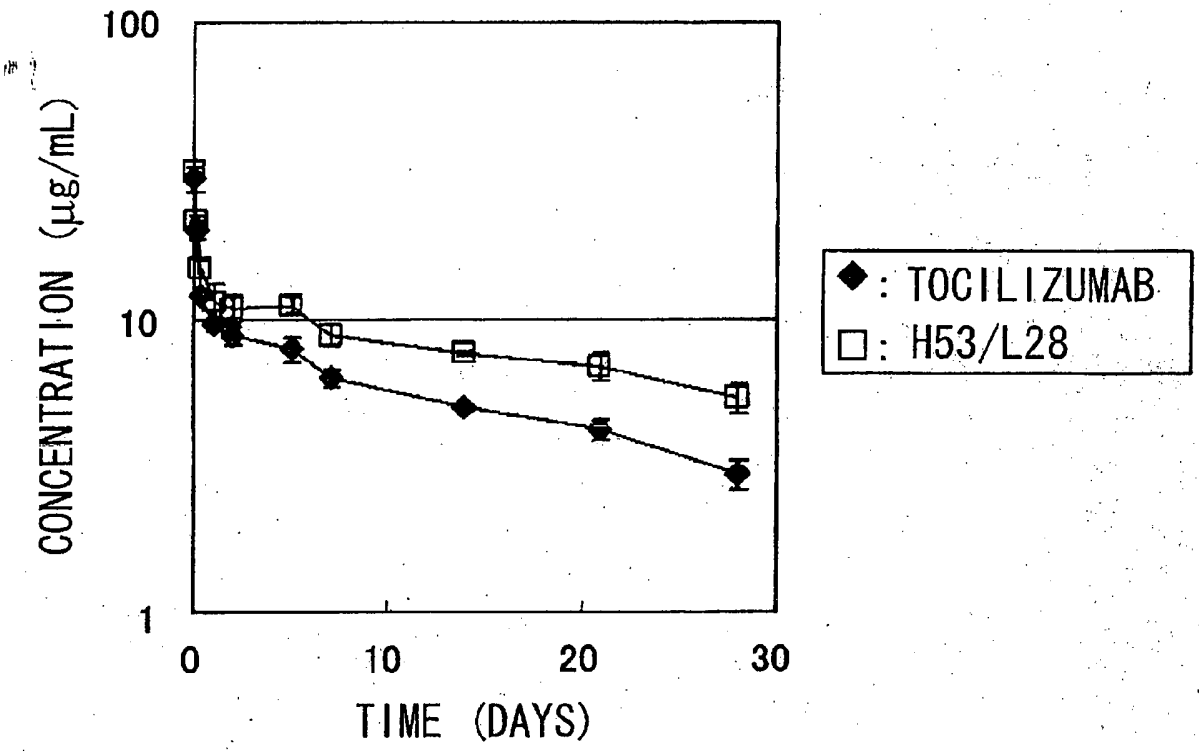
100
10
1
CONCENTRATION (μg/mL)
0
10
20
30
TIME (DAYS)
◆: TOCILIZUMAB
□: H53/L28

FIG. 5

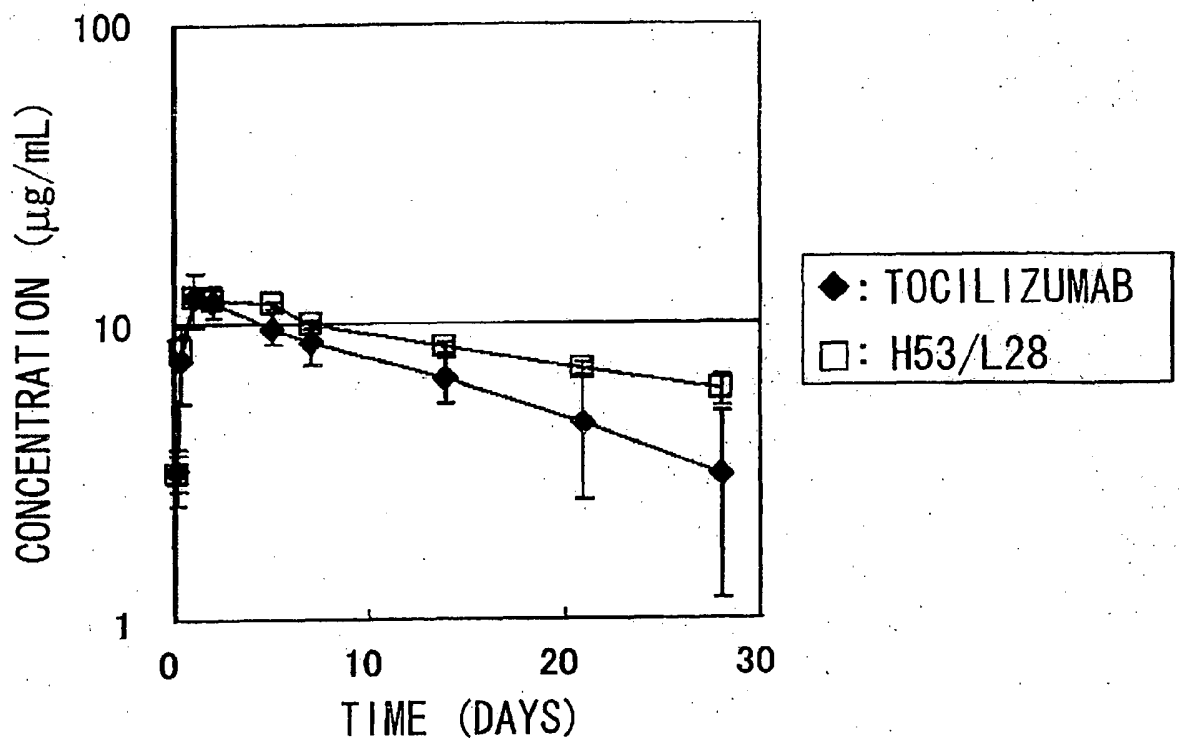
100
10
1
CONCENTRATION (μg/mL)
0
10
20
30
TIME (DAYS)
◆: TOCILIZUMAB
□: H53/L28

FIG. 6

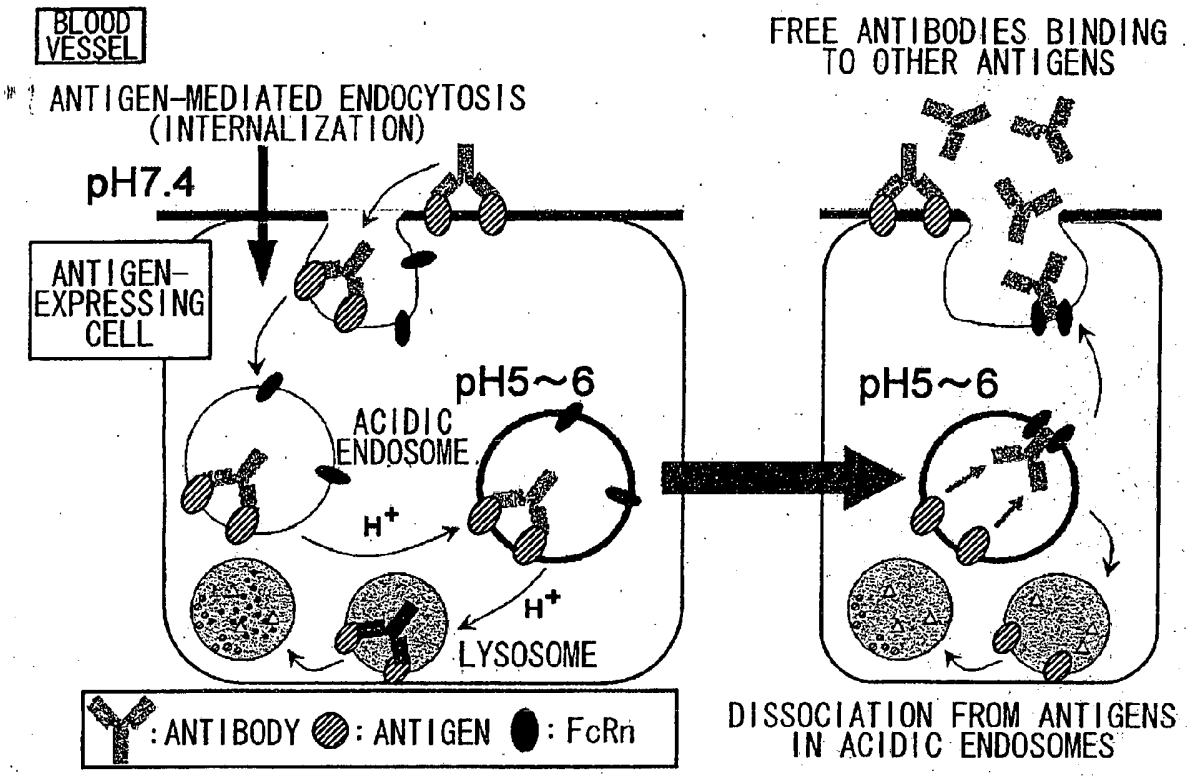
BLOOD VESSEL
ANTIGEN-MEDIATED ENDOCYTOSIS (INTERNALIZATION)
pH7.4
ANTIGEN-EXPRESSING CELL
pH5~6
ACIDIC ENDOSOME
H+
H+
LYSOSOME
:ANTIBODY :ANTIGEN :FcRn
FREE ANTIBODIES BINDING TO OTHER ANTIGENS
pH5~6
DISSOCIATION FROM ANTIGENS IN ACIDIC ENDOSOMES

FIG. 7

| CLASSIFICATION | TOCILIZUMAB SEQUENCE | MUTATION SITE (Kabat No.) | AMINO ACID OF TOCILIZUMAB | AMINO ACID AFTER MUTATION | SEQUENCE AFTER MUTATION |
|---|---|---|---|---|---|
| HFR1 | QVQLQESGPGLVRPSQTLS LTCTVSGYSIT (SEQ ID NO: 93) | 27 | Y | H | QVQLQESGPGLVRPSQTLS LTCTVSGHSIT (SEQ ID NO: 114) |
| HCDR1 | SDHAWS (SEQ ID NO: 95) | 31 | S | H | HDHAWS (SEQ ID NO: 115) |
| LCDR1 | RASQDISSYLN (SEQ ID NO: 87) | 32 | Y | H | RASQDISSHLN (SEQ ID NO: 116) |
| LCDR2 | YTSRLHS (SEQ ID NO: 107) | 53 | R | H | YTSHLHS (SEQ ID NO: 117) |

FIG. 8

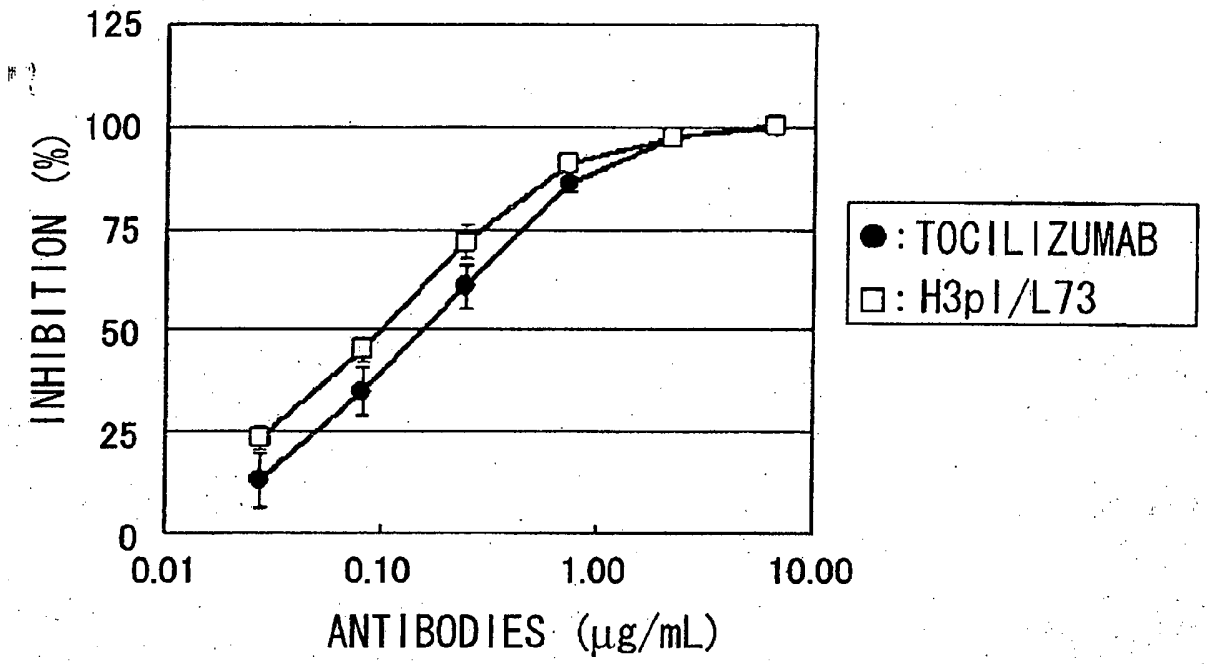
125
100
75
50
25
0
INHIBITION (%)
0.01
0.10
1.00
10.00
ANTIBODIES (μg/mL)
●: TOCILIZUMAB
□: H3pI/L73

FIG. 9

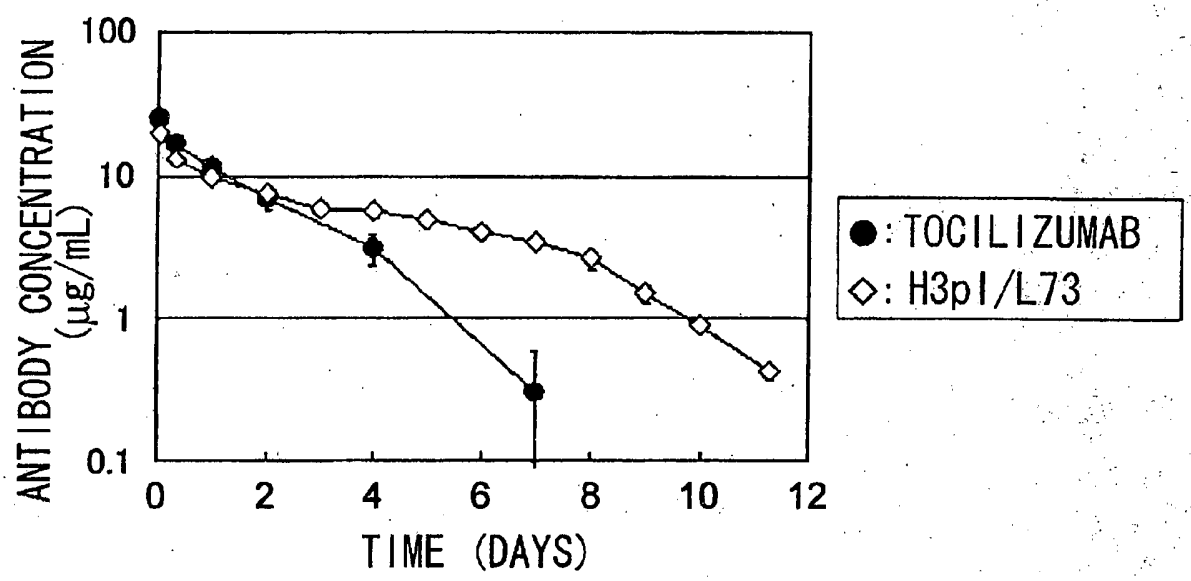
ANTIBODY CONCENTRATION (μg/mL)
100
10
1
0.1
0
2
4
6
8
10
12
TIME (DAYS)
●: TOCILIZUMAB
◇: H3pI/L73

FIG. 10

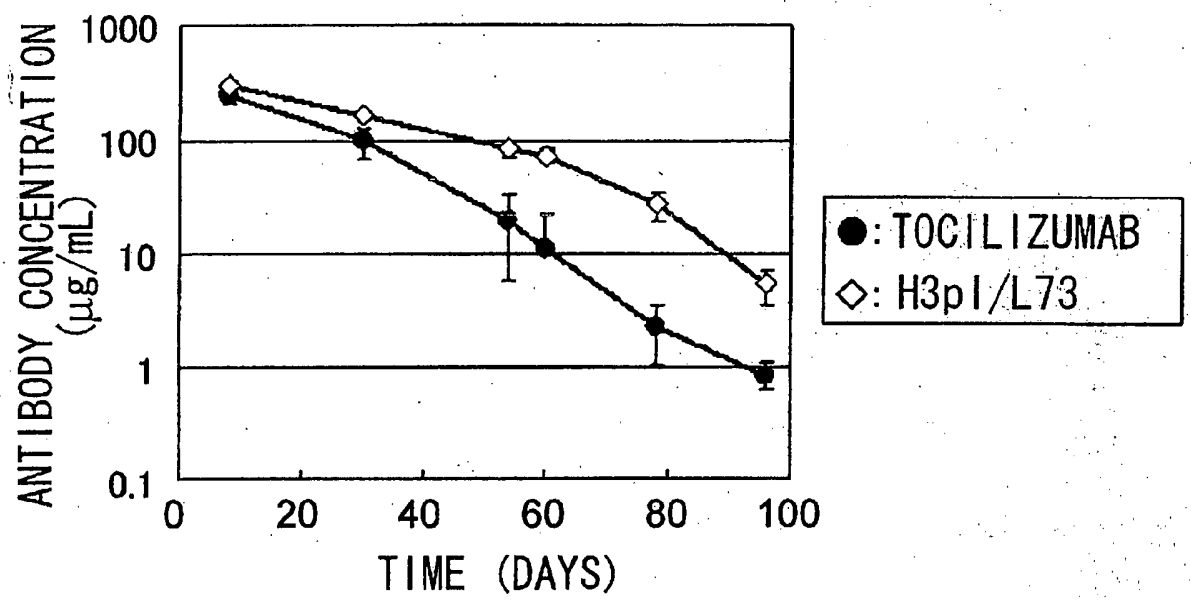
ANTIBODY CONCENTRATION (μg/mL)
1000
100
10
1
0.1
0
20
40
60
80
100
TIME (DAYS)
●: TOCILIZUMAB
◇: H3pI/L73

FIG. 11

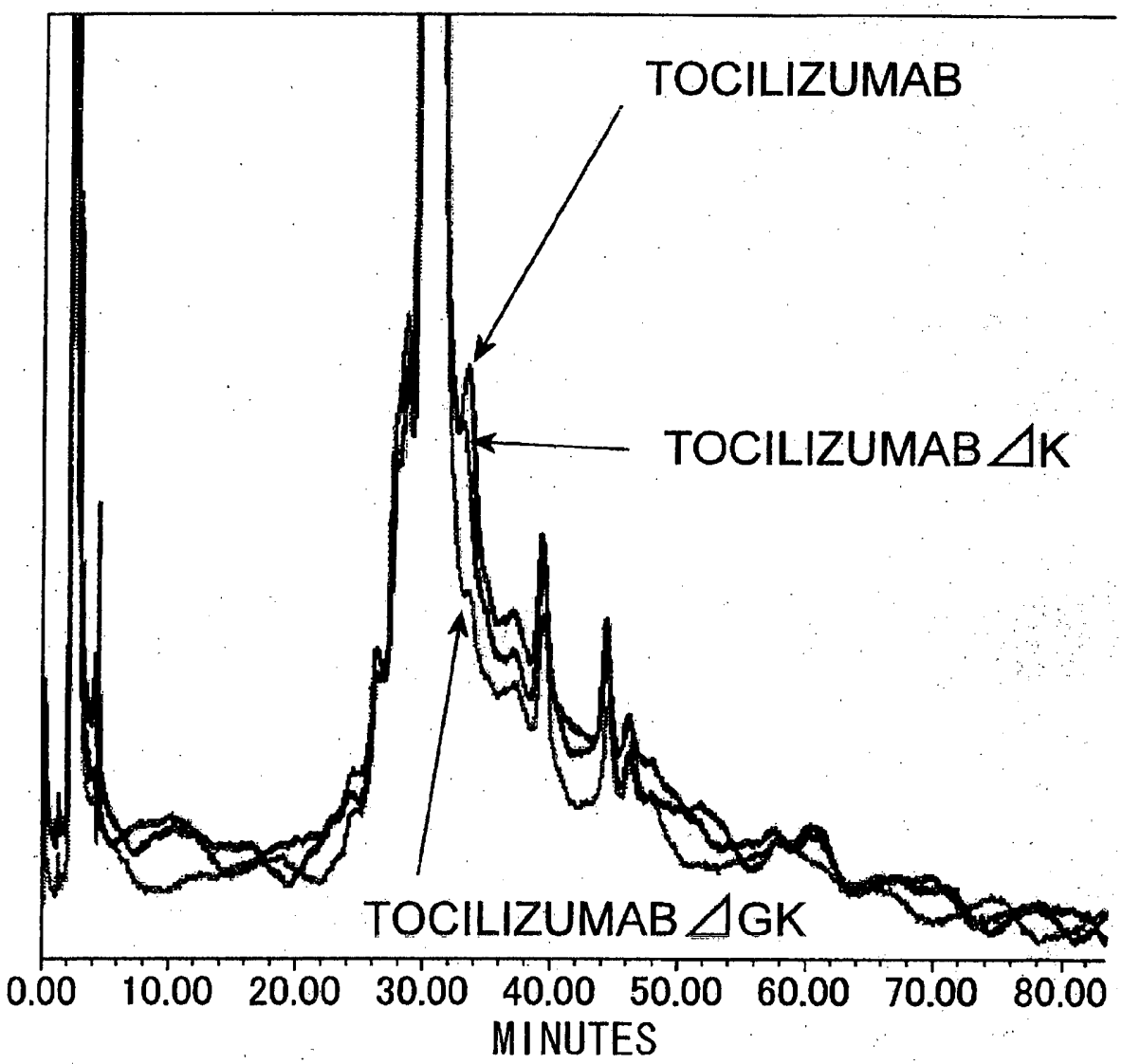
TOCILIZUMAB
TOCILIZUMAB ⊿K
TOCILIZUMAB ⊿GK
0.00
10.00
20.00
30.00
40.00
50.00
60.00
70.00
80.00
MINUTES

FIG. 12

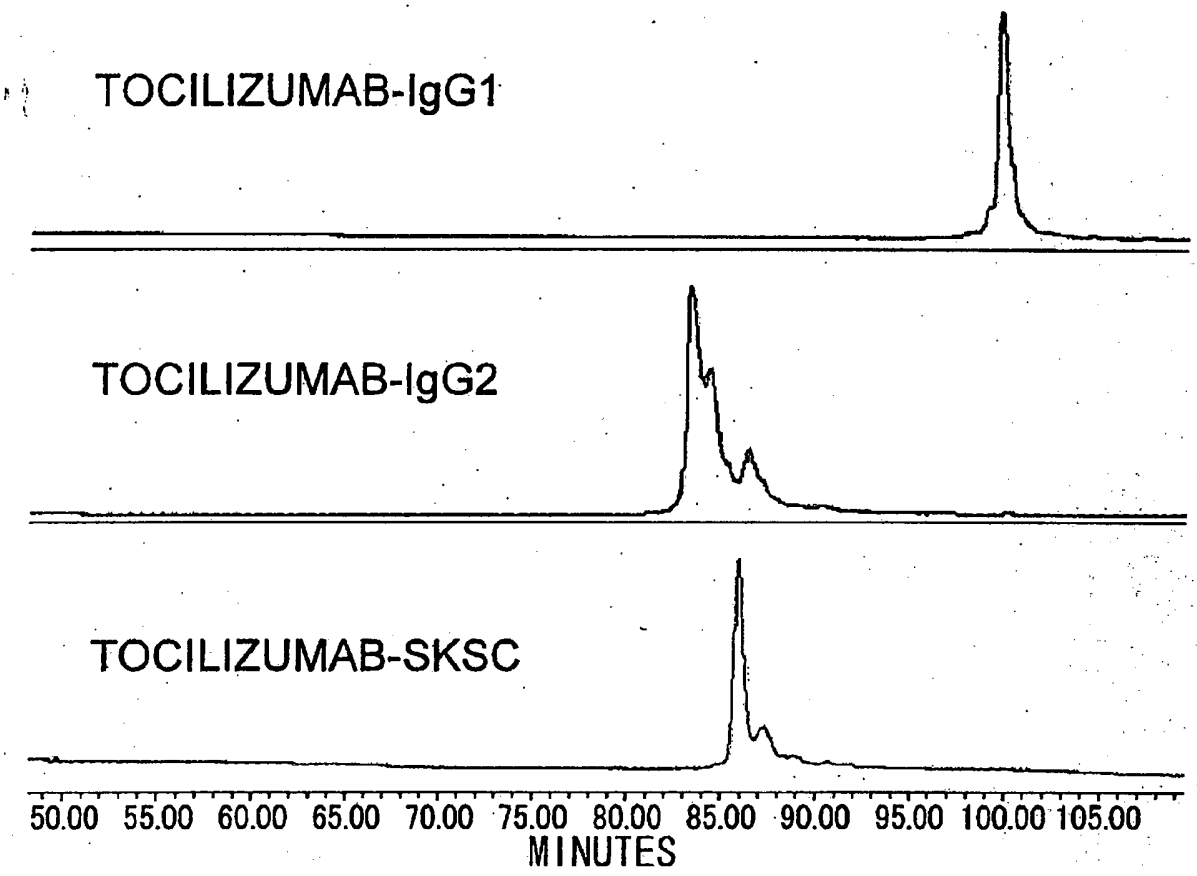
TOCILIZUMAB-IgG1
TOCILIZUMAB-IgG2
TOCILIZUMAB-SKSC
50.00 55.00 60.00 65.00 70.00 75.00 80.00 85.00 90.00 95.00 100.00 105.00
MINUTES

FIG. 13

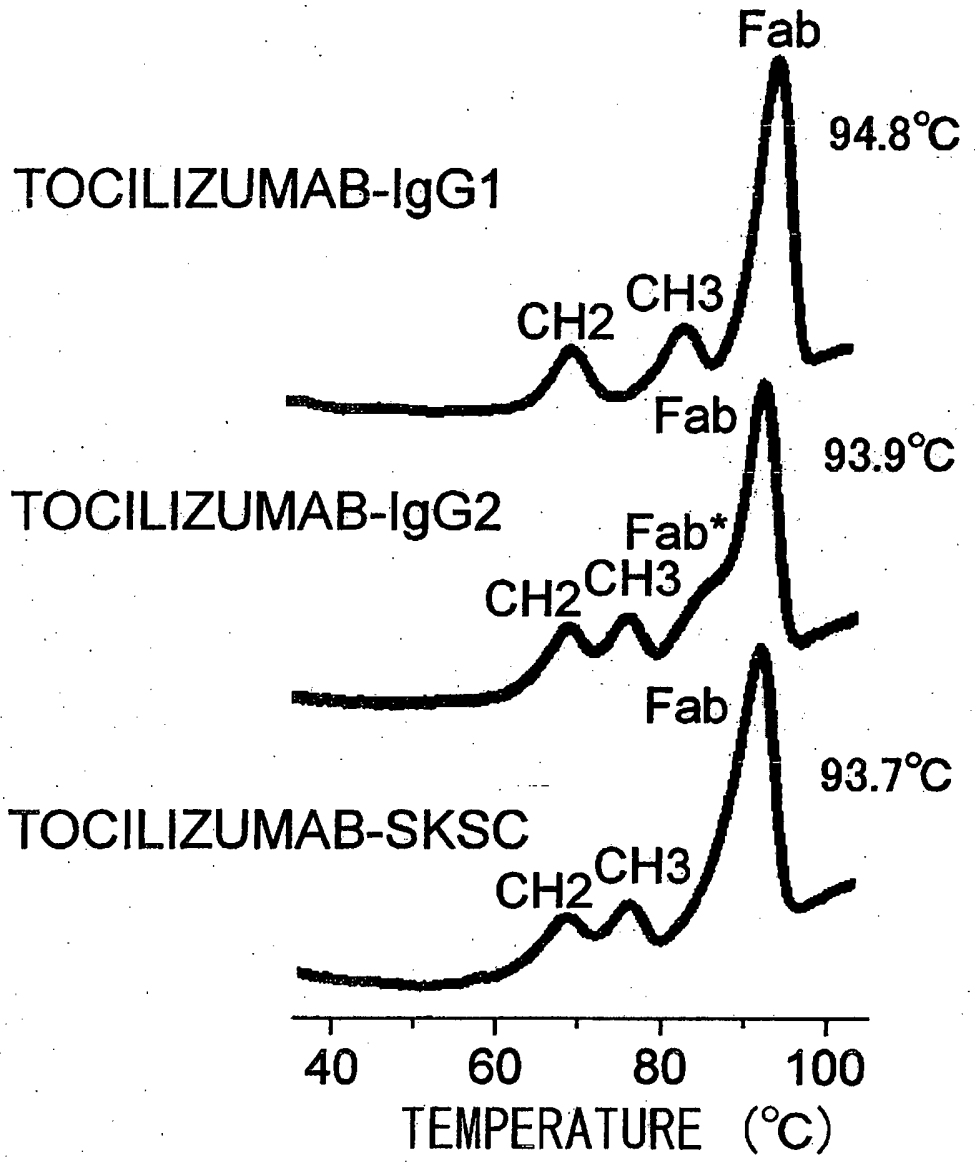
Fab
94.8℃
TOCILIZUMAB-IgG1
CH2
CH3
Fab
93.9℃
TOCILIZUMAB-IgG2
Fab*
CH2
CH3
Fab
93.7℃
TOCILIZUMAB-SKSC
CH2
CH3
40
60
80
100
TEMPERATURE (℃)

FIG. 14

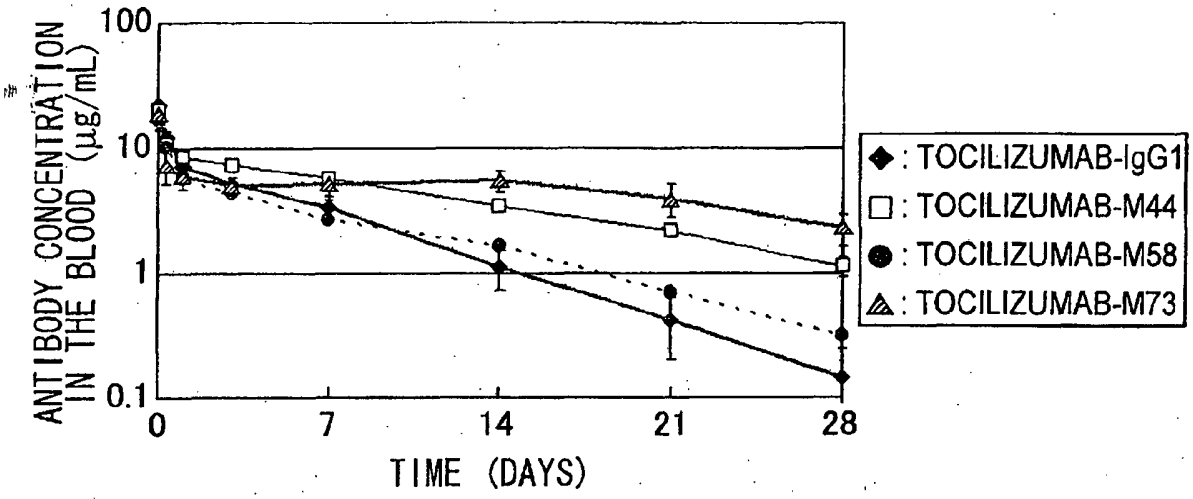
ANTIBODY CONCENTRATION IN THE BLOOD (μg/mL)
100
10
1
0.1
0
7
14
21
28
TIME (DAYS)
◆: TOCILIZUMAB-IgG1
□: TOCILIZUMAB-M44
●: TOCILIZUMAB-M58
△: TOCILIZUMAB-M73

FIG. 15

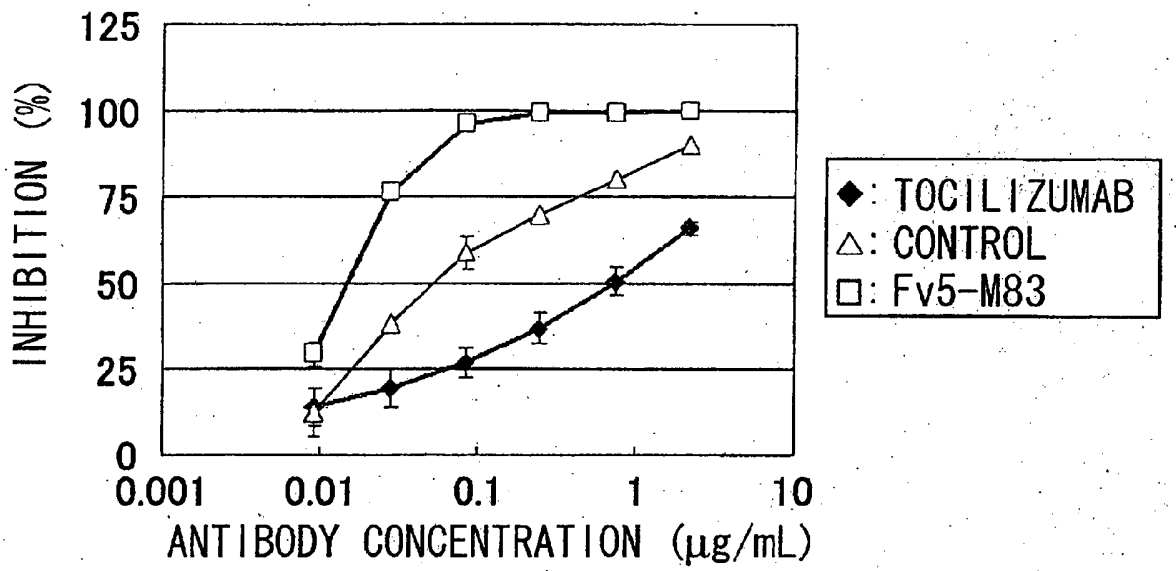
125
100
75
50
25
0
INHIBITION (%)
0.001
0.01
0.1
1
10
ANTIBODY CONCENTRATION (μg/mL)
◆: TOCILIZUMAB
△: CONTROL
□: Fv5-M83

FIG. 16

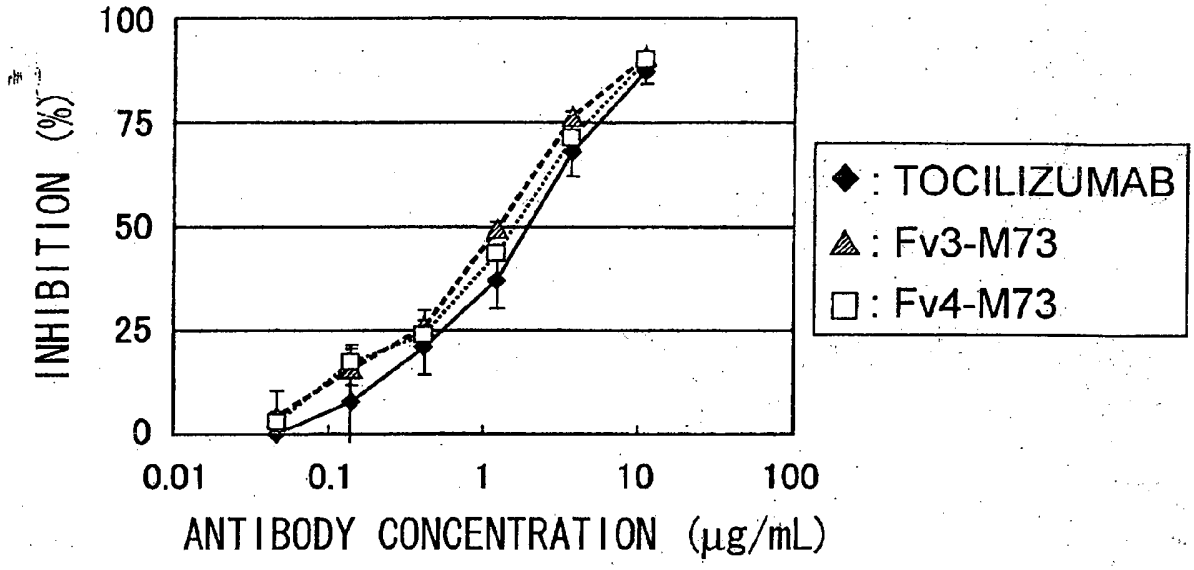
100
75
50
25
0
INHIBITION (%)
0.01
0.1
1
10
100
ANTIBODY CONCENTRATION (μg/mL)
◆ : TOCILIZUMAB
△ : Fv3-M73
□ : Fv4-M73

FIG. 17

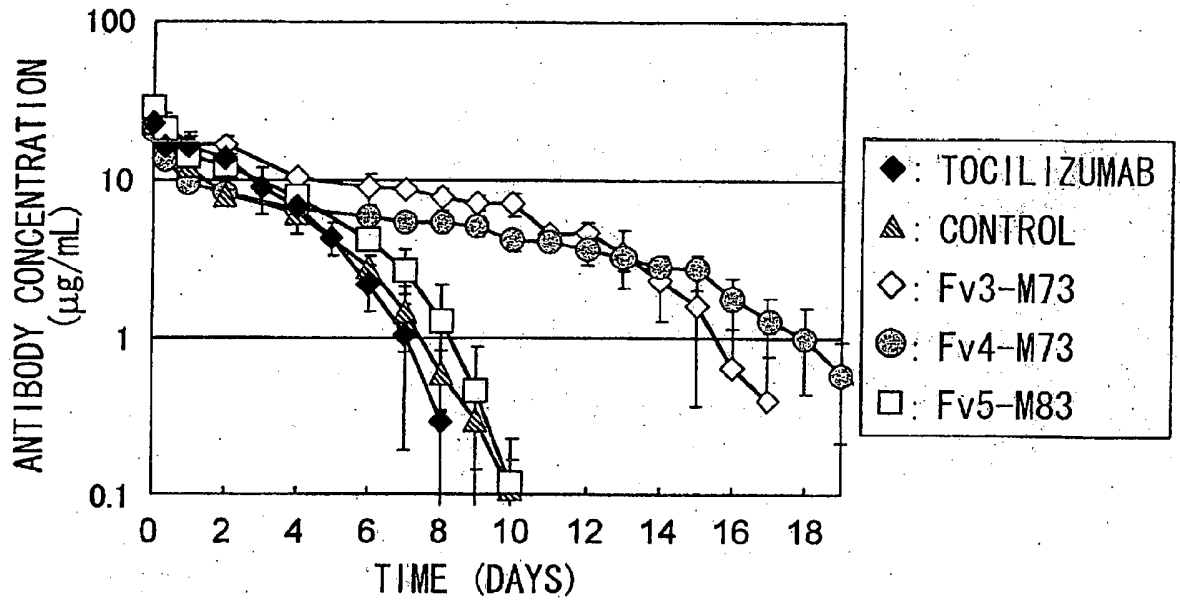
100
10
1
0.1
ANTIBODY CONCENTRATION (μg/mL)
0
2
4
6
8
10
12
14
16
18
TIME (DAYS)
◆: TOCILIZUMAB
△: CONTROL
◇: Fv3-M73
Fv4-M73
□: Fv5-M83

FIG. 18

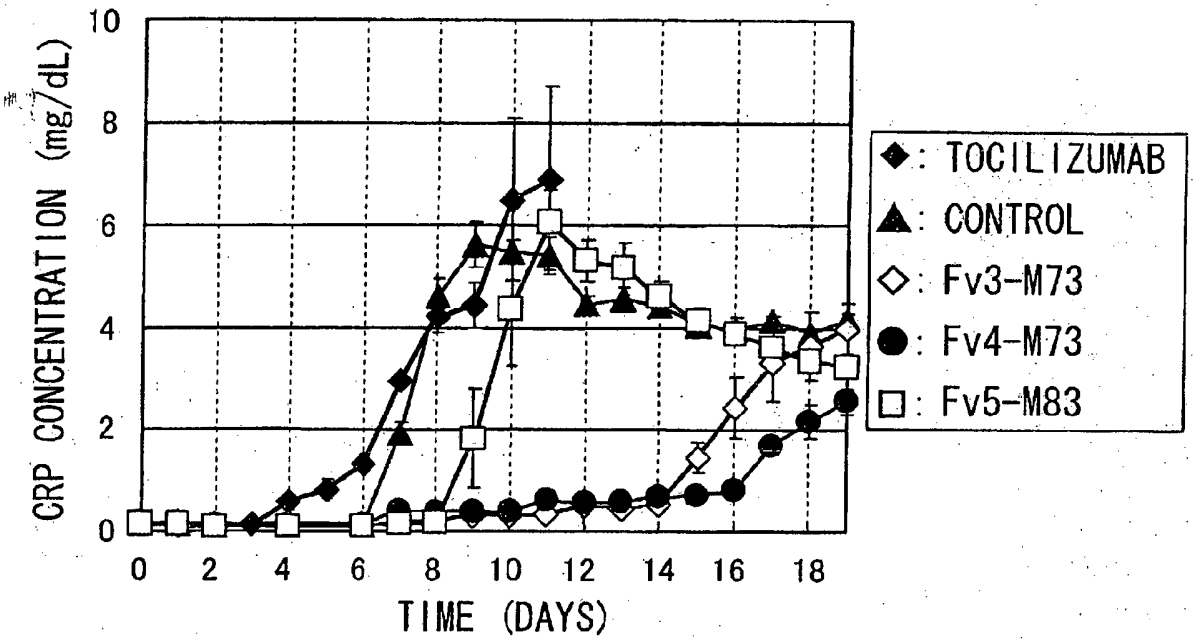
CRP CONCENTRATION (mg/dL)
10
8
6
4
2
0
0
2
4
6
8
10
12
14
16
18
TIME (DAYS)
◆: TOCILIZUMAB
▲: CONTROL
◇: Fv3-M73
●: Fv4-M73
□: Fv5-M83

FIG. 19

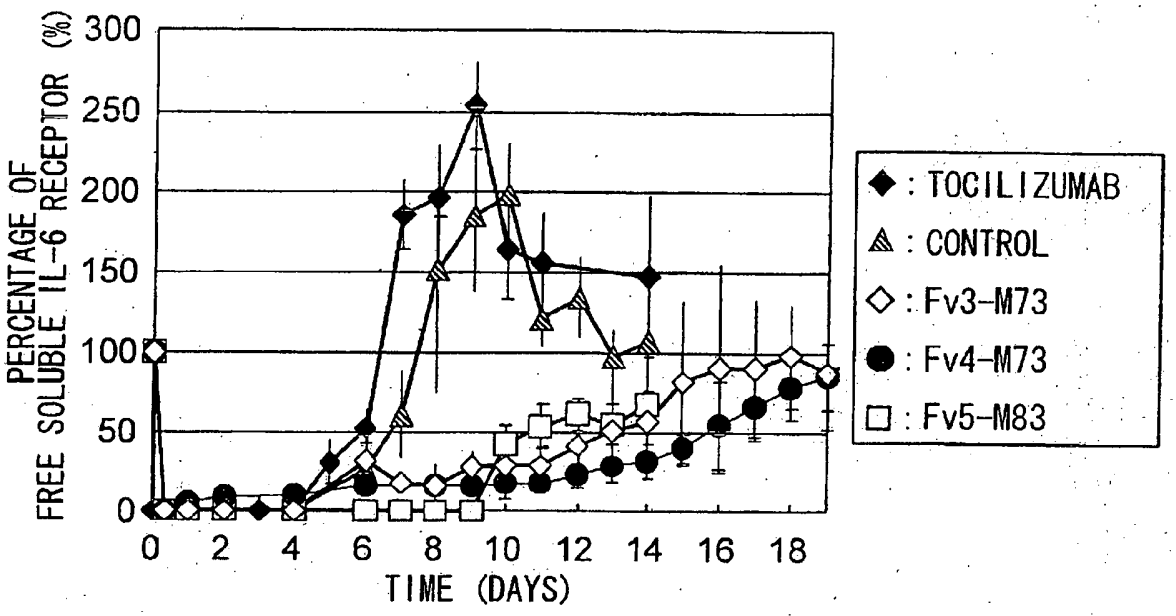
PERCENTAGE OF FREE SOLUBLE IL-6 RECEPTOR (%)
300
250
200
150
100
50
0
0
2
4
6
8
10
12
14
16
18
TIME (DAYS)
: TOCILIZUMAB
: CONTROL
: Fv3-M73
: Fv4-M73
: Fv5-M83

FIG. 20

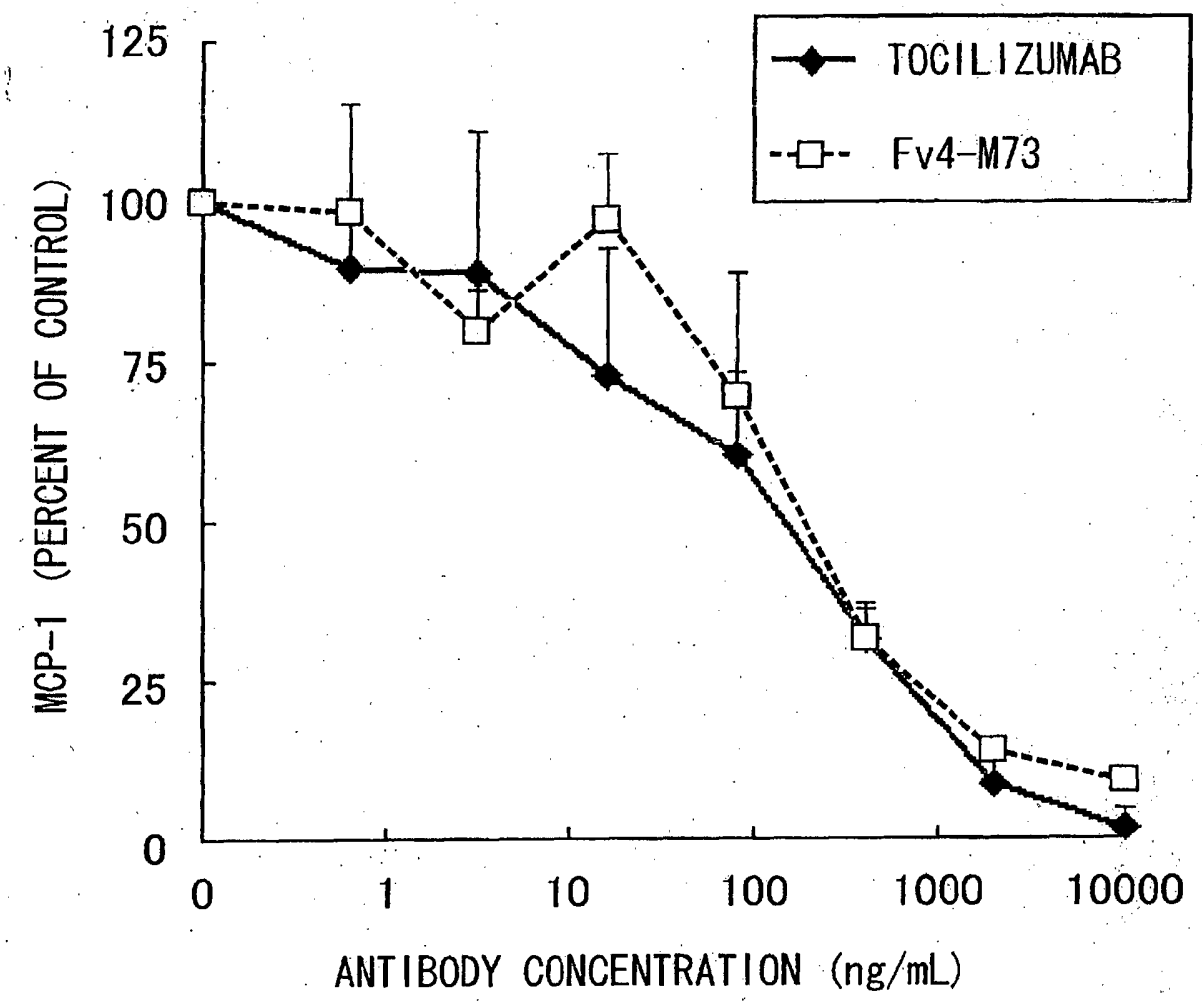
TOCILIZUMAB
Fv4-M73
MCP-1 (PERCENT OF CONTROL)
125
100
75
50
25
0
0
1
10
100
1000
10000
ANTIBODY CONCENTRATION (ng/mL)

FIG. 21

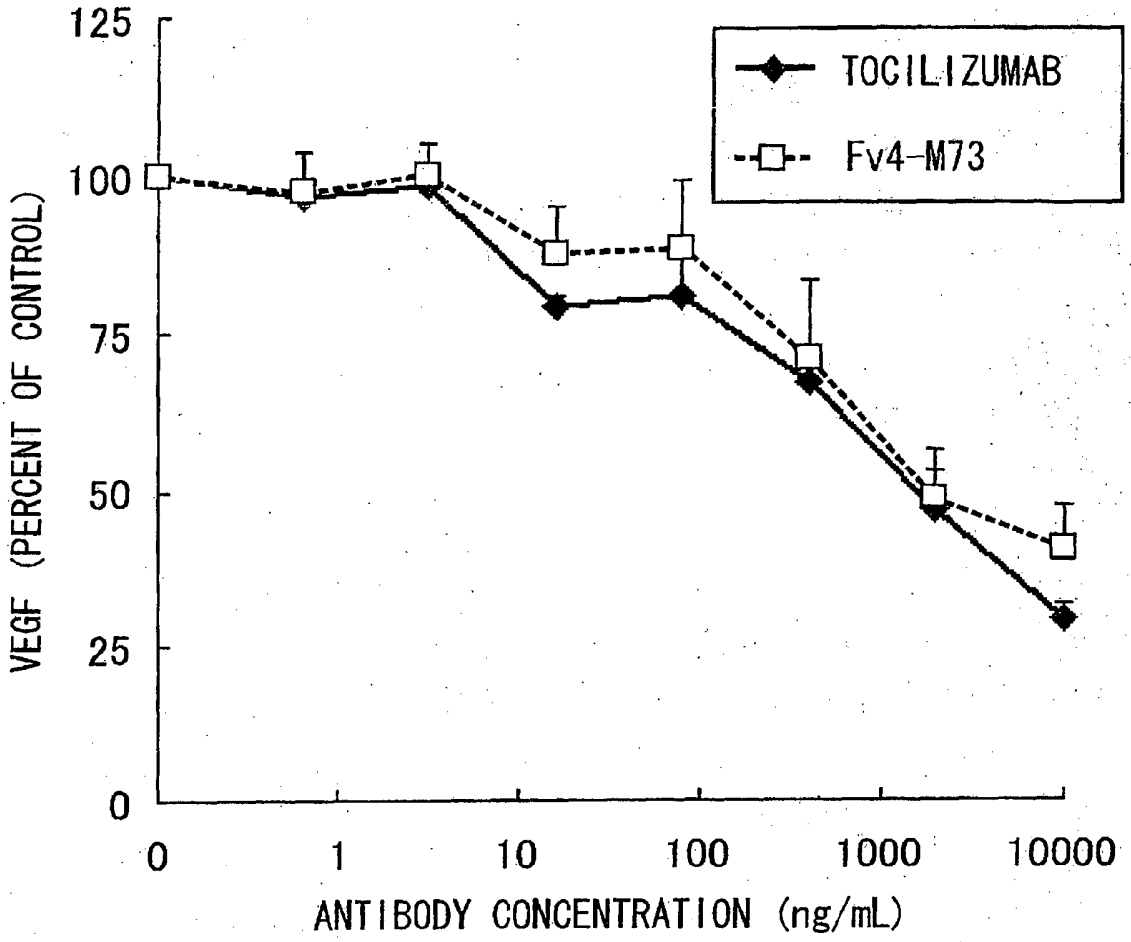
125
100
75
50
25
0
VEGF (PERCENT OF CONTROL)
0
1
10
100
1000
10000
ANTIBODY CONCENTRATION (ng/mL)
TOCILIZUMAB
Fv4-M73

FIG. 22

## REFERENCES CITED IN THE DESCRIPTION

*This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.*

### Patent documents cited in the description


- WO 9219759 A **[0012]**
- WO 9611020 A **[0012]**
- WO 9612503 A **[0012]**
- WO 2007143168 A **[0012]**
- WO 2007114319 A **[0012]**
- WO 2004096273 A **[0012]**
- EP 2206775 A **[0012]**
- EP 2194066 A **[0012]**
- EP 2275443 A **[0012]**
- EP 2202245 A **[0012]**
- JP 2163096 A **[0012]**
- US 20060122377 A **[0026]**
- EP 125023 A **[0050]**
- WO 9602576 A **[0050] [0051]**
- WO 9813388 A **[0051]**
- EP 239400 A **[0051]**
- EP 404097 A **[0066]**
- WO 9311161 A **[0066]**
- US 5057313 A **[0083]**
- US 5156840 A **[0083]**
- US 3773919 A **[0104]**
- EP 58481 A **[0104]**
- EP 133988 A **[0104]**
- WO 2004078140 A **[0104]**
- US 20070280945 A **[0142]**
- US 20070280945 A1 **[0158]**
- JP 2008248213 A **[0172]**
- JP 2009060806 A **[0172]**
- JP 2009067925 A **[0172]**


### Non-patent literature cited in the description


- **JANICE M REICHERT ; CLARK J ROSENSWEIG ; LAURA B FADEN ; MATTHEW C DEWITZ.** Monoclonal antibody successes in the clinic. *Nature Biotechnology,* 2005, vol. 23, 1073-1078 **[0013]**
- **PAVLOU AK ; BELSEY MJ.** The therapeutic antibodies market to 2008. *Eur J Pharm Biopharm.,* April 2005, vol. 59 (3), 389-96 **[0013]**
- **NISHIMOTO N ; KISHIMOTO T.** Interleukin 6: from bench to bedside. *Nat Clin Pract Rheumatol.,* November 2006, vol. 2 (11), 619-26 **[0013]**
- **MAINI RN ; TAYLOR PC ; SZECHINSKI J ; PAVELKA K ; BROLL J ; BALINT G ; EMERY P ; RAEMEN F ; PETERSEN J ; SMOLEN J.** CHARISMA Study Group., Double-blind randomized controlled clinical trial of the interleukin-6 receptor antagonist, Tocilizumab, in European patients with rheumatoid arthritis who had an incomplete response to methotrexate. *Arthritis Rheum.,* September 2006, vol. 54 (9), 2817-29 **[0013]**
- **NISHIMOTO N ; KANAKURA Y ; AOZASA K ; JOHKOH T ; NAKAMURA M ; NAKANO S ; NAKANO N ; IKEDA Y ; SASAKI T ; NISHIOKA K.** Humanized anti-interleukin-6 receptor antibody treatment of multicentric Castleman disease. *Blood,* 15 October 2005, vol. 106 (8), 2627-32 **[0013]**
- **KIM SJ ; PARK Y ; HONG HJ.** Antibody engineering for the development of therapeutic antibodies. *Mol Cells,* 31 August 2005, vol. 20 (1), 17-29 **[0013]**
- **ROTHE A ; HOSSE RJ.** Power BE. Ribosome display for improved biotherapeutic molecules. *Expert Opin Biol Ther.,* February 2006, vol. 6 (2), 177-87 **[0013]**
- **RAJPAL A ; BEYAZ N ; HABER L ; CAPPUCCILLI G ; YEE H ; BHATT RR ; TAKEUCHI T ; LERNER RA ; CREA R.** A general method for greatly improving the affinity of antibodies by using combinatorial libraries. *Proc Natl Acad Sci USA.,* 06 June 2005, vol. 102 (24), 8466-71 **[0013]**
- **WU H ; PFARR DS ; JOHNSON S ; BREWAH YA ; WOODS RM ; PATEL NK ; WHITE WI ; YOUNG JF ; KIENER PA.** Development of Motavizumab, an Ultra-potent Antibody for the Prevention of Respiratory Syncytial Virus Infection in the Upper and Lower Respiratory Tract. *J Mol Biol.,* 2007, vol. 368, 652-665 **[0013]**
- **SHIRE SJ ; SHAHROKH Z ; LIU J.** Challenges in the development of high protein concentration formulations. *J Pharm Sci.,* June 2004, vol. 93 (6), 1390-402 **[0013]**
- **SALFELD JG.** Isotype selection in antibody engineering. *Nat Biotechnol.,* December 2007, vol. 25 (12), 1369-72 **[0013]**
- **HINTON PR ; XIONG JM ; JOHLFS MG ; TANG MT ; KELLER S ; TSURUSHITA N.** An engineered human IgG1 antibody with longer serum half-life. *J Immunol.,* 01 January 2006, vol. 176 (1), 346-56 **[0013]**

- **GHETIE V ; POPOV S ; BORVAK J ; RADU C ; MATESOI D ; MEDESAN C ; OBER RJ ; WARD ES.** Increasing the serum persistence of an IgG fragment by random mutagenesis. *Nat Biotechnol.,* July 1997, vol. 15 (7), 637-40 **[0013]**
- **HWANG WY ; ALMAGRO JC ; BUSS TN ; TAN P ; FOOTE J.** Use of human germline genes in a CDR homology-based approach to antibody humanization. *Methods,* May 2005, vol. 36 (1), 35-42 **[0013]**
- **BARTELDS GM ; WIJBRANDTS CA ; NURMOHAMED MT ; STAPEL S ; LEMS WF ; AARDEN L ; DIJKMANS BA ; TAK P ; WOLBINK GJ.** Clinical response to adalimumab: The relationship with anti-adalimumab antibodies and serum adalimumab concentrations in rheumatoid arthritis. *Ann Rheum Dis.,* 09 March 2007 **[0013]**
- **BENDER NK ; HEILIG CE ; DROLL B ; WOHLGEMUTH J ; ARMBRUSTER FP ; HEILIG B.** Immunogenicity, efficacy and adverse events of adalimumab in RA patients. *Rheumatol Int.,* January 2007, vol. 27 (3), 269-74 **[0013]**
- **VAN WALLE I ; GANSEMANS Y ; PARREN PW ; STAS P ; LASTERS I.** Immunogenicity screening in protein drug development. *Expert Opin Biol Ther.,* March 2007, vol. 7 (3), 405-18 **[0013]**
- **JONES TD ; PHILLIPS WJ ; SMITH BJ ; BAMFORD CA ; NAYEE PD ; BAGLIN TP ; GASTON JS ; BAKER MP.** Identification and removal of a promiscuous CD4+ T cell epitope from the C1 domain of factor VIII. *J Thromb Haemost.,* May 2005, vol. 3 (5), 991-1000 **[0013]**
- **CHIRINO AJ ; ARY ML ; MARSHALL SA.** Minimizing the immunogenicity of protein therapeutics. *Drug Discov Today,* 15 January 2004, vol. 9 (2), 82-90 **[0013]**
- **SATO K ; TSUCHIYA M ; SALDANHA J ; KOISHIHARA Y ; OHSUGI Y ; KISHIMOTO T ; BENDIG MM.** Reshaping a human antibody to inhibit the interleukin 6-dependent tumor cell growth. *Cancer Res.,* 15 February 1993, vol. 53 (4), 851-6 **[0013]**
- **STRAND V ; KIMBERLY R ; ISAACS JD.** Biologic therapies in rheumatology: lessons learned future directions. *Nat Rev Drug Discov.,* January 2007, vol. 6 (1), 75-92 **[0013]**
- **GESSNER JE ; HEIKEN H ; TAMM A ; SCHMIDT RE.** The IgG Fc receptor family. *Ann Hematol.,* June 1998, vol. 76 (6), 231-48 **[0013]**
- **DILION TM ; RICCI MS ; VEZINA C ; FLYNN GC ; LIU YD ; REHDER DS ; PLANT M ; HENKLE B ; LI Y ; DEECHONGKIT S.** Structural and functional characterization of disulfide isoforms of the human IgG2 subclass. *J Biol Chem.,* 06 June 2008, vol. 283 (23), 16206-15 **[0013]**
- **JOHNSON KA ; PAISLEY-FLANGO K ; TANGARONE BS ; PORTER TJ ; ROUSE JC.** Cation exchange-HPLC and mass spectrometry reveal C-terminal amidation of an IgG1 heavy chain. *Anal Biochem.,* 01 January 2007, vol. 360 (1), 75-83 **[0013]**
- **YOSHIYUKI O.** *Gekkan Pharm Stage,* 2007, vol. 7 (5), 13-18 **[0013]**
- **ONDA M et al.** Lowering the isoelectric point of the Fv portion of recombinant immunotoxins leads to decreased nonspecific animal toxicity without affecting antitumor activity. *Cancer Res.,* 2001, vol. 61, 5070-77 **[0013]**
- **ITO W. et al.** The His-probe method: effects of histidine residues introduced into the complementary-determining regions of antibodies on antigen-antibody interactions at different pH values. *FEBS Letter,* 1992, vol. 309 (1), 85-88 **[0013]**
- **SATO, K. et al.** *Cancer Res.,* 1993, vol. 53, 851-856 **[0024] [0029]**
- **HASHIMOTO-GOTOH, T ; MIZUNO, T ; OGASAHARA, Y ; NAKAGAWA, M.** An oligodeoxyribonucleotide-directed dual amber method for site-directed mutagenesis. *Gene,* 1995, vol. 152, 271-275 **[0026]**
- **ZOLLER, MJ ; SMITH, M.** Oligonucleotide-directed mutagenesis of DNA fragments cloned into M13 vectors. *Methods Enzymol.,* 1983, vol. 100, 468-500 **[0026]**
- **KRAMER, W ; DRUTSA, V ; JANSEN, HW ; KRAMER, B ; PFLUGFELDER, M ; FRITZ, HJ.** The gapped duplex DNA approach to oligonucleotide-directed mutation construction. *Nucleic Acids Res.,* 1984, vol. 12, 9441-9456 **[0026]**
- **KRAMER W ; FRITZ HJ.** Oligonucleotide-directed construction of mutations via gapped duplex DNA Methods. *Enzymol.,* 1987, vol. 154, 350-367 **[0026]**
- **KUNKEL, TA.** Rapid and efficient site-specific mutagenesis without phenotypic selection. *Proc Natl Acad Sci U. S. A.,* 1985, vol. 82, 488-492 **[0026]**
- *Mol. Immunol.,* April 2007, vol. 44 (11), 3049-60 **[0026]**
- **CO, M.S. et al.** *J. Immunol.,* 1994, vol. 152, 2968-2976 **[0062]**
- **BETTER, M. ; HORWITZ, A. H.** *Methods in Enzymology,* 1989, vol. 178, 476-496 **[0062]**
- **PLUCKTHUN, A. ; SKERRA, A.** *Methods in Enzymology,* 1989, vol. 178, 476-496 **[0062]**
- **LAMOYI, E.** *Methods in Enzymology,* 1989, vol. 121, 652-663 **[0062]**
- **ROUSSEAUX, J. et al.** *Methods in Enzymology,* 1989, vol. 121, 663-669 **[0062]**
- **BIRD, R. E. et al.** *TIBTECH,* 1991, vol. 9, 132-137 **[0062]**
- **HOLLIGER P et al.** *Proc. Natl. Acad. Sci. USA,* 1993, vol. 90, 6444-6448 **[0066]**
- **HUSTON, J. S. et al.** *Proc. Natl. Acad. Sci. U.S.A.,* 1988, vol. 85, 5879-5883 **[0067]**
- **PLUCKTHUN.** The Pharmacology of Monoclonal Antibodies. Springer Verlag, 1994, vol. 113, 269-315 **[0067]**
- **HUDSON et al.** *J Immunol. Methods,* 1999, vol. 231, 177-189 **[0072]**
- *Protein Engineering,* 1996, vol. 9 (3), 299-305 **[0075]**

- **HOPP, T. P. et al.** *BioTechnology,* 1988, vol. 6, 1204-1210 **[0082]**
- **CO, M. S. et al.** *J. Immunol.,* 1994, vol. 152, 2968-2976 **[0089]**
- **BETTER, M. ; HORWITZ, A. H.** *Methods Enzymol.,* 1989, vol. 178, 476-496 **[0089]**
- **PLUCKTHUN, A. ; SKERRA, A.** *Methods Enzymol.,* 1989, vol. 178, 497-515 **[0089]**
- **LAMOYI, E.** *Methods Enzymol.,* 1986, vol. 121, 652-663 **[0089]**
- **ROUSSEAUX, J. et al.** *Methods Enzymol.,* 1986, vol. 121, 663-669 **[0089]**
- **BIRD, R. E. ; WALKER, B. W.** *Trends Biotechnol.,* 1991, vol. 9, 132-137 **[0089]**
- **WARD et al.** *Nature,* 1989, vol. 341, 544-546 **[0092]**
- *FASEB J.,* 1992, vol. 6, 2422-2427 **[0092]**
- **BETTER et al.** *Science,* 1988, vol. 240, 1041-1043 **[0092]**
- **LEI, S. P. et al.** *J. Bacteriol.,* 1987, vol. 169, 4379 **[0093]**
- *Nucleic Acids. Res.,* 1990, vol. 18 (17), 5322 **[0094]**
- **MULLIGAN et al.** *Nature,* 1979, vol. 277, 108 **[0095]**
- **MIZUSHIMA et al.** *Nucleic Acids Res.,* 1990, vol. 18, 5322 **[0095]**
- Molecular Cloning. Cold Spring Harbor Laboratory Press, 1989 **[0096]**
- Strategies for Protein Purification and Characterization: A Laboratory Course Manual. Cold Spring Harbor Laboratory Press, 1996 **[0098]**
- Remington's Pharmaceutical Science. Mark Publishing Company **[0103]**
- Remington's Pharmaceutical Science. 1980 **[0104]**
- **LANGER et al.** *J. Biomed. Mater. Res.,* 1981, vol. 15, 167-277 **[0104]**
- **LANGER.** *Chem. Tech.,* 1982, vol. 12, 98-105 **[0104]**
- **SIDMAN et al.** *Biopolymers,* 1983, vol. 22, 547-56 **[0104]**
- *Methods,* December 2004, vol. 34 (4), 468-75 **[0115] [0145]**
- *Cancer Res.,* 15 February 1993, vol. 53 (4), 851-6 **[0117]**
- **KABAT EA et al.** Sequences of Proteins of Immunological Interest. NIH, 1991 **[0117]**
- *Mol. Immunol.,* 2007, vol. 44 (4), 412-422 **[0119]**
- *Nat Rev Mol Cell Biol.,* February 2004, vol. 5 (2), 121-32 **[0122]**
- *Eur. J. Immunol.,* 1992, vol. 22, 1719-1728 **[0124]**
- *Proc Natl Acad Sci U S A.,* 23 May 1995, vol. 92 (11), 4862-6 **[0129]**
- *Anal Biochem.,* 01 January 2007, vol. 360 (1), 75-83 **[0131]**
- Sequences of proteins of immunological interest. NIH Publication No.91-3242 **[0132]**
- *Ann Hematol.,* June 1998, vol. 76 (6), 231-48 **[0133]**
- *Nat Biotechnol.,* December 2007, vol. 25 (12), 1369-72 **[0133]**
- *J Biol Chem.,* 06 June 2008, vol. 283 (23), 16206-15 **[0133]**
- *J. Clin. Invest.,* September 1992, vol. 90 (3), 772-779 **[0150]**
- *Inflamm. Allergy Drug Targets,* March 2008, vol. 7 (1), 53-66 **[0150]**
- *J. Rheumatol.,* September 1995, vol. 22 (9), 1624-1630 **[0151]**
- *Arthritis Rheum.,* June 2003, vol. 48 (6), 1521-1529 **[0151]**
- *Arthritis Rheum.,* September 2001, vol. 44 (9), 2055-2064 **[0151]**
- *Mod. Rheumatol.,* 2009, vol. 19 (1), 12-19 **[0151]**
- *Mediators Inflamm. 2008,* 2008, 129873 **[0151]**
- *J. Biochem.,* 1990, vol. 108, 673-676 **[0155]**
- **YAMASAKI et al.** *Science,* 1988, vol. 241, 825-828 **[0155]**
- **BIRNEY et al.** *Nucleic Acids Res.,* 01 January 2006, vol. 34, D556-61 **[0156]**
- *Protein Science,* 1995, vol. 4, 2411-2423 **[0159]**
- **HIBI et al.** *Cell,* 1990, vol. 63, 1149-1157 **[0161]**
- **HIRATA et al.** *FEBS Letter,* 1994, vol. 356, 244-248 **[0161]**
- *J. Exp. Med.,* 1994, vol. 180 (6), 2377-2381 **[0166]**
- *Proc. Natl. Acad. Sci. USA.,* 2002, vol. 99 (26), 16899-16903 **[0166]**
- *J. Immunol.,* 01 November 2002, vol. 169 (9), 5171-80 **[0167]**

## Szabadalmi igénypontok

1. Egy IL-6-receptor elleni antitest, mely az alábbiak egyike:
   (a) egy antitest, amely tartalmaz egy nehézlánc variábilis régiót, mely a SEQ ID NO: 20 szerinti szekvenciát tartalmazza (VH3-M73 variábilis régiója) és egy könnyűlánc variábilis régiót, mely a SEQ ID NO: 23 szerinti szekvenciát tartalmazza (VL3 variábilis régiója);
   (b) egy antitest, amely tartalmaz egy nehézlánc variábilis régiót, mely a SEQ ID NO: 19 szekvenciát tartalmazza (VH4-M73 variábilis régiója) és egy könnyűlánc variábilis régiót, mely a SEQ ID NO: 22 szekvenciát tartalmazza (VL1 variábilis régiója); és
   (c) egy antitest, amely tartalmaz egy nehézlánc variábilis régiót, mely a SEQ ID NO: 21 szekvenciát tartalmazza (VH5-M83 variábilis régiója) és egy könnyűlánc variábilis régiót, mely a SEQ ID NO: 24 szekvenciát tartalmazza (VL5 variábilis régiója).
2. Egy IL-6-receptor elleni antitest, mely az alábbiak egyike:
   (a) egy antitest, amely tartalmaz egy nehézláncot, mely a SEQ ID NO: 26 szekvenciát (VH3-M73) tartalmazza és egy könnyűláncot, mely a SEQ ID NO: 29 szekvenciát (VL3) tartalmazz;
   (b) egy antitest, amely tartalmaz egy nehézláncot, mely a SEQ ID NO: 25 szekvenciát (VH4-M73) tartalmazza és egy könnyűláncot, mely a SEQ ID NO: 28 szekvenciát (VL1) tartalmazza; és
   (c) egy antitest, amely tartalmaz egy nehézláncot, mely a SEQ ID NO: 27 szekvenciát (VH5-M83) tartalmazza és egy könnyűláncot, mely a SEQ ID NO: 30 szekvenciát (VL5) tartalmazza.
3. Egy gén, amely az 1. vagy 2. igénypont szerinti antitestet kódolja.
4. Egy vektor, amely a 3. igénypont szerinti gént hordozza.
5. Egy gazdasejt, amely a 4. igénypont szerinti vektort hordozza.
6. Egy eljárás az 1. vagy 2. igénypont szerinti antitest termelésére az 5. igénypont szerinti gazdasejt tenyésztésével.
7. Egy gyógyszerkészítmény, amely tartalmazza az 1. vagy 2. igénypont szerinti antitestet vagy egy, a 6. igénypont szerinti eljárással termelt antitestet.

A meghatalmazott:

Dr. Láng Tivadar
SBGK Szabadalmi Ügyvivői Iroda
H-1062 Budapest, Andrássy út 113.
Telefon: 461-1070 Fax: 461-1099
Email: lang@sbgk.hu