

(19) **DANMARK**



Patent- og
Varemærkestyrelsen

(10) **DK/EP 2781221 T3**

(12) Oversættelse af
europæisk patentskrift

-
- (51) Int.Cl.: **A 61 P 31/04 (2006.01)** **A 61 K 38/36 (2006.01)**
- (45) Oversættelsen bekendtgjort den: **2020-06-02**
- (80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2020-04-08**
- (86) Europæisk ansøgning nr.: **12850047.7**
- (86) Europæisk indleveringsdag: **2012-11-14**
- (87) Den europæiske ansøgnings publiceringsdag: **2014-09-24**
- (86) International ansøgning nr.: **JP2012079449**
- (87) Internationalt publikationsnr.: **WO2013073545**
- (30) Prioritet: **2011-11-15 US 201161559864 P**
- (84) Designerede stater: **AL 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 RS SE SI SK SM TR**
- (73) Patenthaver: **Asahi Kasei Pharma Corporation, 1-1-2 Yurakucho, Chiyoda-ku, Tokyo 100-0006, Japan**
- (72) Opfinder: **TSURUTA, Kazuhisa, c/o 1-105, Kanda Jinbocho, Chiyoda-ku, Tokyo 101-8101, Japan**
AOKI, Yoshikazu, c/o 1-105, Kanda Jinbocho, Chiyoda-ku, Tokyo 101-8101, Japan
OSAWA, Yutaka, c/o 1-105, Kanda Jinbocho, Chiyoda-ku, Tokyo 101-8101, Japan
KAUL, Inder, 200 Fifth Avenue, Waltham, MA 02451, USA
- (74) Fuldmægtig i Danmark: **Zacco Denmark A/S, Arne Jacobsens Allé 15, 2300 København S, Danmark**
- (54) Benævnelse: **Medicin til behandling og/eller forbedring af sepsis**
- (56) Fremdragne publikationer:
WO-A1-2008/038777
JP-A- 2005 515 214
WITTEBOLE X ET AL: "Adjunctive therapies for severe sepsis", INTERNATIONAL JOURNAL OF ANTIMICROBIAL AGENTS, ELSEVIER SCIENCE, AMSTERDAM, NL, vol. 32, 1 November 2008 (2008-11-01), pages S34-S38, XP025560112, ISSN: 0924-8579, DOI: 10.1016/J.IJANTIMICAG.2008.06.005 [retrieved on 2008-08-19]
"AMERICA COLLEGE OF CHEST PHYSICIANS/SOCIETY OF CRITICAL CARE MEDICINE CONSENSUS CONFERENCE: DEFINITIONS FOR SEPSIS AND ORGAN FAILURE AND GUIDELINES FOR THE USE OF INNOVATIVE THERAPIES IN SEPSIS", CRITICAL CARE MEDICINE, LIPPINCOTT WILLIAMS & WILKINS, US, vol. 20, no. 6, 1 June 1992 (1992-06-01), pages 864-874, XP008036965, ISSN: 0090-3493
TOMOHIRO KOGA ET AL: "Successful treatment of sepsis-induced disseminated intravascular coagulation in a patient with idiopathic thrombocytopenic purpura using recombinant human soluble thrombomodulin", RHEUMATOLOGY INTERNATIONAL ; CLINICAL AND EXPERIMENTAL INVESTIGATIONS, SPRINGER, BERLIN, DE, vol. 31, no. 12, 15 January 2011 (2011-01-15), pages 1657-1659, XP019980006, ISSN: 1437-160X, DOI: 10.1007/S00296-010-1754-1
ABRAHAM, E. ET AL.: 'Assessment of the safety of recombinant tissue factor pathway inhibitor in patients with

Fortsættes ...

severe sepsis: a multicenter, randomized, placebo-controlled, single-blind, dose escalation study' **CRITICAL CARE MEDICINE** vol. 29, no. 11, 2001, pages 2081 - 9, XP009077243

YAMAKAWA, K. ET AL.: 'Treatment effects of recombinant human soluble thrombomodulin in patients with severe sepsis: a historical control study' **CRITICAL CARE** vol. 15, no. 3, May 2011, page R123, XP021104493

SAITO, H. ET AL.: 'Efficacy and safety of recombinant human soluble thrombomodulin (ART- 123) in disseminated intravascular coagulation: results of a phase III, randomized, double- blind clinical trial' **JOURNAL OF THROMBOSIS AND HAEMOSTASIS** vol. 5, no. L, 2007, pages 31 - 41, XP055154592

KAZUMA YAMAKAWA ET AL.: 'Haikessho Shinryo ni Okeru DIC Chiryoyaku no Yukosei ni Kansuru Evidence - Idenshi Kumikaegata Hito Thrombomodulin Seizai no Koka' **JOURNAL OF JAPANESE ASSOCIATION FOR ACUTE MEDICINE** vol. 22, no. 8, August 2011, page 369, XP008174278

NAKAHARA, M. ET AL.: 'Efficacy of recombinant soluble thrombomodulin in disseminated intravascular coagulation associated with sepsis' **EUROPEAN JOURNAL OF ANAESTHESIOLOGY** vol. 28, June 2011, page 177, XP008174075

ABRAHAM, E. ET AL.: 'Efficacy and safety of tifacogin (recombinant tissue factor pathway inhibitor) in severe sepsis. A randomized controlled trial' **JAMA** vol. 290, no. 2, 2003, pages 238 - 247, XP009077208

DESCRIPTION

Technical Field

[0001] The present invention relates to a medicament for therapeutic treatment and/or improvement of sepsis in a severe septic patient.

Background Art

[0002] Sepsis is a systemic inflammatory response syndrome (SIRS) induced by infection. Specifically, sepsis is defined as a pathological condition that meets, in addition to the presence of infection, two or more of the SIRS items ((1) body temperature $> 38^{\circ}\text{C}$ or $< 36^{\circ}\text{C}$, (2) heart rate $> 90/\text{minute}$, (3) respiration rate $> 20/\text{minute}$, or $\text{PaCO}_2 < 32$ torr, and (4) leucocyte count $> 12,000/\mu\text{L}$ or $< 4000/\mu\text{L}$, or immature leucocytes $> 10\%$). Although presence of bacteria in blood (bacteremia) has been significantly focused so far, bacteria-positive result of blood culture is not necessarily required according to the above definition. Among sepsis, a condition presenting organ dysfunction, organ hypoperfusion, or hypotension is called severe sepsis. The organ hypoperfusion or abnormal perfusion includes lactic acidosis, oliguria, mental clouding, and the like. Among the severe sepsis, a condition continuously presenting hypotension despite of sufficient load of fluid therapy is called septic shock (Non-patent document 1). It is considered that the circulatory failure observed in these pathological conditions is caused by malfunction of the sympathetic nervous system or a mediator released from neutrophils and the like, and the organ dysfunction is caused by tissue hypoxia (dysoxia).

[0003] Thrombomodulin has been known as a substance that acts to specifically bind to thrombin so as to inhibit the blood coagulation activity of thrombin, and at the same time, exerts anticoagulant activity so as to significantly promote the ability of thrombin to activate Protein C. It has also been known that thrombomodulin exerts to prolong the clotting time by thrombin, or suppresses platelet aggregation by thrombin. Protein C is a vitamin K-dependent protein that plays an important role in a blood coagulation and fibrinolysis, and activated by the action of thrombin to be converted as activated Protein C. It has been known that the activated Protein C inactivates activated blood coagulation factor V and activated blood coagulation factor VIII *in vivo*, and is involved in generation of a plasminogen activator having thrombolytic action (Non-patent document 2). Accordingly, it has been considered that thrombomodulin promotes the activation of Protein C by thrombin, and therefore is useful as an anticoagulant or a thrombolytic agent. It has also been reported that, in an animal experiment, thrombomodulin is effective for therapy or prophylaxis of diseases associated with hypercoagulable state (Non-patent document 3).

[0004] Thrombomodulin was first discovered and obtained as a glycoprotein expressed on the

vascular endothelial cells of various animal species including humans, and then successfully cloned. Specifically, a gene of a human thrombomodulin precursor including a signal peptide was cloned from a human lung cDNA library by genetic engineering techniques and the entire gene sequence of thrombomodulin was analyzed, and as a result, an amino acid sequence consisting of 575 residues containing a signal peptide (in general, 18 amino acid residues are exemplified) was revealed (Patent document 1). It is known that a mature thrombomodulin, from which the signal peptide is cleaved, is composed of 5 regions, namely, an N-terminal region (amino acid residues 1 to 226, these positions are defined under an assumption that the signal peptide consists of 18 amino acid residues, and the same shall apply to the following descriptions), a region having six EGF-like structures (amino acid residues 227 to 462), an O-linked glycosylation region (amino acid residues 463 to 498), a transmembrane region (amino acid residues 499 to 521), and a cytoplasmic region (amino acid residues 522 to 557), from the N-terminal side of the mature peptide. It is also known that a partial protein having the same activity as that of the entire length thrombomodulin (i.e., a minimal active unit) is mainly consisting of the 4th, 5th, and 6th EGF-like structures from the N-terminal side in the region having six EGF-like structures (Non-patent document 4).

[0005] The entire length thrombomodulin is hardly dissolved in the absence of a surfactant, and addition of a surfactant is essential for manufacturing an entire thrombomodulin preparation. A soluble thrombomodulin is also available that can be fully dissolved even in the absence of a surfactant. The soluble thrombomodulin may be prepared by removing at least a part of the transmembrane region or the entire transmembrane region. For example, it has been confirmed that a soluble thrombomodulin consisting of only 3 regions, namely, the N-terminal region, the region having six EGF-like structures, and the O-linked glycosylation region (i.e., a soluble thrombomodulin having an amino acid sequence consisting of amino acid residues 19 to 516 of SEQ ID NO:9) can be obtained by applying recombination techniques, and that the resulting recombinant soluble thrombomodulin has the same activity as that of an entire thrombomodulin (Patent document 1). Some other reports are also available regarding soluble thrombomodulins (Patent documents 2 to 9). A human urine-derived soluble thrombomodulin and the like are also exemplified as native thrombomodulins (Patent documents 10 and 11).

[0006] As recognized in many cases, as a result of spontaneous mutations or mutations occurring at the time of obtainment, polymorphic mutations have been found in the human genes. At present, thrombomodulin proteins in which the amino acid at the position 473 of human thrombomodulin precursor having the amino acid sequence consisting of 575 amino acid residues is converted to Val or Ala have been identified. In the nucleotide sequence encoding the amino acid sequence, this variation of amino acid residue corresponds to mutation to T or C at the position 1418 (Non-patent document 5). However, the two types of thrombomodulins are completely identical in terms of their activity and physicochemical properties, and it can be considered that they are substantially identical.

[0007] It has been reported that thrombomodulin is effective for a therapeutic treatment of disseminated intravascular coagulation (henceforth also referred to as DIC) (Non-patent

document 6). As for use of thrombomodulin, in addition to the aforementioned use, thrombomodulin is expected to be used in therapeutic and prophylactic treatments of various diseases such as acute coronary syndrome (ACS), thrombosis, peripheral vessel obstruction, obstructive arteriosclerosis, vasculitis, functional disorder occurring after heart surgery, complication caused by organ transplantation, angina pectoris, transient ischemic attack, toxemia of pregnancy, diabetes, liver VOD (liver veno-occlusive disease, e.g., fulminant hepatitis, veno occlusive disease of liver occurring after bone marrow transplantation), and deep venous thrombosis (DVT), and further, sepsis and adult respiratory distress syndrome (ARDS) (Patent document 12).

[0008] Non-patent document 7 Non-patent document 7 discloses the successful treatment of sepsis-induced disseminated intravascular coagulation in a patient-, with idiopathic thrombocytopenic purpura using human soluble thrombomodulin.

[Prior art references]

[Patent documents]

[0009]

Patent document 1: Japanese Patent Unexamined Publication (Kokai) No. 64-6219

Patent document 2: Japanese Patent Unexamined Publication No. 5-213998

Patent document 3: Japanese Patent Unexamined Publication No. 2-255699

Patent document 4: Japanese Patent Unexamined Publication No. 3-133380

Patent document 5: Japanese Patent Unexamined Publication No. 3-259084

Patent document 6: Japanese Patent Unexamined Publication No. 4-210700

Patent document 7: WO92/00325

Patent document 8: WO92/03149

Patent document 9: WO93/15755

Patent document 10: Japanese Patent Unexamined Publication No. 3-86900

Patent document 11: Japanese Patent Unexamined Publication No. 3-218399

Patent document 12: WO03/061687

Non-patent Documents

[0010]

Non-patent document 1: American College of Chest Physicians, CHEST/101/6-/JUNE, 1992:1481-1483

Non-patent document 2: Koji Suzuki, Igaku no Ayumi (Progress of Medicine), Vol. 125, 901(1983)

Non-patent document 3: K. Gomi et al., Blood, 75, 1396-1399 (1990)

Non-patent document 4: M. Zushi et al., J. Biol. Chem., 246, 10351-10353 (1989)

Non-patent document 5: D.Z. Wen et al., Biochemistry, 26, 4350-4357 (1987)

Non-patent document 6: S.M. Bates et al., Br. J. Pharmacol., 144, 1017-1028 (2005)

Non-patent document 7: T. KOGA et. al., RHEUMATOLOGY INTERNATIONAL CLINICAL AND EXPERIMENTAL INVESTIGATIONS, 31, 1657-1659 (2011)

Summary of the Invention

Object to be Achieved by the Invention and its solution

[0011] An object of the present invention is to provide a medicament or method for effective therapeutic treatment or improvement of sepsis in a severe septic patient.

Means for Achieving the Objects

[0012] International Normalized Ratio (henceforth also abbreviated as "INR") in a plasma specimen of a patient with sepsis is known to mean coagulopathy. For example, in Congress of Critical Care Medicine (CCM) held in 2003, INR > 1.5 is reported as well as aPPT > 60 seconds as criteria of coagulopathy (Crit. Care Med., 31, pp.1250-1256 (2003)). However, the value of INR has not yet been authorized as a clear criterion, because the value has not been verified through clinical trials and the like. Actually, through Phase III clinical trial for treatment of patients with severe sepsis, Tifagofin as a tissue factor pathway inhibitor in a class of anticoagulants is reported to have achieved more favorable result in a group of patients with $INR \leq 1.2$ than a group of patients with $INR > 1.2$ as a result of clinical test in which patients

with INR > 1.2 were mainly targeted (JAMA, July 9, Vol. 290, No. 2, pp.238-247 (2003)). Whilst as another result of the aforementioned clinical test, among the class of patients with INR > 1.2, the drug is reported to have achieved higher effect in patients with INR > 1.5 than in patients with INR > 1.2. Further, with Xigris, which is the only drug that has been verified to be effective against sepsis through clinical study, prolongation of prothrombin-time (PT) was observed in most of the patients in the study (93.4%).

[0013] As explained above, in the therapeutic treatment of patients with sepsis by an anticoagulant, high efficacy is expected by choosing a class of patients accompanied with coagulopathy in view of some of the clinical study results. However, it is The object is solved by the embodiments of the invention, which are as follows:

1. 1. A medicament which comprises thrombomodulin as an active ingredient for use in therapeutic treatment and/or improvement of sepsis, wherein said medicament is to be administered to a patient with severe sepsis accompanied with one or more organ dysfunctions, wherein a value of International Normalized Ratio (INR) of a plasma specimen obtained from said patient is more than 1.4.
2. 2. A medicament which comprises thrombomodulin as an active ingredient for use in decreasing the mortality of a patient with sepsis, wherein said medicament is to be administered to a patient with severe sepsis accompanied with one or more organ dysfunctions, wherein a value of International Normalized Ratio (INR) of a plasma specimen obtained from said patients is more than 1.4.
3. 3. The medicament for use according to embodiment 1 or 2, wherein said medicament is to be administered to a patient with severe sepsis accompanied with one or more organ dysfunctions, wherein a value of International Normalized Ratio (INR) of a plasma specimen obtained from said patient is more than 1.4 and equal to or less than 1.6.
4. 4. The medicament for use according to any one of embodiments 1 to 3, wherein the patient with severe sepsis is a severe septic patient who is not a septic patient with organ dysfunction limited to the liver or kidney.
5. 5. The medicament for use according to any one of embodiments 1 to 4, wherein said medicament is to be administered to a patient with severe sepsis accompanied with one or more organ dysfunctions selected from the group consisting of liver dysfunction, kidney dysfunction, respiratory organ dysfunction, and circulatory organ dysfunction.
6. 6. The medicament for use according to any one of embodiments 1 to 4, wherein said medicament is to be administered to a patient with severe sepsis accompanied with one or more organ dysfunctions selected from the group consisting of respiratory organ dysfunction and circulatory organ dysfunction.
7. 7. The medicament for use according to any, one of embodiments 1 to 6, wherein the thrombomodulin is a soluble thrombomodulin.
8. 8. The medicament for use according to any one of embodiments 1 to 7, wherein the thrombomodulin is a peptide obtainable from a transformed cell prepared by transfecting a host cell with a DNA coding for the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 11.
9. 9. The medicament for use according to any one of embodiments 1 to 8, wherein the

thrombomodulin is a peptide containing the amino acid sequence of (i-1);

(i-1) the amino acid sequence of the positions 19 to 516 in the amino acid sequence of SEQ ID NO: 9 or 11.

10. 10. The medicament for use according to any one of embodiments 1 to 9, wherein the thrombomodulin is to be administered at a dose of 0.005 to 1 mg/kg within 5 minutes by intravenous bolus administration.
11. 11. The medicament for use according to any one of embodiments 1 to 10, wherein sepsis is severe sepsis with coagulopathy.
12. 12. The medicament for use according to any one of embodiments 1 to 11, wherein said circulatory organ dysfunction is shock.
13. 13. The medicament for use according to any one of embodiments 1 to 12, wherein said organ dysfunctions are induced by sepsis.
14. 14. The medicament for use according to any one of embodiments 1 to 13, wherein said medicament is to be administered to a patient with severe sepsis wherein a patient with an INR value of 1.7 is excluded.
15. 15. The medicament for use according to any one of embodiments 1 to 14, wherein said thrombomodulin is administered to said patient intravenously.
16. 16. The medicament for use according to any one of embodiments 1 to 15, wherein said thrombomodulin is administered to said patient at a dose of 0.04 - 0.08 mg/kg/day.

considered that the definition of coagulopathy has not yet been authorized, because, for example, clinical results to the contrary were also obtained. In other words, how an excellent result can be obtained by choosing target patients by means of the INR value has not been clarified, and there is no common technical knowledge that what level of the INR value of a patient with sepsis assures particular effectiveness of the drug. With regard to correlation of the INR value with clinical effectiveness, it is considered that only a limited part of the correlation has been known as case-by-case basis as for some of individual drugs.

[0014] Under the circumstances, the inventors devoted their attention to thrombomodulin among anticoagulants, and conducted various researches on therapeutic and/or improving effect against sepsis. As a result, they unexpectedly found that sepsis can be therapeutically treated and/or improved more effectively in severe septic patients with one or more organ dysfunctions (except severe septic patient with organ dysfunction limited to the liver or kidney) than in severe septic patients without organ dysfunction when the INR value of the patients is more than 1.4, in other words, as for therapeutic treatment and/or improvement of sepsis by thrombomodulin, the inventors found that there is a particular correlation between severe septic patients with one or more organ dysfunctions, among the class of septic patients, and the INR value more than 1.4, which is unexpected by one of ordinary skill in the art. Further surprisingly, the inventors found that, for severe septic patients with the INR value more than 1.4 and equal to or less than 1.6, particularly remarkable effect was achieved in that a difference in mortality rate between thrombomodulin group and placebo group was higher than 15%, and as a result, they accomplished the present invention. In view of the difference in mortality rate of about 6% between drug group and placebo group achieved by Xigris (N. Engl. J. Med., 344, No. 10, March 8, pp.699-709 (2001)), which is a sole commercially available drug

in Europe for treatment of sepsis, the value of 15% as the difference in mortality rate is a remarkable value as high as about 2.5-fold, and therefore, it can be recognized that one of embodiments of the present invention achieves unexpectedly surprising effect.

[0015] Specifically, the present invention includes the followings:

[A1] A medicament which comprises thrombomodulin as an active ingredient for use in therapeutic treatment and/or improvement of sepsis, wherein said medicament is to be administered to a patient with severe sepsis accompanied with one or more organ dysfunctions, wherein a value of International Normalized Ratio (INR) of a plasma specimen obtained from said patient is more than 1.4.

[A1-2] A medicament which comprises thrombomodulin as an active ingredient for use in decreasing the mortality of a patient with sepsis, wherein said medicament is to be administered to a patient with severe sepsis accompanied with one or more organ dysfunctions, wherein a value of International Normalized Ratio (INR) of a plasma specimen obtained, from said patients is more than 1.4.

[A2] The medicament for use according to [A1] or [A1-2] mentioned above, wherein said medicament is to be administered to a patient with severe sepsis accompanied with one or more organ dysfunctions, wherein a value of International Normalized Ratio (INR) of a plasma specimen obtained from said patient is more than 1.4 and equal to or less than 1.6.

[A3] The medicament for use according to any one of [A1] to [A2] mentioned above, wherein the patient with severe sepsis is a severe septic patient who is not a septic patient with organ dysfunction limited to the liver or kidney.

[0016] When the referred item numbers are indicated with such a range as "[A1] to [A2]" mentioned above, and the range includes an item indicated with a number having a subnumber such as [A1-2], it is meant that the item indicated with the number having a subnumber such as [A1-2] is also cited. The same shall apply to the following definitions.

[A4] The medicament for use according to any one of [A1] to [A3] mentioned above, wherein said medicament is to be administered to a patient with severe sepsis accompanied with one or more organ dysfunctions selected from the group consisting of liver dysfunction, kidney dysfunction, respiratory organ dysfunction, and circulatory organ dysfunction.

[A5] The medicament for use according to any one of [A1] to [A4] mentioned above, wherein the thrombomodulin is a soluble thrombomodulin.

[A5-2] The medicament for use according to any one of [A1] to [A5] mentioned above, wherein the soluble thrombomodulin has the following properties (1) to (4):

1. (1) an action of selectively binding to thrombin,
2. (2) an action of promoting activation of Protein C by thrombin,

3. (3) an action of extending thrombin clotting time, and
4. (4) an action of suppressing platelet aggregation caused by thrombin.

[A5-3] The medicament for use according to any one of [A1] to [A5] mentioned above, wherein the soluble thrombomodulin has the following properties (1) to (5):

1. (1) an action of selectively binding to thrombin,
2. (2) an action of promoting activation of Protein C by thrombin,
3. (3) an action of extending thrombin clotting time,
4. (4) an action of suppressing platelet aggregation caused by thrombin, and
5. (5) anti-inflammatory action.

[A6] The medicament for use according to any one of [A1] to [A5-3] mentioned above, wherein the thrombomodulin is a peptide obtainable from a transformed cell prepared by transfecting a host cell with a DNA coding for the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 11.

[A7] The medicament for use according to any one of [A1] to [A6] mentioned above, wherein the soluble thrombomodulin is a peptide containing the amino acid sequence of (i-1) or (i-2) mentioned below, and said peptide is a peptide having the thrombomodulin activities;

(i-1) the amino acid sequence of the positions 19 to 516 in the amino acid sequence of SEQ ID NO: 9 or 11, or

(i-2) the amino acid sequence of (i-1) mentioned above, further including substitution, deletion or addition of one or more amino acid residues.

[A7-2] The medicament for use according to any one of [A1] to [A6] mentioned above, wherein the soluble thrombomodulin is a peptide containing:

1. (i) the amino acid sequence of the positions 367 to 480 in the amino acid sequence of SEQ ID NO: 9 or 11, and the amino acid sequence of (ii-1) or (ii-2) mentioned below, and said peptide is a peptide having the thrombomodulin activities:

(ii-1) the amino acid sequence of the positions 19 to 244 in the amino acid sequence of SEQ ID NO: 9 or 11, or

(ii-2) the amino acid sequence of (ii-1) mentioned above, further including substitution, deletion or addition of one or more amino acid residues,

[A8] The medicament for use according to any one of [A1] to [A7-2] mentioned above, wherein the thrombomodulin is to be administered at a dose of 0.005 to 1 mg/kg within 5 minutes by intravenous bolus administration.

[B] Illustrated are:

[0017]

[B1] A method for therapeutic treatment and/or improvement of sepsis, which comprises the step of administering thrombomodulin to a patient with severe sepsis, wherein a value of International Normalized Ratio (INR) of a plasma specimen obtained from said patient is more than 1.4.

[B1-2] A method for therapeutic treatment and/or improvement of sepsis accompanied with coagulopathy, which comprises the step of administering thrombomodulin to a patient with severe sepsis, wherein a value of International Normalized Ratio (INR) of a plasma specimen obtained from said patient is more than 1.4.

[B-2] The method according to [B1] or [B1-2] mentioned above, which comprises the step of administering thrombomodulin to a patient with severe sepsis accompanied with one or more organ dysfunctions, wherein a value of International Normalized Ratio (INR) of a plasma specimen obtained from said patient is more than 1.4 and equal to or less than 1.6.

[B3] The method according to any one of [B1] to [B-2] mentioned above, wherein the patient with severe sepsis is a severe septic patient who is not a septic patient with organ dysfunction limited to the liver or kidney.

[B4] The method according to any one of [B1] to [B3] mentioned above, which comprises the step of administering thrombomodulin to a patient with severe sepsis accompanied with one or more organ dysfunctions selected from the group consisting of liver dysfunction, kidney dysfunction, respiratory organ dysfunction, and circulatory organ dysfunction.

[B5] The method according to any one of [B1] to [B4] mentioned above, wherein the thrombomodulin is a soluble thrombomodulin.

[B6] The method according to any one of [B1] to [B5] mentioned above, wherein the thrombomodulin is a peptide obtainable from a transformed cell prepared by transfecting a host cell with a DNA coding for the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 11.

[B7] The method according to any one of [B1] to [B6] mentioned above, wherein the soluble thrombomodulin is a peptide containing the amino acid sequence of (i-1) or (i-2) mentioned below, and said peptide is a peptide having the thrombomodulin activities;

(i-1) the amino acid sequence of the positions 19 to 516 in the amino acid sequence of SEQ ID NO: 9 or 11, or

(i-2) the amino acid sequence of (i-1) mentioned above, further including substitution, deletion or addition of one or more amino acid residues.

[B8] The method according to any one of [B1] to [B7] mentioned above, wherein the thrombomodulin is administered at a dose of 0.005 to 1 mg/kg within 5 minutes by intravenous bolus administration.

[B8-2] The method according to any one of [B1] to [B8] mentioned above, wherein the

thrombomodulin is a thrombomodulin having the characteristic features mentioned in [A5-2], [A5-3] or [A7-2] mentioned above.

[B9] Use of thrombomodulin as a medicament for therapeutic treatment and/or improvement of sepsis, wherein said medicament is to administer to a patient with severe sepsis accompanied with one or more organ dysfunctions, wherein a value of International Normalized Ratio (INR) of a plasma specimen obtained from said patient is more than 1.4.

[B9-2] The use according to [B9] mentioned above, wherein the thrombomodulin is a thrombomodulin having the characteristic features mentioned in [A5-2], [A5-3] or [A7-2] mentioned above.

[B9-3] The use according to [B9] mentioned above, which has the characteristic features mentioned in any one of [A1] to [A8] mentioned above.

[C] Illustrated are:

[0018]

[C1] A medicament for therapeutic treatment and/or improvement of disseminated intravascular coagulation, wherein said medicament is to administer to a patient with disseminated intravascular coagulation accompanied with one or more organ dysfunctions, wherein a value of International Normalized Ratio (INR) of a plasma specimen obtained from said patient is higher than 1.4.

[C2] The medicament according to [C1] mentioned above, which has the characteristic features mentioned in any one of [A1] to [A8] mentioned above.

Effect of the Invention

[0019] With the medicament for use of the present invention containing thrombomodulin, sepsis in a severe septic patient, wherein a value of International Normalized Ratio (INR) of a plasma specimen obtained from said patient is more than 1.4, can be effectively treated and/or improved.

Modes for Carrying out the Invention

[0020] Hereafter, several preferred embodiments of the present invention (preferred modes

for carrying out the invention, henceforth also referred to as "embodiments" in the specification) will be specifically explained. However, the scope of the present invention as defined in the claims is not limited to the specific embodiments explained below.

[0021] The thrombomodulin used for the present invention preferably is known to have an action of (1) selectively binding to thrombin (2) to promote activation of Protein C by thrombin. In addition, it is preferred that the thrombomodulin is confirmed to generally have (3) an action of extending thrombin clotting time, (4) an action of suppressing platelet aggregation caused by thrombin, and/or (5) anti-inflammatory action. Such actions possessed by thrombomodulin may be referred to as thrombomodulin activities.

[0022] As the thrombomodulin activities, thrombomodulin preferably has the actions of (1) and (2) mentioned above, and more preferably has the actions of (1) to (4) mentioned above. As the thrombomodulin activities, thrombomodulin more preferably has all of the actions of (1) to (5) mentioned above.

[0023] The action of thrombomodulin to bind with thrombin can be confirmed by the study methods described in various known publications such as *Thrombosis and Haemostasis*, 70(3):418-422 (1993) and *The Journal of Biological Chemistry*, 264, 9, pp.4872-4876 (1989). As for the action of promoting activation of Protein C by thrombin, degree of the activity of promoting the activation of Protein C by thrombin or presence or absence of the action can be easily confirmed by the study methods clearly described in various known publications including, for example, Japanese Patent Unexamined Publication No. 64-6219. Further, the action of extending thrombin clotting time, and/or the action of suppressing platelet aggregation caused by thrombin can be similarly and easily confirmed. Furthermore, the anti-inflammatory action can also be confirmed by the study methods described in various known publications including, for example, *Blood*, 112:3361-3670 (2008) and *The Journal of Clinical Investigation*, 115, 5:1267-1274 (2005).

[0024] The thrombomodulin used for the present invention is not particularly limited so far as having the thrombomodulin activities. The thrombomodulin is preferably a soluble thrombomodulin under the condition without surfactants. The solubility of the soluble thrombomodulin in water such as distilled water used for injection (in the absence of a surfactant such as Triton X-100 or polidocanol, and generally around the neutral pH range) is preferably, for example, 1 mg/mL or more or 10 mg/mL or more; preferably 15 mg/mL or more or 17 mg/mL or more; more preferably 20 mg/mL or more, 25 mg/mL or more, or 30 mg/mL or more; particularly preferably 60 mg/mL or more. In some cases, the solubility is, for example, 80 mg/mL or more, or 100 mg/mL or more. For determining whether or not a soluble thrombomodulin is successfully dissolved in water, it is understood that clear appearance of a solution and the absence of apparently observable insoluble substances is served as simple criteria, after the soluble thrombomodulin is dissolved in water and the solution is observed by visual inspection, for example, just under a white light at a position corresponding to an illumination of approximately 1000 luxes. It is also possible to observe the presence or absence of any residue after filtration.

[0025] The molecular weight of the thrombomodulin is not limited so far that it has the thrombomodulin activities as described above. The molecular weight is preferably 100,000 or smaller, more preferably 90,000 or smaller, still more preferably 80,000 or smaller, most preferably 70,000 or smaller, and the molecular weight is preferably 50,000 or larger, most preferably 60,000 or larger. The molecular weight of the soluble thrombomodulin can be easily measured by ordinary methods for measuring molecular weight of protein. Measurement by mass spectrometry is preferred, and MALDI-TOF-MS method is more preferred. For obtaining soluble thrombomodulin having a molecular weight within a desired range, a soluble thrombomodulin, which is obtained by culturing a transformant cell prepared by transfecting a host cell with a DNA encoding soluble thrombomodulin using a vector, can be subjected to fractionation using column chromatography or the like as described later.

[0026] The thrombomodulin used for the present invention preferably comprises the amino acid sequence consisting of the amino acid residues at the positions 19 to 132 of SEQ ID NO:1, which has been known as the central portion of the thrombomodulin activities of human thrombomodulin, and the thrombomodulin is not particularly limited, so long as the thrombomodulin comprises the amino acid sequence consisting of the amino acid residues at the positions 19 to 132 of SEQ ID NO: 1. The amino acid sequence consisting of the amino acid residues at the positions 19 to 132 of SEQ ID NO: 1 may be naturally or artificially mutated, so long as the sequence has an action to promote the activation of Protein C by thrombin, namely, one of the thrombomodulin activities. Specifically, the sequence may comprise substitution, deletion, or addition of one or more amino acid residue in the amino acid sequence consisting of the amino acid residues at the positions 19 to 132 of SEQ ID NO:1. Acceptable level of the mutation is not particularly limited, so long as the amino acid sequence has the thrombomodulin activities. An example includes a homology 50% or more as amino acid sequences, and the homology is preferably 70% or more, more preferably 80% or more, further preferably 90% or more, particularly preferably 95% or more, and most preferably 98% or more. Such mutated amino acid sequence including substitution, deletion or addition of one or more amino acid residues is referred to as homologous mutation sequence. As described later, these mutated amino acid sequences can be easily produced by using ordinary gene manipulation techniques. The thrombomodulin is not particularly limited so far that it has the aforementioned sequence and the action of selectively binding to thrombin to promote activation of Protein C by thrombin at least as the whole thrombomodulin, but the thrombomodulin preferably also has the anti-inflammatory action.

[0027] The amino acid sequence of SEQ ID NO: 3 comprises the mutation of Val as the amino acid at the position 125 of the sequence of SEQ ID NO:1 to Ala. The thrombomodulin used for the present invention also preferably comprises the amino acid sequence from the position 19 to 132 of SEQ ID NO: 3.

[0028] As described above, although the thrombomodulin used for the present invention is not particularly limited so long that the thrombomodulin has at least the amino acid sequence from the position 19 to 132 of SEQ ID NO: 1 or 3, or a homologous mutation sequence thereof, and

comprises at least a peptide sequence having the thrombomodulin activities, preferred examples of the thrombomodulin include a peptide consisting of the sequence from the position 19 to 132 or 17 to 132 in either of SEQ ID NO: 1 or SEQ ID NO: 3, and a peptide consisting of a homologous mutation sequence of the aforementioned sequence and having at least the thrombomodulin activities. A peptide consisting of the sequence from the position 19 to 132 in either of SEQ ID NO:1 or SEQ ID NO:3 is more preferred. In another embodiment, a peptide consisting of a homologous mutation sequence of the sequence from the position 19 to 132 or 17 to 132 in either of SEQ ID NO: 1 or SEQ ID NO: 3 and having at least the thrombomodulin activities is more preferred.

[0029] As another embodiment of the thrombomodulin used for the present invention, the thrombomodulin preferably comprises the amino acid sequence from the positions 19 to 480 of SEQ ID NO:5, which is not particularly limited so long as the thrombomodulin comprises the amino acid sequence from the position 19 to 480 of SEQ ID NO:5. The amino acid sequence from the positions 19 to 480 of SEQ ID NO:5 may be a homologous mutation sequence thereof, so long as the sequence has an action to promote the activation of Protein C by thrombin, i.e., one of the thrombomodulin activities.

[0030] The sequence of SEQ ID NO:7 comprises the mutation of Val as the amino acid at the position 473 of the sequence of SEQ ID NO:5 to Ala. The thrombomodulin used for the present invention also preferably comprises the amino acid sequence from the position 19 to 480 of SEQ ID NO: 7.

[0031] As described above, although the thrombomodulin used for the present invention is not particularly limited so long as the thrombomodulin has at least the sequence from the position 19 to 480 in either of SEQ ID NO:5 or SEQ ID NO: 7, or a homologous mutation sequence thereof, and comprises at least a peptide sequence having the thrombomodulin activities, preferred examples of the thrombomodulin include a peptide consisting of the sequence from the position 19 to 480 or 17 to 480 in either of SEQ ID NO: 5 or SEQ ID NO:7, and a peptide consisting of a homologous mutation sequence of the aforementioned sequence and having at least the thrombomodulin activities. A peptide consisting of the sequence from the position 19 to 480 of SEQ ID NO: 5 or 7 is more preferred. In another embodiment, a peptide consisting of a homologous mutation sequence of the sequence from the position 19 to 480 or 17 to 480 in either of SEQ ID NO: 5 or SEQ ID NO: 7, and having the thrombomodulin activities is more preferred.

[0032] As another embodiment of the thrombomodulin used for the present invention, the thrombomodulin preferably comprises the amino acid sequence from the position 19 to 515 of SEQ ID NO: 9, which is not particularly limited so long as the thrombomodulin comprises the amino acid sequence from the position 19 to 515 of SEQ ID NO:9. The amino acid sequence from the position 19 to 515 of SEQ ID NO: 9 may be a homologous mutation sequence thereof, so long as the sequence has an action to promote the activation of Protein C by thrombin, i.e., the thrombomodulin activities.

[0033] The amino acid sequence of SEQ ID NO:11 comprises the mutation of Val as the amino acid at the position 473 of SEQ ID NO: 9 to Ala. The thrombomodulin used for the present invention also preferably comprises the amino acid sequence from the position 19 to 515 of SEQ ID NO: 11.

[0034] As described above, although the thrombomodulin used for the present invention is not particularly limited so long as the thrombomodulin has at least the sequence from the position 19 to 515 in either of SEQ ID NO:9 or SEQ ID NO:11, or a homologous mutation sequence thereof, and comprises a peptide sequence having at least the thrombomodulin activities, more preferred examples include a peptide having the sequence from position 19 to 516, 19 to 515, 17 to 516, or 17 to 515 in either of SEQ ID NO:9 or SEQ ID NO:11, and a peptide consisting of a homologous mutation sequence of the aforementioned sequence and having at least the thrombomodulin activities. A peptide having the sequence from the position 19 to 516, 19 to 515, 17 to 516, or 17 to 515 of SEQ ID NO:9 is particularly preferred. A mixture thereof is also a preferred example. In another embodiment, a peptide having the sequence from the position 19 to 516, 19 to 515, 17 to 516, or 17 to 515 of SEQ ID NO: 11 is particularly preferred. A mixture thereof is also a preferred example. Further, a peptide consisting of a homologous mutation sequence thereof and having at least the thrombomodulin activities is also a preferred example. It is preferred that the soluble thrombomodulin also has the anti-inflammatory action.

[0035] A peptide having a homologous mutation sequence is as described above, and means a peptide that may comprise substitution, deletion, or addition of at least one, namely, one or more, preferably several (for example, 1 to 20, preferably 1 to 10, more preferably 1 to 5, particularly preferably 1 to 3) amino acid residues, in the amino acid sequence of the subjected peptide. Although acceptable level of mutation is not particularly limited so long as the peptide has the thrombomodulin activities, an example of the acceptable level of homology includes 50% or more as an amino acid sequences, and the homology may be preferably 70% or more, more preferably 80% or more, further preferably 90% or more, particularly preferably 95% or more, and most preferably 98% or more.

[0036] Preferred examples of the thrombomodulin used for the present invention also include the peptide consisting of the sequence of SEQ ID NO: 14 (462 amino acid residues), the peptide consisting of the sequence of SEQ ID NO: 8 (272 amino acid residues), and the peptide consisting of the sequence of SEQ ID NO: 6 (236 amino acid residues) described in Japanese Patent Unexamined Publication No. 64-6219.

[0037] The thrombomodulin used for the present invention is not particularly limited so long as the thrombomodulin has at least the amino acid sequence from the position 19 to 132 in either of SEQ ID NO:1 or SEQ ID NO:3. As such a thrombomodulin, a peptide having at least the amino acid sequence from the position 19 to 480 in either of SEQ ID NO:5 or SEQ ID NO:7 is preferred, and a peptide having at least the amino acid sequence from the position 19 to 515 in either of SEQ ID NO:9 or SEQ ID NO:11 is more preferred. A more preferred example of the peptide having at least the amino acid sequence from the position 19 to 515 in either of SEQ

ID NO:9 or SEQ ID NO:11 is a peptide having the sequence from the position 19 to 516, 19 to 515, 19 to 514, 17 to 516, 17 to 515, or 19 to 514 in either of SEQ ID NO: 9 or SEQ ID NO: 11. Furthermore, a mixture of peptides each consisting of the sequence from the position 19 to 516, 19 to 515, 19 to 514, 17 to 516, 17 to 515, or 19 to 514 in either of SEQ ID NO:9 or SEQ ID NO:11 is also a preferred example.

[0038] In the case of the aforementioned mixture, the mixing ratio of a peptide that starts from the position 17 and a peptide that starts from the position 19 for each of SEQ ID NOS: 9 and 11 is, for example, 30:70 to 50:50, preferably 35:65 to 45:55.

[0039] Further, the mixing ratio of a peptide that terminates at the position 514, a peptide that terminates at the position 515, and a peptide that terminates at the position 516 for each of SEQ ID NOS: 9 and 11 is, for example, 0:0:100 to 0:90:10, or 0:70:30 to 10:90:0, or 10:0:90 to 20:10:70, if desired.

[0040] The mixing ratio of the peptides can be determined by an ordinary method.

[0041] The sequence of the positions 19 to 132 in SEQ ID NO: 1 corresponds to the sequence of the positions 367 to 480 in SEQ ID NO:9, and the sequence of the positions 19 to 480 in SEQ ID NO: 5 corresponds to the sequence of the positions 19 to 480 in SEQ ID NO:9. Further, the sequence of the positions 19 to 132 in SEQ ID NO: 3 corresponds to the sequence of the positions 367 to 480 in SEQ ID NO:11, and the sequence of the positions 19 to 480 in SEQ ID NO:7 corresponds to the sequence of the positions 19 to 480 in SEQ ID NO: 11. Furthermore, all the sequences of the positions 1 to 18 in SEQ ID NOS: 1, 3, 5, 7, 9 and 11 are identical sequences.

[0042] As described below, these thrombomodulins used for the present invention can be obtained from transformant cells prepared by transfecting host cells with a DNA encoding the peptide (specifically, the nucleotide sequences of SEQ ID NOS: 2, 4, 6, 8, 10, 12, and the like) by using a vector.

[0043] It is sufficient that these peptides only have the aforementioned amino acid sequences, and a sugar chain may be attached or not attached, which is not particularly limited. In gene manipulation techniques, a type of a sugar chain, a position to which a sugar chain is added, and a level of addition thereof differ depending on a type of host cells used, and any techniques may be used. As for binding position of a sugar chain and a type thereof, facts described in Japanese Patent Unexamined Publication No. 11-341990 are known, and the thrombomodulins according to the present invention may be added with the same sugar chain at the same position. Two types of N-linked sugar chains, those of fucosyl biantennary type and fucosyl triantennary type, may bind to the thrombomodulin of this embodiment, and ratio thereof is, for example, 100:0 to 60:40, preferably 95:5 to 60:40, more preferably 90:10 to 70:30. The ratio of these sugar chains can be measured on a two-dimensional sugar chain map described in Biochemical Experimental Methods, Vol. 23, Methods of Researches on Glycoprotein Sugar Chains, Japan Scientific Societies Press (1990), and the like. Furthermore,

when a sugar composition of the thrombomodulin of this embodiment is examined, neutral saccharides, aminosaccharides, and sialic acid are detected, of which content may be, each independently for example, 1 to 30%, preferably 2 to 20%, more preferably 5 to 10%, in terms of weight ratio based on the protein content. The sugar contents can be measured by the methods described in Lecture of New Biochemical Experiments, Vol. 3, Sugar I, Glycoprotein (Book 1), Tokyo Kagaku Dojin (1990) (neutral saccharides: phenol-sulfuric acid method, aminosaccharides: Elson-Morgan method, sialic acid: periodic acid-resorcinol method).

[0044] Although the method for obtaining thrombomodulin is not limited to obtaining it by genetic manipulation as described later, as a signal sequence that can be used for expression where the thrombomodulin is obtained by gene manipulation, a nucleotide sequence encoding the amino acid sequence of the positions 1 to 18 in SEQ ID NO:9, and a nucleotide sequence encoding the amino acid sequence of the positions 1 to 16 in SEQ ID NO:9 can be used, and other known signal sequences such as the signal sequence of human tissue plasminogen activator can also be used (International Publication WO88/9811).

[0045] When a DNA sequence encoding thrombomodulin is introduced into a host cell, examples of preferred methods include a method of incorporating a DNA sequence encoding thrombomodulin into, preferably, a vector, more preferably an expression vector capable of being expressed in animal cells, and then introducing the DNA with the vector. An expression vector is a DNA molecule that is constituted with a promoter sequence, a sequence for adding a ribosome binding site to mRNA, a DNA sequence encoding a protein to be expressed, a splicing signal, a terminator sequence for transcription termination, a replication origin sequence, and the like. Examples of preferred animal cell expression vector include pSV2-X reported by Mulligan R.C. et al. (Proc. Natl. Acad. Sci. U.S.A., 78, 2072 (1981)); pBP69T (69-6) reported by Howley P.M. et al. (Methods in Enzymology, 101, 387 (1983), Academic Press), and the like. Further, there is also another preferred embodiment in which DNA is introduced into an expression vector expressible in a microorganism.

[0046] Examples of host cell that can be used in production of such peptides as mentioned above include animal cells. Examples of the animal cells include Chinese hamster ovary (CHO) cells, COS-1 cells, COS-7 cells, VERO (ATCC CCL-81) cells, BHK cells, canine kidney-derived MDCK cells, hamster AV-12-664 cells, and the like. In addition, examples of host cell derived from human include HeLa cells, WI38 cells, human 293 cells, and PER.C6 cells. Of these cells, CHO cells are very common and preferred, and among the CHO cells, dihydrofolate reductase (DHFR)-deficient CHO cells are more preferred.

[0047] In a gene manipulation process or a peptide production process, microorganisms such as *Escherichia coli* are also often used. A host-vector system suitable for each process is preferably used, and an appropriate vector system can also be selected for the aforementioned host cells. A thrombomodulin gene used in a genetic recombination technique has been cloned. Examples of production of thrombomodulin by such a gene recombination technique have been disclosed, and further, methods for purifying thrombomodulin to obtain a purified product thereof are also known (Japanese Patent Unexamined Publication Nos. 64-

6219, 2-255699, 5-213998, 5-310787, 7-155176; and J. Biol. Chem., 264:10351-10353 (1989)). Therefore, the thrombomodulin used for the present invention can be produced by using the methods described in the aforementioned reports, or by similar methods. For example, Japanese Patent Unexamined Publication No. 64-6219 discloses the *Escherichia coli* K-12 strain DH5 (ATCC Accession No. 67283) containing a plasmid pSV2TMJ2 that contains a DNA encoding the full-length thrombomodulin. This strain re-deposited at the former National Institute of Bioscience and Human-Technology (currently Independent Administrative Institution, National Institute of Advanced Industrial Science and Technology, International Patent Organism Depositary) (*Escherichia coli* DH5/pSV2TMJ2) (FERM BP-5570) can also be used. The thrombomodulin according to the present invention can be prepared by a known gene manipulation technique using a DNA encoding the full-length thrombomodulin as a starting material.

[0048] The thrombomodulin used in this embodiment may be prepared by a conventionally known method or a similar method. For example, the aforementioned method of Yamamoto et al. (Japanese Patent Unexamined Publication No. 64-6219) or the method described in Japanese Patent Unexamined Publication No. 5-213998 can be referred to. Specifically, for example, a DNA encoding the amino acid sequence of SEQ ID NO: 9 is prepared from a human-derived thrombomodulin gene by a gene manipulation technique, and may be further modified as required. For such modification, in order to obtain a DNA encoding the amino acid sequence of SEQ ID NO:11 (which specifically consists of the nucleotide sequence of SEQ ID NO: 12), codons encoding the amino acid at the position 473 in the amino acid sequence of SEQ ID NO: 9 (in particular, the nucleotide at the position 1418 in SEQ ID NO:10) are mutated by site-directed mutagenesis according to the method described by Zoller M.J. et al. (Method in Enzymology, 100:468-500 (1983), Academic Press). For example, by using a synthetic DNA for mutation having the nucleotide sequence of SEQ ID NO: 13, the nucleotide T at the position 1418 in SEQ ID NO: 10 may be converted to the nucleotide C to obtain a mutated DNA.

[0049] The DNA prepared as described above is incorporated into, for example, Chinese hamster ovary (CHO) cells to obtain transformant cells. Such cells are subjected to appropriate selection, and thrombomodulin purified by a known method can be produced from a culture solution obtained by culturing a selected cell. As described above, the DNA (SEQ ID NO:10) encoding the amino acid sequence of SEQ ID NO: 9 is preferably transfected into the aforementioned host cell.

[0050] The method for producing thrombomodulin of this embodiment is not limited to the aforementioned method. For example, it is also possible to extract and purify the thrombomodulin from urine, blood, other body fluids and the like, or extract and purify the thrombomodulin from a tissue producing thrombomodulin or a culture of the aforementioned tissue and the like. Further, the thrombomodulin may be further subjected to a cleavage treatment using a protease, as required.

[0051] For the culture of the aforementioned transformant cell, a medium used for ordinary cell culture may be used, and it is preferable to culture the transformant cell in various kinds of

media in advance to choose an optimal medium. For example, a known medium such as MEM medium, DMEM medium, and 199 medium may be used as a base medium, and a further improved medium or a medium added with supplements for various media may be used. Examples of the culture method include serum culture, in which culture is performed in a medium containing blood serum, and serum-free culture, in which culture is performed in a medium not containing blood serum. Although the culture method is not particularly limited, the serum-free culture is preferred.

[0052] When serum is added to a medium in the case of the serum culture, bovine serum is preferred. Examples of bovine serum include fetal bovine serum, neonate bovine serum, calf bovine serum, adult bovine serum, and the like, and any of these examples may be used so far that the serum is suitable for the cell culture. As the serum-free medium used in the serum-free culture, commercially available media can be used. Serum-free media suitable for various cells are marketed, and for example, for the CHO cell, CD-CHO, CHO-S-SFMII and CHO-III-PFM are sold by Invitrogen, and IS CHO, IS CHO-CD medium, and the like are sold by Irvine Scientific. These media may be used without any treatment, or they may be improved or added with supplements and used. Examples of the serum-free medium further include the DMEM medium containing 5 mg/L each of insulin, transferrin, and selenious acid. As described above, the medium is not particularly limited so far that the medium can be used to produce the thrombomodulin of this embodiment. The culture method is not particularly limited, and any of batch culture, repetitive batch culture, fed-batch culture, perfusion culture, and the like may be used.

[0053] When the thrombomodulin used for the present invention is prepared by the aforementioned cell culture method, diversity may be observed in the N-terminus amino acid due to posttranslational modification of the protein. For example, the amino acid of the position 17, 18, 19 or 22 in SEQ ID NO: 9 may serve as the N-terminus amino acid. Further, for example, the N-terminus amino acid may be modified so that the glutamic acid at the position 22 is changed to pyroglutamic acid. It is preferred that the amino acid of the position 17 or 19 serves as the N-terminus amino acid, and it is more preferred that the amino acid of the position 19 serves as the N-terminus amino acid. Further, there is also another embodiment in which the amino acid of the position 17 serves as the N-terminus amino acid, which is a preferred embodiment. As for the modification, diversity and the like mentioned above, similar examples can be mentioned for the sequence of SEQ ID NO: 11.

[0054] Further, when the soluble thrombomodulin is prepared by using a DNA having the nucleotide sequence of SEQ ID NO: 10, diversity of the C-terminus amino acid may be observed, and a peptide shorter by one amino acid residue may be produced. Specifically, the C-terminus amino acid may be modified so that the amino acid of the position 515 serves as the C-terminus amino acid, and further the position 515 is amidated. Further, a peptide shorter by two amino acid residues may be produced. Specifically, the amino acid of the position 514 may serve as the C-terminus amino acid. Therefore, any of peptides having significant diversity of the N-terminus amino acid and C-terminus amino acid, or a mixture of them may be produced. It is preferred that the amino acid of the position 515 or the amino acid of the

position 516 serves as the C-terminus amino acid, and it is more preferred that the amino acid of the position 516 serves as the C-terminus amino acid. Further, there is also another embodiment in which the amino acid of the position 514 serves as the C-terminus amino acid, which is a preferred embodiment. Concerning the modification, diversity and the like described above, the same shall apply to a DNA having the nucleotide sequence of SEQ ID NO: 12.

[0055] The thrombomodulin obtained by the method described above may be a mixture of peptides having diversity in the N-terminus and C-terminus amino acids. Specific examples include a mixture of peptides having the sequences of the positions 19 to 516, positions 19 to 515, positions 19 to 514, positions 17 to 516, positions 17 to 515, and positions 17 to 514 in SEQ ID NO: 9.

[0056] Then, isolation and purification of thrombomodulin from a culture supernatant or culture obtained as described above can be carried out by known methods [edited by Takeichi Horio, Tanpakushitsu/Koso no Kiso Jikken Ho (Fundamental Experimental Methods for Proteins and Enzymes) (1981)]. For example, it is preferable to use ion exchange chromatography or adsorption chromatography, which utilizes an interaction between thrombomodulin and a chromatographic carrier on which functional groups having a charge opposite to that of thrombomodulin are immobilized. Another preferred example is affinity chromatography utilizing specific affinity with thrombomodulin. Preferred examples of adsorbent include thrombin that is a ligand of thrombomodulin and an anti-thrombomodulin antibody. As the antibody, anti-thrombomodulin antibodies having appropriate properties or recognizing appropriate epitopes can be used. Examples include, for example, those described in Japanese Patent Publication (Kokoku) No. 5-42920, Japanese Patent Unexamined Publication Nos. 64-45398 and 6-205692 and the like. Other examples include gel filtration chromatography and ultrafiltration, which utilize the molecular size of thrombomodulin. Other examples further include hydrophobic chromatography that utilizes hydrophobic bond between a chromatographic carrier on which hydrophobic groups are immobilized, and a hydrophobic portion of thrombomodulin. Furthermore, hydroxyapatite may be used as a carrier in adsorption chromatography, of which examples include, for example, those described in Japanese Patent Unexamined Publication No. 9-110900. These means may be used in combination, as required. Although degree of purification can be selected depending on a purpose of use and the like, it is desirable to purify thrombomodulin until a single band is obtained as a result of electrophoresis, preferably SDS-PAGE, or a single peak is obtained as a result of gel filtration HPLC or reverse phase HPLC of the isolated and purified product. It should be understood that, when two or more types of thrombomodulins are used, it is preferred that only the bands of the thrombomodulins are substantially obtained, and it is not required to obtain one single band.

[0057] Specific examples of the purification method used in the present invention include a purification method using the thrombomodulin activities as a criterion, for example, a purification method comprising roughly purifying a culture supernatant or a culture product with an ion exchange column Q-Sepharose Fast Flow to collect a fraction having the thrombomodulin activities; then purifying the fraction with an affinity column, DIP-thrombin-

agarose (diisopropylphosphorylthrombin agarose) column, as the main purification step to recover a fraction having potent thrombomodulin activities; then concentrating the recovered fraction and followed by gel filtration to obtain a thrombomodulin active fraction as a purified product (Gomi K. et al., Blood, 75: 1396-1399 (1990)). An example of the thrombomodulin activities used as the criterion is an activity of promoting the activation of Protein C by thrombin. Other preferred examples of the purification method will be exemplified below.

[0058] An appropriate ion exchange resin having good adsorptive condition for thrombomodulin is selected and purification by ion exchange chromatography is performed. A particularly preferred example is a method comprising the use of Q-Sepharose Fast Flow equilibrated with a 0.02 mol/L Tris-HCl buffer (pH 7.4) containing 0.18 mol/L NaCl. After washing as required, elution can be performed with a 0.02 mol/L Tris-HCl buffer (pH 7.4) containing 0.3 mol/L NaCl, for example, to obtain thrombomodulin as a roughly purified product.

[0059] Then, for example, a substance having specific affinity to thrombomodulin can be immobilized on a resin to perform purification by affinity chromatography. Preferred examples include a DIP-thrombin-agarose column and an anti-thrombomodulin monoclonal antibody column. In the case of the DIP-thrombin-agarose column, the column is equilibrated beforehand with a 20 mmol/L Tris-HCl buffer (pH 7.4) containing 100 mmol/L NaCl and 0.5 mmol/L calcium chloride, and the aforementioned roughly purified product is then charged on the column, washed as required, and then eluted with, for example, a 20 mmol/L Tris-HCl buffer (pH 7.4) containing 1.0 mol/L NaCl and 0.5 mmol/L calcium chloride to obtain thrombomodulin as a purified product. In the case of the anti-thrombomodulin monoclonal antibody column, an example of the method comprises: contacting an anti-thrombomodulin monoclonal antibody solution in a 0.1 mol/L NaHCO₃ buffer (pH 8.3) containing 0.5 mol/L NaCl with Sepharose 4FF (GE Health Care Biosciences) activated with CNBr beforehand to obtain the resin Sepharose 4FF coupled with the anti-thrombomodulin monoclonal antibodies, equilibrating the resin filled in a column beforehand with, for example, a 20 mmol/L phosphate buffer (pH 7.3) containing 0.3 mol/L NaCl, washing as required, and then performing elution with a 100 mmol/L glycine-HCl buffer (pH 3.0) containing 0.3 mol/L NaCl. An effluent may be neutralized with an appropriate buffer to obtain a product as a purified product.

[0060] Subsequently, the purified product is adjusted to pH 3.5, and then charged on a cation exchanger, preferably SP-Sepharose FF (GE Health Care Biosciences) as a strong cation exchanger, equilibrated with a 100 mmol/L glycine-HCl buffer (pH 3.5) containing 0.3 mol/L NaCl, and washing is performed with the same buffer to obtain a non-adsorptive fraction. The resulting fraction is neutralized with an appropriate buffer to obtain a highly purified product. These products are preferably concentrated by ultrafiltration.

[0061] Further, it is also preferable to exchange the buffer by gel filtration. For example, a highly purified product concentrated by ultrafiltration can be charged on a Sephacryl S-300 column or S-200 column equilibrated with a 20 mmol/L phosphate buffer (pH 7.3) containing 50 mmol/L NaCl, and then developed for fractionation with a 20 mmol/L phosphate buffer (pH

7.3) containing 50 mmol/L NaCl. The activity for promoting the activation of Protein C by thrombin can be confirmed to collect an active fraction and thereby obtain a buffer-exchanged highly purified product. In order to improve safety, a highly purified product obtained as described above is preferably filtered through an appropriate filter for eliminating viruses such as Planova 15N (Asahi Kasei Medical Co., Ltd.), and then the resultant can be concentrated by ultrafiltration to a desired concentration. Finally, the product is preferably filtered through an aseptic filtration filter.

[0062] According to the present invention, there is provided a medicament for use in therapeutic treatment and/or improvement of sepsis which comprises thrombomodulin as an active ingredient, wherein said medicament is to administer to a patient with severe sepsis accompanied with one or more organ dysfunctions, wherein a value of International Normalized Ratio (INR) of a plasma specimen obtained from said patient is more than 1.4.

[0063] That is, the medicament for use in therapeutic treatment and/or improvement of sepsis according to this embodiment is a medicament for therapeutic treatment and/or improvement of sepsis in a patient with severe sepsis accompanied with one or more organ dysfunctions, wherein a value of International Normalized Ratio (INR) of a plasma specimen obtained from said patient is more than 1.4.

[0064] There is also provided a medicament for use in decreasing mortality of a human patient with severe sepsis accompanied with one or more organ dysfunctions, wherein a value of International Normalized Ratio (INR) of a plasma specimen obtained from said patient is more than 1.4.

[0065] As for the therapeutic treatment and/or improvement of sepsis, examples of preferred effects thereof include, for example, "prevention of death of a patient from sepsis". Examples also include "prevention of aggravation of general conditions of a patient by sepsis".

[0066] The sepsis referred to in this embodiment is known as a severe systemic infectious disease wherein microorganisms continuously or intermittently invade into blood from an infection focus, which disease is induced by a disease such as infectious diseases, malignant tumors, hepatic cirrhosis, renal failure, diabetes, and dystocia, or a therapeutic treatment for injury or disease such as use of indwelling catheter, infusion device, dialysis, and the like and tracheostomy. If the symptoms advance, a systemic shock is induced by septic shock, i.e., rapid decrease of blood pressure and peripheral circulatory failure, and lethality is provided by organ dysfunctions of vital organs, such as lung, kidney, liver, heart, alimentary canal, and central nervous system. As a complication accompanying sepsis, there is induced adult respiratory distress syndrome (ARDS) characterized by edema of lung stroma, hemorrhage and acute respiratory failure due to lung capillary obstruction associated with DIC or activation of neutrophils and migration and accumulation thereof in lung parenchyma, which results in extremely bad prognosis.

[0067] The sepsis referred to in this embodiment is the systemic inflammatory response

syndrome (SIRS) induced by infection. More specifically, it includes a pathological condition that meets, in addition to the presence of infection, two or more of the SIRS items ((1) body temperature $> 38^{\circ}\text{C}$ or $< 36^{\circ}\text{C}$, (2) heart rate $> 90/\text{minute}$, (3) respiration rate $> 20/\text{minute}$, or $\text{PaCO}_2 < 32$ torr, and (4) leucocyte count $> 12,000/\mu\text{L}$ or $< 4000/\mu\text{L}$, or bademia $> 10\%$), and sepsis can be basically diagnosed on the basis of such a pathological condition.

[0068] There are several methods for diagnosing sepsis, and they are summarized in Levy M. et al., Crit. Care. Med., 31:1250-1256. For example, there are a method based on diagnosis performed by a medical practitioner, and a method of using test values and the like. Examples of the latter include a method in which when two items are fulfilled among the four items of (1) body temperature $> 38^{\circ}\text{C}$ or $< 36^{\circ}\text{C}$, (2) heart rate $> 90/\text{minute}$, (3) respiration rate $> 20/\text{minute}$, or necessity of artificial respiration, and (4) leucocyte count $> 12,000/\mu\text{L}$ or $< 4000/\mu\text{L}$, or bandemia $> 10\%$, diagnosis of SIRS is established, and SIRS for which a microorganism is identified or suspected as the cause thereof is diagnosed as sepsis [LaRosa S., the homepage of The Cleveland Clinic]. Another method similar to the above method is described in Members of the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: Crit. Care Med., 20, 864-874 (1992).

[0069] Examples of the symptoms of sepsis include, for example, bacteriemia, septicemia, systemic inflammatory response syndrome (SIRS), sepsis (SIRS for which a microorganism is identified or suspected as the cause thereof), severe sepsis, septic shock, intractable septic shock, and multiple organ dysfunction syndrome (henceforth also referred to as MODS) (Harrison's Principles of Internal Medicine, 15th edition of original work, Section 124, pp.828-833, Medical Science International, Ltd.). The aforementioned conditions are exemplified as symptoms on which the medicament for use of the present invention for therapeutic treatment and/or improvement is effective.

[0070] Although the sepsis is not particularly limited so long as a disease is diagnosed as sepsis on the basis of the aforementioned diagnosis criteria, it is preferably sepsis accompanied by abnormal coagulation (sepsis with coagulopathy). Although the coagulopathy is not particularly limited so long as INR of a plasma specimen obtained from patient is more than 1.4.

[0071] Examples of the bacteriemia include a condition that presence of bacteria in blood is verified by a positive result of blood culture.

[0072] Examples of the septicemia include a condition that presence of microorganisms or other toxins in blood is confirmed.

[0073] Examples of the systemic inflammatory response syndrome (SIRS) include a condition of a preliminary stage of DIC, as described above.

[0074] Examples of the severe sepsis include sepsis accompanied by one or more symptoms including organ dysfunction such as metabolic acidosis, organ hypoperfusion, acute

encephalopathy, oliguria, hypoxemia or disseminated intravascular coagulation, and hypotension. As sepsis, one presenting organ dysfunction, organ hypoperfusion, or hypotension is called severe sepsis. The organ hypoperfusion or abnormal perfusion includes lactic acidosis, oliguria, mental clouding, and the like. Among the severe sepsis, a condition persistently presenting hypotension despite of sufficient load of fluid therapy is called as septic shock.

[0075] More specifically, the severe sepsis referred to in this embodiment is as follows.

[0076] Examples of the septic shock include a condition with hypotension (blood pressure of 90 mmHg or lower or lower than usual blood pressure by 40 mmHg or more), not responding to resuscitation by fluid replacement, and accompanied by organ failure.

[0077] Examples of the intractable septic shock include a condition with septic shock continuing over 1 hour or longer, and not responding to a hypertensor with fluid therapy.

[0078] Examples of the multiple organ dysfunction syndrome (MODS) include a condition with malfunction of one or more organs, and requiring medical intervention for maintaining homeostasis.

[0079] INR referred to in this embodiment is an examination criterion that defines blood coagulopathy. INR means a prothrombin time (henceforth also abbreviated as PT) normalized as for differences between manufacturing lots of thromboplastin preparations. INR is generally defined as follows:

$$\text{INR value} = (\text{Coagulation time (sec) of test specimen} / \text{Coagulation time (sec) of control specimen})^{\text{ISI value}}$$

[0080] In the equation, coagulation time (sec) of test specimen represents PT of test plasma specimen of a subject to be examined, and ISI represents International Sensitivity Index.

[0081] Examples of the severe sepsis referred to in this embodiment include sepsis accompanied by one or more symptoms including organ dysfunction such as metabolic acidosis, acute encephalopathy, oliguria, hypoxemia or disseminated intravascular coagulation, and hypotension, as described above. The term severe means that the disease is in a critical condition for life support. Examples of the severe sepsis include, in particular, sepsis accompanied by one or more organ dysfunctions. Although the organ dysfunction is not particularly limited so far that the organ dysfunction is induced by sepsis, the organ dysfunction preferably includes failure of an organ that is essential for supporting life. Examples of the one or more organ dysfunctions include one or more organ dysfunctions selected from the group consisting of circulatory organ dysfunction, respiratory organ dysfunction, kidney dysfunction and liver dysfunction, preferred examples include one or more organ dysfunctions selected from the group consisting of respiratory organ dysfunction, circulatory organ dysfunction, and kidney dysfunction, and more preferred examples include one or more organ dysfunctions

selected from the group consisting of respiratory organ dysfunction, and circulatory organ dysfunction. Although number of the organ dysfunctions is not particularly limited so far that the number is one or more, the number may be preferably two or more. In particular, it is preferred that there are two kinds of organ dysfunctions of respiratory organ dysfunction and circulatory organ dysfunction.

[0082] The circulatory organ dysfunction is not particularly limited so long as a generally known circulatory organ dysfunction, and examples include, for example, blood pressure decrease and shock.

[0083] The respiratory organ dysfunction is not particularly limited so long as a generally known respiratory organ dysfunction, and examples include, for example, hypoxemia, acute lung injury and dyspnea.

[0084] The kidney dysfunction is not particularly limited so long as a generally known kidney dysfunction, and examples include, for example, renal function disorder, oliguria, and renal failure.

[0085] The liver dysfunction is not particularly limited so long as a generally known liver dysfunction, and examples include, for example, hepatic function disorder, jaundice, hepatic failure, and the like.

[0086] These organ dysfunctions are generally known as described in publications published before the application date of this application, for example, Funada H., "Elucidation and Treatment Strategy for Sepsis", Iyaku Journal Co., Ltd., p.38- (2006)), "Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock 2008" (Crit. Care Med., 2008 Jan;36(1):296-327), and the like.

[0087] It is supposed that organ dysfunction may be induced by a factor other than sepsis, such as in the case of drug-induced organ dysfunction, and accordingly, it is desirable that patients with organ dysfunction limited to the liver or kidney are excluded from the severe septic patients. It is also known that thrombocytopenia may be developed as a result of organ dysfunction. Although platelet count in patients to be administered with the medicament of this embodiment is not particularly limited so far that the platelet count is less than 300,000/ μ L, the count is preferably less than 200,000/ μ L, more preferably less than 150,000/ μ L.

[0088] In this embodiment, the value of INR in a plasma specimen of a sepsis patient is not particularly limited so far that the value is more than 1.4, and when INR is more than 1.4, thrombomodulin is more effective for sepsis patients with one or more organ dysfunctions. The maximum INR may be, for example, 2.0 or lower, preferably 1.9 or lower, more preferably 1.8 or lower, still more preferably 1.7 or lower, most preferably 1.6 or lower. The value may also be preferably 1.5 or lower. It may also be preferred that a patient with an INR value of 1.7 is excluded.

[0089] The expression "INR more than 1.4" may also be indicated as "INR > 1.4".

[0090] In this embodiment, DIC is a disease or syndrome whereby large quantities of blood coagulation-accelerating substances are generated as a result of tissue damage caused by various diseases, so that the function of a coagulation system is excessively accelerated, and small thrombuses are formed in generalized blood vessel (microthrombus formation) and they clog small vessels, and at the same time, thrombocytes or coagulation factors necessary for the control of bleeding are consumed, thereby causing clotting abnormality. Specifically, as a result of fibrin formation in vascular vessel, bleeding due to consumption coagulopathy or organ failure due to microthrombus formation occurs. DIC is also referred to as disseminated intravascular coagulation syndrome or diffuse intravascular coagulation syndrome.

[0091] DIC has various types of clinical symptoms depending on the type of underlying pathogenic condition. In addition to observation of bleeding or organ symptoms, a preferred method for diagnosing an illness as DIC comprises keeping the score of DIC on the basis of several test values as described below and then diagnosing the illness as DIC when the DIC score has reached a certain level. Examples of such test values include the number of blood platelets, the concentration of fibrin/fibrinogen degradation products (hereinafter abbreviated as FDP, at times) decomposed by plasmin, a D-dimer concentration, a fibrinogen concentration, and a prothrombin time. Moreover, it is also possible to diagnose a certain condition as preDIC based on a decrease in platelets, an increase in the D-dimer or FDP concentration, and the like without keeping the DIC score (Masao Nakagawa, "Search report regarding use of criteria of disseminated intravascular coagulation (DIC)," Research Study Team of Intractable Disease (Blood Coagulation Abnormality), the Ministry of Health and Welfare, Study report 1999, 1999:65-72; Katsumi Deguchi, "Tentative plan regarding standards for initiation of early treatment of DIC," Research Study Team of Intractable Disease (Blood Coagulation Abnormality), the Ministry of Health and Welfare, Study report 1999, 1999:73-77; and Katsumi Nakagawa & Hajime Tsuji, "Current diagnosis of DIC-Reports on results of inquiry survey" Clinical Blood. 1999, 40:362-364).

[0092] In this embodiment, a sepsis patient with an INR value more than 1.4, in a plasma specimen of the patient can be called a DIC patient in a broad sense, and the medicament for therapeutic treatment and/or improvement of sepsis according to this embodiment may be used as a medicament for therapeutic treatment and/or improvement of DIC.

[0093] The medicament for use of this embodiment may also be used for DIC. Sepsis is also regarded as SIRS induced by critical clinical invasion from infection, and closely relates to DIC of which causative disease is an infectious disease. DIC is often developed simultaneously with sepsis, and the medicament of this embodiment may also be used for such a sepsis patient simultaneously developing DIC. In other words, the medicament for use of this embodiment may be used for a patient suffering from or suspected to suffer from either one of DIC and sepsis, or the both.

[0094] In this embodiment, INR can be measured, for example, as follows. Specifically, tissue

thromboplastin and Ca^{2+} are added to plasma (test specimen) obtained by adding sodium citrate, time (PT) required for coagulation (precipitation of fibrin) is measured, and evaluation is established on the basis of relative ratio of the time in terms of second with respect to that of a control specimen (activity ratio). The activity ratio can be obtained as "coagulation time (second) of test specimen/coagulation time (second) of control specimen", but the ratio may vary among laboratories in which the test is implemented due to difference in sensitivity of used tissue thromboplastin. The INR value was devised in order to eliminate such variation, and by evaluating PT using the INR value corrected with an international sensitivity index (henceforth also abbreviated as ISI), variation caused by difference of laboratories can be eliminated to obtain a standard result. ISI represents difference from the international standard sample. ISI is determined for every tissue thromboplastin reagent, and is attached to the reagent. Examples of the thromboplastin reagent include Thromborel S (registered trademark, Sysmex Corp.), Thromboplastin C+ (registered trademark, Sysmex Corp.), and the like, but not limited to these examples. Thromborel S (registered trademark) uses human placenta thromboplastin (ISI value is around 1.0), and thromboplastin C+ (registered trademark) uses rabbit brain thromboplastin (ISI value is about 1.8).

[0095] ISI is attached to each tissue thromboplastin reagent, and the INR value is calculated in accordance with Equation 1 mentioned above.

[0096] Although the control specimen is not particularly limited so far that the specimen is a commercially available pooled normal human plasma, there can be used commercially available pooled citrated (Na citrate) normal human plasma, and the like, available from, for example, Kojin-Bio Co., Ltd. or International Bioscience Inc.

[0097] As therapeutic treatments of sepsis, such basic treatments as mentioned below are generally performed by referring to known publications (Surviving Sepsis Campaign: International guidelines for management of severe sepsis and septic shock: Crit. Care Med., 2008, 36:296-327; Crit. Care Med., 32(3), 1250-56 (2003)), and thrombomodulin and another medicament may be used in combination. However, the other medicament used in combination is not limited to those mentioned below.

[0098] When hypotension continues in a septic shock patient even after the central venous pressure (CVP) rises to a desired value, dopamine may be administered in order to raise the average blood pressure to at least 60 mmHg. When the dopamine dose exceeds 20 $\mu\text{g}/\text{kg}/\text{minute}$, another vasopressor (usually norepinephrine) may be additionally administered.

[0099] For therapeutic treatment against causative bacteria of sepsis, an antibiotic is generally used. For the selection of the antibiotic, there is required well-grounded estimation based on suspected cause, clinical sign, knowledge concerning microorganisms and knowledge concerning pattern of sensitivity common to a specific hospital ward for inpatients, results of preliminary culture test, and the like. Intensive normalization of blood sugar level in sepsis patients improves clinical outcome of the patients in critical conditions.

[0100] When an antibiotic is used, a specimen such as blood, body fluid or wound part can be investigated, and a drug effective for the causative bacterium can be chosen. For example, in the case of septic shock of unknown cause, gentamycin or tobramycin and a third generation cephalosporin may be administered in combination. Further, when infection of resistant *Staphylococcus* or *Enterococcus* bacteria is suspected, vancomycin is additionally administered.

[0101] In general, the dose is adjusted to maintain the blood sugar level at 80 to 110 mg/dL (4.4 to 6.1 mmol/L) by continuous intravenous injection of insulin.

[0102] Since corticosteroid therapy is effective for the therapeutic treatment of sepsis, it may be administered at a supplemental dose.

[0103] To a patient of high death risk (APACHE II score ≥ 25 , multiple organ failure due to sepsis, ARDS due to septic shock or sepsis), a recombinant activated Protein C (rhAPC, drotrecogin α) may be administered when there are no contraindications (hemorrhage and the like).

[0104] Although target patients are limited, packed red blood cell transfusion may be performed aiming at Hb 7.0 to 9.0 g/dL.

[0105] In the case of impaired erythropoiesis in a sepsis patient due to renal failure, erythropoietin (EPO) may be administered.

[0106] In the case of severe sepsis, heparin may be administered at a low dose unfractionated heparin or low molecular weight heparin may be administered for prevention of DVT.

[0107] The medicament for use of the present invention may contain a carrier. As the carrier usable in the present invention, a water-soluble carrier is preferred, and tonicity agent, buffering agent, viscosity enhancer, surfactant, preservative, antiseptic, soothing agent, pH modifier, or the like acceptable as pharmaceutical additives is usually preferred. For example, the medicament for use of the present invention can be prepared by adding sucrose, glycerin, pH modifier consisting of an inorganic salt, or the like as additives. Further, if necessary, amino acids, salts, carbohydrates, surfactants, albumin, gelatin or the like may be added as disclosed in Japanese Patent Unexamined Publication Nos. 64-6219 and 6-321805. Method for adding these additives is not particularly limited. However, in the case of preparing a lyophilized product, examples include, for example, a method of mixing a solution containing at least one therapeutic agent selected from an immunosuppressant and a therapeutic agent for hematological malignancy, and a solution containing thrombomodulin, then adding additives to the mixture, and mixing the resulting mixture, and a method of mixing additives with at least one therapeutic agent selected from an immunosuppressant and a therapeutic agent for hematological malignancy dissolved in water, water for injection, or an appropriate buffer beforehand, adding a solution containing thrombomodulin to the mixture, mixing the resulting mixture to prepare a solution, and lyophilizing the solution, in manners as those commonly

employed. When the medicament of the present invention is a medicament comprising a combination of the components of the medicament, each component is preferably prepared by adding a carrier according to an appropriate preparation method. The medicament for use of the present invention may be provided in the form of an injection, or in the form of a lyophilized preparation to be dissolved upon use.

[0108] As for preparation of the medicament for use of the present invention, an aqueous solution for injection can be prepared by filling a solution containing 0.1 to 10 mg of thrombomodulin, water for injection, and additives in an ampoule or vial in a volume of, for example, 0.5 to 10 mL. Examples of the preparation method also include a method of freezing such a solution, and drying the frozen solution under reduced pressure to prepare a lyophilized preparation.

[0109] The medicament for use of the present invention is desirably administered by parenteral administration such as intravenous administration, intramuscular administration, and subcutaneous administration. The medicament may also be administered by oral administration, intrarectal administration, intranasal administration, sublingual administration or the like. When the medicament for use of the present invention is a medicament comprising a combination of multiple active ingredients, each active ingredient of the medicament is preferably administered by an administration method suitable for the ingredient.

[0110] Examples of method for the intravenous administration include a method of administering a desired dose of the medicament at one time (intravenous bolus administration), and intravenous administration by drip infusion.

[0111] The method of administering a desired dose of the medicament at one time (intravenous bolus administration) is preferred from the viewpoint that the method requires only a short time for administration. Especially it is preferred in case of sepsis patients who need the urgent treatment. When the medicament is administered at one time, a period required for administration by using an injectable syringe may generally varies. In general, the period of time required for the administration is, for example, 5 minutes or shorter, preferably 3 minutes or shorter, more preferably 2 minutes or shorter, still more preferably 1 minute or shorter, particularly preferably 30 seconds or shorter, although it depends on a volume to be administered. Although the minimum administration time is not particularly limited, the period is preferably 1 second or longer, more preferably 5 seconds or longer, still more preferably 10 seconds or longer. The dose is not particularly limited so long that the dose is within the aforementioned preferred dose. Intravenous administration by drip infusion is also preferred from a viewpoint that blood level of thrombomodulin can be easily kept constant.

[0112] A daily dose of the medicament for use of the present invention may vary depending on age, body weight of patients, severity of disease, administration route and the like. In general, the maximum dose is preferably 20 mg/kg or less, more preferably 10 mg/kg or less, still more preferably 5 mg/kg or less, particularly preferably 2 mg/kg or less, and most preferably 1 mg/kg or less, and the minimum dose is preferably 0.001 mg/kg or more, more preferably 0.005

mg/kg or more, still more preferably 0.01 mg/kg or more, particularly preferably 0.02 mg/kg or more, and most preferably 0.05 mg/kg or more, in terms of the amount of thrombomodulin.

[0113] In the case of intravenous bolus administration, although the dose is not particularly limited so long as the dose is within the aforementioned preferred dose, the maximum daily dose is preferably 1 mg/kg or less, more preferably 0.5 mg/kg or less, still more preferably 0.1 mg/kg or less, particularly preferably 0.08 mg/kg or less, and most preferably 0.06 mg/kg or less, and the minimum dose is preferably 0.005 mg/kg or more, more preferably 0.01 mg/kg or more, still more preferably 0.02 mg/kg or more, and particularly preferably 0.04 mg/kg or more.

[0114] When the medicament for use of the present invention is administered to a patient having a body weight exceeding 100 kg, it may be preferably administered at a fixed dose of 6 mg, since blood volume is not proportional to the body weight, and blood volume is relatively reduced with respect to the body weight in such a patient.

[0115] In the case of continuous intravenous infusion, although the dose is not particularly limited so long as the dose is within the aforementioned preferred dose, the maximum daily dose is preferably 1 mg/kg or less, more preferably 0.5 mg/kg or less, still more preferably 0.1 mg/kg or less, particularly preferably 0.08 mg/kg or less, and most preferably 0.06 mg/kg or less, and the minimum dose is preferably 0.005 mg/kg or more, more preferably 0.01 mg/kg or more, still more preferably 0.02 mg/kg or more, and particularly preferably 0.04 mg/kg or more.

[0116] When the medicament for use of the present invention is administered to a patient having a body weight exceeding 100 kg, it may be preferably administered at a fixed dose of 6 mg, since blood volume is not proportional to the body weight, and blood volume is relatively reduced with respect to the body weight in such a patient.

[0117] The medicament is administered once or several times a day as required. As for administration interval, the medicament may be administered once in 2 to 14 days, preferably once in 2 to 7 days, more preferably once in 3 to 5 days.

[Explanation of Sequence listing]

[0118]

SEQ ID NO: 1: Amino acid sequence encoded by the gene used in production of TME456

SEQ ID NO:2: Nucleotide sequence encoding the amino acid sequence of SEQ ID NO:1

SEQ ID NO:3: Amino acid sequence encoded by the gene used in production of TME456M

SEQ ID NO: 4: Nucleotide sequence encoding the amino acid sequence of SEQ ID NO:3

SEQ ID NO: 5: Amino acid sequence encoded by the gene used in production of TMD12

SEQ ID NO: 6: Nucleotide sequence encoding the amino acid sequence of SEQ ID NO:5

SEQ ID NO:7: Amino acid sequence encoded by the gene used in production of TMD12M

SEQ ID NO: 8: Nucleotide sequence encoding the amino acid sequence of SEQ ID NO:7

SEQ ID NO: 9: Amino acid sequence encoded by the gene used in production of TMD123

SEQ ID NO: 10: Nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 9

SEQ ID NO:11: Amino acid sequence encoded by the gene used in production of TMD123M

SEQ ID NO: 12: Nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 11

SEQ ID NO:13: Synthetic DNA for mutation used for carrying out site-directed mutagenesis

Examples

[0119] The present invention will be explained in detail with reference to examples and test examples. However, the present invention is not limited by these examples.

[0120] The thrombomodulin of the present invention used in the test examples was prepared according to the aforementioned method of Yamamoto et al. (the method described in Japanese Patent Unexamined Publication No. 64-6219). Preparation examples thereof are described below. Safety of the thrombomodulins obtained in these preparation examples was confirmed by single and repetitive intravenous administration tests using rats and monkeys, mouse reproduction test, local irritation test, pharmacological safety test, virus inactivation test, and the like.

[Preparation Example 1]

<Obtaining thrombomodulin>

[0121] A highly purified product was obtained by the aforementioned method. Specifically, Chinese hamster ovary (CHO) cells were transfected with a DNA encoding the amino acid sequence of SEQ ID NO:9 (which specifically consisted of the nucleotide sequence of SEQ ID NO:10). From the culture of the above transformant cells, a highly purified product was obtained by collecting an active fraction with a 20 mmol/L phosphate buffer (pH 7.3) containing 50 mmol/L NaCl according to the aforementioned conventional purification method. The product was further concentrated by using an ultrafiltration membrane to obtain a thrombomodulin solution having a concentration of 11.0 mg/mL (henceforth also abbreviated

as TMD123 in the specification).

<Preparation of polysorbate solution>

[0122] Polysorbate 80 was weighed (0.39 g) in a glass beaker, added with water for injection (30 mL), and dissolved.

<Preparation and filling of drug solution>

[0123] The TMD123 solution obtained above (2239 mL, corresponding to 24.63 g of soluble thrombomodulin protein, added in a 5% excess amount) was put into a 5-L stainless steel vessel. The polysorbate solution obtained above was further added, and sodium chloride (27.9 g) was added. Water for injection (600 mL) was added, and the mixture was stirred. The mixture was adjusted to pH 6.0 by adding a 1 mol/L hydrochloric acid solution. Water for injection was further added to the mixture up to a total amount of 3940 g, and the mixture was uniformly mixed and stirred. This drug solution was subjected to filtration sterilization using a filter having a pore diameter of 0.22 μm (MCGL10S, manufactured by Millipore). The filtrate was filled in ampoules in an amount of 1.1 g each to obtain a TMD123 preparation.

[Preparation Example 2]

[0124] Chinese hamster ovary (CHO) cells are transfected with a DNA encoding the amino acid sequence of SEQ ID NO:11 (which specifically consists of the nucleotide sequence of SEQ ID NO:12), a solution of thrombomodulin purified from a culture of the above transformant cells (henceforth also abbreviated as TMD123M in the specification) by the aforementioned conventional purification method is obtained, and a TMD123M preparation is obtained in the same manner as that described above.

[Preparation Example 3]

[0125] Chinese hamster ovary (CHO) cells are transfected with a DNA encoding the amino acid sequence of SEQ ID NO: 1 (which specifically consists of the nucleotide sequence of SEQ ID NO: 2), thrombomodulin purified from a culture of the above transformant cells (henceforth also abbreviated as TME456 in the specification) by the aforementioned conventional purification method is obtained, and a TME456 preparation is obtained in the same manner as that described above.

[Preparation Example 4]

[0126] Chinese hamster ovary (CHO) cells are transfected with a DNA encoding the amino acid sequence of SEQ ID NO: 3 (which specifically consists of the nucleotide sequence of SEQ ID NO: 4), thrombomodulin purified from a culture of the above transformant cells (henceforth also abbreviated as TME456M in the specification) by the aforementioned conventional purification method is obtained, and a TME456M preparation is obtained in the same manner as that described above.

[Preparation Example 5]

[0127] Chinese hamster ovary (CHO) cells are transfected with a DNA encoding the amino acid sequence of SEQ ID NO: 5 (which specifically consists of the nucleotide sequence of SEQ ID NO: 6), thrombomodulin purified from a culture of the above transformant cells (henceforth also abbreviated as TMD12 in the specification) by the aforementioned conventional purification method is obtained, and a TMD12 preparation is obtained in the same manner as that described above.

[Preparation Example 6]

[0128] Chinese hamster ovary (CHO) cells are transfected with a DNA encoding the amino acid sequence of SEQ ID NO: 7 (which specifically consists of the nucleotide sequence of SEQ ID NO: 8), thrombomodulin purified from a culture of the above transformant cells (henceforth also abbreviated as TMD12M in the specification) by the aforementioned conventional purification method is obtained, and a TMD12M preparation is obtained in the same manner as that described above.

[Preparation Example 7]

Preparation of placebo formulation

<Preparation of polysorbate solution>

[0129] Polysorbate 80 was weighed (0.4 g) in a glass beaker, added with water for injection (30 mL), and dissolved.

<Preparation and filling of drug solution>

[0130] Water for injection (2000 mL) was put into a 5-L stainless steel vessel. The polysolvate solution obtained above was further added. Water for injection was further added to the mixture up to a total amount of 4000 g, and the mixture was uniformly mixed and stirred. This drug solution was subjected to filtration sterilization using a filter having a pore diameter of 0.22 μm (MCGL10S, manufactured by Millipore). The filtrate was filled in ampoules in an amount of 1.1 g each to obtain a placebo preparation.

[Example 1]

<Method for Experiment>

[0131] By using TMD-123 prepared according to Preparation Example 1 as thrombomodulin, a randomized double-blind placebo-controlled study was conducted for patients with sepsis and DIC. Targeted patient number is 750 in total, and among them, 741 patients were administered with investigational drug (TMD-123 for 370 patients and placebo for 371 patients). TMD-123 was administered once a day at 0.06 mg/kg for successive 6 days via intravenous bolus administration. As the placebo, the preparation manufactured according to Preparation Example 7 was used.

[0132] For patients over the body weight of 100 kg, a fix dose of 6 mg was evenly administered once a day for successive 6 days via intravenous bolus administration in order to suppress side effects due to overdose.

[0133] A plasma INR value of a patient before the administration of the test drug was measured by the above method described as Equation 1.

[0134] Severe septic patients with organ dysfunction limited to the liver or kidney were excluded for the analysis. In patients with organ dysfunction limited to the liver or kidney, the organ dysfunction may possibly be occurred on the basis of causes not limited to sepsis such as drug-induced organ dysfunction.

[0135] Outcome after 28 days from the start of the administration was observed and a mortality rate (Mortality) of each patent group was calculated.

[0136] Further, a difference in mortality rate between TMD-123 group and placebo group was calculated as "Difference".

<Study Results>

[0137] In the group of patient without organ dysfunction, patients with the INR value of INR > 1.5 in plasma before the administration of the test drug gave maximum difference (Difference: 6.1%) between the mortality rate of the group administered with TMD-123 and that of the group administered with placebo, and the group of patient with INR > 1.6 gave the second highest difference in mortality rate (4.5%). In the group of patient with INR > 1.4, no significantly high difference in mortality rate was absolutely or relatively observed, i.e., difference in mortality rate was about 1.7% (Table 1).

[0138] From Table 1, it is recognized that the maximum result can be found between 1.5 and 1.6 of the lower limits of INR, and when the lower limits of INR are outside the range of from 1.5 to 1.6, the difference in mortality rate was significantly decreased.

[0139] Whilst, in the group of severe septic patients having one or more organ dysfunctions selected from circulatory organ dysfunction, respiratory organ dysfunction, kidney dysfunction, and liver dysfunction, the group of patients with INR > 1.4, not the group with INR > 1.5, gave the maximum difference in mortality rate (9.7%), which is absolutely and relatively high difference in mortality rate as compared to the other INR lower limits (Table 2). The group of severe septic patients having one or more organ dysfunctions selected from circulatory organ dysfunction, respiratory organ dysfunction, kidney dysfunction, and liver dysfunction gave more significant difference in mortality rate between TMD-123 group and placebo group on the whole as compared to the group of patients without organ dysfunction (the former: 5.4%, the latter -1.1%).

[0140] Further, in the group of severe septic patients having one or more organ dysfunctions selected from circulatory organ dysfunction, respiratory organ dysfunction, kidney dysfunction, and liver dysfunction, the patient with $1.4 < \text{INR} \leq 1.6$ gave absolutely remarkable difference in mortality rate as being 16.0% (Table 3). As understood from Table 3, any of the other INR upper limits gave values of the difference in mortality rate in a range of 10 to 12%, which indicates relatively recognizable remarkably high difference in mortality rate of the aforementioned group.

[0141] On the other hand, no tendency of relatively outstanding peak increase in a certain group was observed in the group of patients without organ dysfunction. The difference in mortality rate obtained was found at utmost to be 7.1% (in patient group with $1.4 < \text{INR} \leq 1.7$) (Table 4).

[Table 1]

INR value of plasma before administration	TMD-123			Placebo			Difference
	Number	Mortality		Number	Mortality		
INR >1.2	106	10/106=	9.4%	93	9/93=	9.7%	0.2%
INR >1.3	81	6/81=	7.4%	75	6/75=	8.0%	0.6%
INR >1.4	71	5/71=	7.0%	69	6/69=	8.7%	1.7%
INR >1.5	57	3/57=	5.3%	53	6/53=	11.3%	6.1%

INR value of plasma before administration	TMD-123			Placebo			Difference
	Number	Mortality		Number	Mortality		
INR >1.6	42	3/42=	7.1%	43	5/43=	11.6%	4.5%
INR >1.7	29	3/29=	10.3%	27	1/27=	3.7%	-6.6%
INR >1.8	22	3/22=	13.6%	20	1/20=	5.0%	-8.6%
INR >1.9	14	2/14=	14.3%	12	14/32=	8.3%	-6.0%
INR >2.0	9	1/9=	11.1%	7	1/7=	14.3%	3.2%

[Table 2]

INR value of plasma before administration	TMD-123			Placebo			Difference
	Number	Mortality		Number	Mortality		
INR >1.2	184	44/184=	23.9%	186	55/186=	29.6%	5.7%
INR >1.3	160	40/160=	25.0%	162	52/162=	32.1%	7.1%
INR >1.4	137	33/137=	24.1%	136	46/136=	33.8%	9.7%
INR >1.5	101	26/101=	25.7%	100	34/100=	34.0%	8.3%
INR >1.6	81	23/81=	28.4%	77	26/77=	33.8%	5.4%
INR >1.7	61	19/61=	31.1%	65	25/65=	38.5%	7.3%
INR >1.8	45	15/45=	33.3%	48	19/48=	39.6%	6.3%
INR >1.9	36	14/36=	38.9%	32	14/32=	43.8%	4.9%
INR >2.0	27	13/27=	48.1%	25	14/25=	56.0%	7.9%

[Table 3]

INR value of plasma before administration	TMD-123			Placebo			Difference
	Number	Mortality		Number	Mortality		
1.6>=INR >1.4	56	10/56=	17.9 %	59	20/59=	33.9 %	16.0%
1.7>= INR>1.4	76	14/76=	18.4 %	71	21/71=	29.6 %	11.2%
1.8>= INR>1.4	92	18/92=	19.6 %	88	27/88=	30.7 %	11.1%
1.9>= INR>1.4	101	19/101=	18.8 %	104	32/104=	30.8 %	12.0%
2.0>= INR>1.4	110	20/110=	18.2 %	111	32/111=	28.8 %	10.6%

[Table 4]

INR value of plasma before administration	TMD-123			Placebo			Difference
	Number	Mortality		Number	Mortality		
1.6>= INR >1.4	29	2/29=	6.9%	26	1/26=	3.8%	-3.1%
1.7>= INR >1.4	42	2/42=	4.8%	42	5/42=	11.9%	7.1%

INR value of plasma before administration	TMD-123			Placebo			Difference
	Number	Mortality		Number	Mortality		
1.8>= INR >1.4	49	2/49=	4.1%	49	5/49=	10.2%	6.1%
1.9>= INR >1.4	57	3/57=	5.3%	57	5/57=	8.8%	3.5%
2.0>= INR >1.4	62	4/62=	6.5%	62	5/62=	8.1%	1.6%

Industrial Applicability

[0142] The medicament for use of the present invention containing thrombomodulin is useful as a medicament enabling effective therapeutic treatment and/or improvement of sepsis in a severe septic patient, wherein a INR value of a plasma specimen obtained from said patient is more than 1.4.

SEQUENCE LISTING

[0143]

<110> Asahi Kasei Pharma Corporation

<120> Medicament for therapeutic treatment and/or improvement of sepsis

<130> F112127-WO

<150> US 61/559,864

<151> 2011-11-15

<160> 13

<210> 1

<211> 132

<212> PRT

<213> human

<400> 1

```

Met Leu Gly Val Leu Val Leu Gly Ala Leu Ala Leu Ala Gly Leu Gly
1      \201@201@201@5\201@201@201@201@201@201@201@ \201@
10\201@201@201@201@201@201@201@201@201@201@ 15
Phe Pro Asp Pro Cys Phe Arg Ala Asn Cys Glu Tyr Gln Cys Gln Pro
\201@ \201@201@201@201@201@ 20 \201@201@201@201@ \201@201@201@201@25
\201@201@201@201@201@201@201@201@ 30
Leu Asn Gln Thr Ser Tyr Leu Cys Val Cys Ala Glu Gly Phe Ala Pro
\201@201@201@201@ 35 \201@201@201@201@201@201@201@201@201@201@ 40
\201@201@201@ \201@201@201@201@201@201@45
Ile Pro His Glu Pro His Arg Cys Gln Met Phe Cys Asn Gln Thr Ala
50 \201@201@201@201@ \201@201@201@201@55
\201@201@201@201@201@201@201@ 60
Cys Pro Ala Asp Cys Asp Pro Asn Thr Gln Ala Ser Cys Glu Cys Pro

```

```

65\201@\201@\201@ \201@\201@\201@\201@\201@70
\201@\201@\201@\201@\201@\201@\201@\201@ 75 \201@\201@\201@
\201@\201@\201@\201@\201@80
Glu Gly Tyr Ile Leu Asp Asp Gly Phe Ile Cys Thr Asp Ile Asp Glu
\201@\201@\201@ \201@\201@\201@ 85 \201@\201@\201@\201@\201@
\201@\201@90 \201@\201@\201@\201@\201@ \201@\201@ 95
Cys Glu Asn Gly Gly Phe Cys Ser Gly Val Cys His Asn Leu Pro Gly
\201@\201@\201@\201@\201@\201@100 \201@\201@ \201@\201@\201@\201@105
\201@\201@\201@\201@\201@\201@ \201@ 110
Thr Phe Glu Cys Ile Cys Gly Pro Asp Ser Ala Leu Val Arg His Ile
\201@\201@ 115 \201@\201@ \201@\201@\201@\201@\201@120
\201@\201@\201@\201@\201@\201@125
Gly Thr Asp Cys
\201@\201@130

```

<210> 2

<211> 396

<212> DNA

<213> human

<400> 2

```

atgcttggggg tcctgggtcct tggcgcgctg gccctggccg gcctgggggtt ccccgaccgg 60
tgcttcagag ccaactgcga gtaccagtgc cagcccctga accaaactag ctacctctgc 120
gtctgcgccg agggcttcgc gccattccc cagcagccgc acaggtgccg gatgttttgc 180
aaccagactg cctgtccagc cgactgcgac cccaacaccc aggctagctg tgagtgcctt 240
gaaggctaca tcctggacga cggtttcatc tgcacggaca tcgacgagtg cgaaaacggc 300
ggcttctgct ccggggtgtg ccacaacctc cccggtacct tcgagtgcac ctgcggggccc 360
gactcggccc ttgtccgccg cattggcacc gactgt 396

```

<210> 3

<211> 132

<212> PRT

<213> human

<400> 3

```

Met Leu Gly Val Leu Val Leu Gly Ala Leu Ala Leu Ala Gly Leu Gly
1          5          10          15
Phe Pro Asp Pro Cys Phe Arg Ala Asn Cys Glu Tyr Gln Cys Gln Pro
\201@\201@\201@\201@\201@ 20          25          30
Leu Asn Gln Thr Ser Tyr Leu Cys Val Cys Ala Glu Gly Phe Ala Pro
35          40          45
Ile Pro His Glu Pro His Arg Cys Gln Met Phe Cys Asn Gln Thr Ala

50          55          60
Cys Pro Ala Asp Cys Asp Pro Asn Thr Gln Ala Ser Cys Glu Cys Pro
65          70          75          80
Glu Gly Tyr Ile Leu Asp Asp Gly Phe Ile Cys Thr Asp Ile Asp Glu
85          90          95
Cys Glu Asn Gly Gly Phe Cys Ser Gly Val Cys His Asn Leu Pro Gly
100          105          110
Thr Phe Glu Cys Ile Cys Gly Pro Asp Ser Ala Leu Ala Arg His Ile
115          120          125
Gly Thr Asp Cys
130

```

<210> 4

<211> 396

<212> DNA

<213> human

<400> 4

```

atgcttggggg tcctgggtcct tggcgcgctg gccctggccg gcctgggggtt ccccgaccgg 60

```

```

tgcttcagag ccaactgcga gtaccagtgc cagcccctga accaaactag ctacctctgc 120
gtctgcgcgc agggcttcgc gccattccc cagcagccgc acaggtgcc gatgttttgc 180
aaccagactg cctgtccagc cgactgcgac cccaacaccc aggctagctg tgagtgcctt 240
gaaggctaca tcctggacga cggtttcac tgcacggaca tcgacgagtg cgaaaacggc 300
ggcttctgct ccggggtgtg ccacaacct cccggtacct tcgagtgcac ctgcggggcc 360
gactcggccc ttgcccgcca cattggcacc gactgt 396

```

<210> 5

<211> 480

<212> PRT

<213> human

<400> 5

```

Met Leu Gly Val Leu Val Leu Gly Ala Leu Ala Leu Ala Gly Leu Gly
1      5      10      15
Phe Pro Ala Pro Ala Glu Pro Gln Pro Gly Gly Ser Gln Cys Val Glu
20      25      30
His Asp Cys Phe Ala Leu Tyr Pro Gly Pro Ala Thr Phe Leu Asn Ala
35      40      45
Ser Gln Ile Cys Asp Gly Leu Arg Gly His Leu Met Thr Val Arg Ser
50      55      60
Ser Val Ala Ala Asp Val Ile Ser Leu Leu Leu Asn Gly Asp Gly Gly
65      70      75      80
Val Gly Arg Arg Arg Leu Trp Ile Gly Leu Gln Leu Pro Pro Gly Cys
85      90      95
Gly Asp Pro Lys Arg Leu Gly Pro Leu Arg Gly Phe Gln Trp Val Thr
100     105     110
Gly Asp Asn Asn Thr Ser Tyr Ser Arg Trp Ala Arg Leu Asp Leu Asn
115     120     125
Gly Ala Pro Leu Cys Gly Pro Leu Cys Val Ala Val Ser Ala Ala Glu
130     135     140
Ala Thr Val Pro Ser Glu Pro Ile Trp Glu Glu Gln Gln Cys Glu Val
145     150     155     160
Lys Ala Asp Gly Phe Leu Cys Glu Phe His Phe Pro Ala Thr Cys Arg
165     170     175
Pro Leu Ala Val Glu Pro Gly Ala Ala Ala Ala Val Ser Ile Thr
180     185     190
Tyr Gly Thr Pro Phe Ala Ala Arg Gly Ala Asp Phe Gln Ala Leu Pro
195     200     205
Val Gly Ser Ser Ala Ala Val Ala Pro Leu Gly Leu Gln Leu Met Cys
210     215     220
Thr Ala Pro Pro Gly Ala Val Gln Gly His Trp Ala Arg Glu Ala Pro
225     230     235     240
Gly Ala Trp Asp Cys Ser Val Glu Asn Gly Gly Cys Glu His Ala Cys
245     250     255
Asn Ala Ile Pro Gly Ala Pro Arg Cys Gln Cys Pro Ala Gly Ala Ala
260     265     270
Leu Gln Ala Asp Gly Arg Ser Cys Thr Ala Ser Ala Thr Gln Ser Cys

275      280      285
Asn Asp Leu Cys Glu His Phe Cys Val Pro Asn Pro Asp Gln Pro Gly
290     295     300
Ser Tyr Ser Cys Met Cys Glu Thr Gly Tyr Arg Leu Ala Ala Asp Gln
305     310     315     320
His Arg Cys Glu Asp Val Asp Asp Cys Ile Leu Glu Pro Ser Pro Cys
325     330     335
Pro Gln Arg Cys Val Asn Thr Gln Gly Gly Phe Glu Cys His Cys Tyr
340     345     350
Pro Asn Tyr Asp Leu Val Asp Gly Glu Cys Val Glu Pro Val Asp Pro
355     360     365
Cys Phe Arg Ala Asn Cys Glu Tyr Gln Cys Gln Pro Leu Asn Gln Thr
370     375     380
Ser Tyr Leu Cys Val Cys Ala Glu Gly Phe Ala Pro Ile Pro His Glu
385     390     395     400
Pro His Arg Cys Gln Met Phe Cys Asn Gln Thr Ala Cys Pro Ala Asp
405     410     415
Cys Asp Pro Asn Thr Gln Ala Ser Cys Glu Cys Pro Glu Gly Tyr Ile
420     425     430
Leu Asp Asp Gly Phe Ile Cys Thr Asp Ile Asp Glu Cys Glu Asn Gly
435

```

Gly	Phe	Cys	Ser	Gly	Val	Cys	His	Asn	Leu	Pro	Gly	Thr	Phe	Glu	Cys	
	450					455					460					
Ile	Cys	Gly	Pro	Asp	Ser	Ala	Leu	Val	Arg	His	Ile	Gly	Thr	Asp	Cys	
465					470					475					480	

$\langle 210 \rangle$ 6

<211> 1440

<212> DNA

<213> human

<400> 6

atgcttgggg	tcttggctct	tggcgcgctg	gccctggccg	gcttggggtt	ccccgcaccc	60
gcagagccgc	agccgggtgg	cagccagtg	gtcgagcacg	actgcttcgc	gctctaccgc	120
ggccccgcga	ccttcctcaa	tgccagtcat	atctgcgacg	gactcggggg	ccacctaatg	180
acagtgcgtc	cctcgttgcc	gtccgatgtc	attctcttgc	tactgaacgc	cgacggcgccg	240
gttggccgcc	ggcgctctcg	gacggcgctg	cagctgccac	ccggctcgcg	gcaccccaag	300
cgctcggggc	ccctgcgcgg	cttcagtggt	gttacggggag	acaacaacac	cagctatagc	360
aggtgggcac	ggctcgacct	caatggggct	cccctctgcg	gcccgttgtg	cgtcgctgtc	420
tccgttgctg	aggccactgt	gcccagcgag	ccgatctggg	aggagcagca	gtgcgaagtg	480
aagggcggatg	gcttcctctg	cgagctccac	ttccagacca	cctgcaggcc	actggctgtg	540
ggagccggcg	cccgggctcg	cgcgctctcg	atcacctacg	gcaccccggt	cgcgggccgc	600
ggagcggact	tccaggcgct	gccggtgggc	agctccggcg	cggtggctcc	cctcggttta	660
cagctaatgt	gcaccgcgcc	gccccggagcg	gtccaggggc	actgggccag	ggagggcgccg	720
ggcgcttggg	actgcagcgt	ggagaacggc	ggctgcgagc	acgcgtgcaa	tgcgatccct	780
ggggctcccc	gtgtccagtg	cccgccggcg	ggcgccctgc	aggcagacgg	gctgcctcgc	840
accgcatccg	cgacgcagtc	ctgcaacgac	ctctgcgagc	acttctgcgt	tcccaacccc	900
gaccagccgg	gctcctactc	gtgcatgtgc	gagaccggct	accggctggc	ggccgaccaa	960
cacgggtgcg	aggacgttga	tgactgcata	ctggagccca	gtccgtgtcc	gcagcgctgt	1020
gtcaatcacac	agggtggctt	cgagtgccac	tgctacccta	actacgacct	ggtggcaggg	1080
gaagtgtgcg	agcccggtga	cccgtgtctc	agagccaa	gcgagtacca	gtgcgagccc	1140
ctgaaccaa	ctagctacct	ctgcgtctgc	gccgagggct	tcgcgccc	tccccacgag	1200
ccgcacaggt	gccagatgtt	ttgcaaccag	actgcctgtc	cagccgactg	cgaccccaac	1260
accaggctga	gctgtgaagt	ccctgaaggc	tacatcctgg	acgacggttt	catctgcacg	1320
gacatcgac	agtgcaaaa	cggcggtctc	tgtccgggg	tgtgccaca	cctccccggt	1380
accttcgagt	gcattctggg	gcccgactcg	gcccttgc	ggcacattgg	caccgactgt	1440

 $\langle 210 \rangle$ 7

<211> 480

<212> PRT

<213> human

$\langle 400 \rangle$ 7

Met	Leu	Gly	Val	Leu	Val	Leu	Gly	Ala	Leu	Ala	Leu	Ala	Gly	Leu	Gly
1				5					10					15	
Phe	Pro	Ala	Pro	Ala	Glu	Pro	Gln	Pro	Gly	Gly	Ser	Gln	Cys	Val	Glu
			20					25					30		
His	Asp	Cys	Phe	Ala	Leu	Tyr	Pro	Gly	Pro	Ala	Thr	Phe	Leu	Asn	Ala
		35					40					45			
Ser	Gln	Ile	Cys	Asp	Gly	Leu	Arg	Gly	His	Leu	Met	Thr	Val	Arg	Ser
	50					55					60				
Ser	Val	Ala	Ala	Asp	Val	Ile	Ser	Leu	Leu	Leu	Asn	Gly	Asp	Gly	Gly
65					70					75					80
Val	Gly	Arg	Arg	Arg	Leu	Trp	Ile	Gly	Leu	Gln	Leu	Pro	Pro	Gly	Cys
				85					90					95	
Gly	Asp	Pro	Lys	Arg	Leu	Gly	Pro	Leu	Arg	Gly	Phe	Gln	Trp	Val	Thr
			100					105					110		
Gly	Asp	Asn	Asn	Thr	Ser	Tyr	Ser	Arg	Trp	Ala	Arg	Leu	Asp	Leu	Asn
		115					120					125			
Gly	Ala	Pro	Leu	Cys	Gly	Pro	Leu	Cys	Val	Ala	Val	Ser	Ala	Ala	Glu
						135					140				
Ala	Thr	Val	Pro	Ser	Glu	Pro	Ile	Trp	Glu	Glu	Gln	Gln	Cys	Glu	Val
145					150					155					160
Lys	Ala	Asp	Gly	Phe	Leu	Cys	Glu	Phe	His	Phe	Pro	Ala	Thr	Cys	Arg
				165					170					175	

```

Pro Leu Ala Val Glu Pro Gly Ala Ala Ala Ala Val Ser Ile Thr
      180      185      190
Tyr Gly Thr Pro Phe Ala Ala Arg Gly Ala Asp Phe Gln Ala Leu Pro
      195      200      205
Val Gly Ser Ser Ala Ala Val Ala Pro Leu Gly Leu Gln Leu Met Cys
      210      215      220
Thr Ala Pro Pro Gly Ala Val Gln Gly His Trp Ala Arg Glu Ala Pro
      225      230      235      240
Gly Ala Trp Asp Cys Ser Val Glu Asn Gly Gly Cys Glu His Ala Cys
      245      250      255
Asn Ala Ile Pro Gly Ala Pro Arg Cys Gln Cys Pro Ala Gly Ala Ala
      260      265      270
Leu Gln Ala Asp Gly Arg Ser Cys Thr Ala Ser Ala Thr Gln Ser Cys
      275      280      285
Asn Asp Leu Cys Glu His Phe Cys Val Pro Asn Pro Asp Gln Pro Gly
      290      295      300
Ser Tyr Ser Cys Met Cys Glu Thr Gly Tyr Arg Leu Ala Ala Asp Gln
      305      310      315      320
His Arg Cys Glu Asp Val Asp Asp Cys Ile Leu Glu Pro Ser Pro Cys
      325      330      335
Pro Gln Arg Cys Val Asn Thr Gln Gly Gly Phe Glu Cys His Cys Tyr
      340      345      350
Pro Asn Tyr Asp Leu Val Asp Gly Glu Cys Val Glu Pro Val Asp Pro
      355      360      365
Cys Phe Arg Ala Asn Cys Glu Tyr Gln Cys Gln Pro Leu Asn Gln Thr
      370      375      380
Ser Tyr Leu Cys Val Cys Ala Glu Gly Phe Ala Pro Ile Pro His Glu
      385      390      395      400
Pro His Arg Cys Gln Met Phe Cys Asn Gln Thr Ala Cys Pro Ala Asp
      405      410      415
Cys Asp Pro Asn Thr Gln Ala Ser Cys Glu Cys Pro Glu Gly Tyr Ile
      420      425      430
Leu Asp Asp Gly Phe Ile Cys Thr Asp Ile Asp Glu Cys Glu Asn Gly
      435      440      445
Gly Phe Cys Ser Gly Val Cys His Asn Leu Pro Gly Thr Phe Glu Cys
      450      455      460
Ile Cys Gly Pro Asp Ser Ala Leu Ala Arg His Ile Gly Thr Asp Cys
      465      470      475      480

```

<210> 8

<211> 1440

<212> DNA

<213> human

<400> 8

```

atgcttgggg tcttggtcct tggcgcgctg gccctggccg gcctgggggt ccccgcaccc 60
gcagagccgc agccgggtgg cagccagtgc gtcgagcacg actgcttcgc gctctaccgc 120

```

```

ggccccgcga ccttcctcaa tgccagtcag atctgcgacg gactgcgggg ccacctaattg 180
acagtgcgct cctcggtggc tgccgatgtc atttccttgc tactgaacgg cgacggcggc 240
gttgcccgcc ggccctctcg gatcgccctg cagctgccac ccggtgctcg cgacccaag 300
cgccctcgggc ccctgcgcgg cttccagtgg gttacgggag acaacaacac cagctatagc 360
aggtgggcac ggctcgacct caatggggct cccctctgcg gccggttgtg cgtcgctgtc 420
tccgctgctg aggccactgt gccagcgag ccgatctggg aggagcagca gtgcgaagtg 480
aaggccgatg gcttcctctg cgagttccac ttcccagcca cctgcaggcc actggctgtg 540
gagcccgccg ccgcggtgcg cgcgtctcg atcacctacg gcaccccggt cgcggcccgc 600
ggagcggact tccaggcgct gccggtgggc agctccgcgg cggtaggtcc cctcggctta 660
cagctaattg gcaccgcgcc gcccgagcg gtccaggggc actgggccag ggaggcgccg 720
ggcgcttggg actgcagcgt ggagaacggc ggctgcgagc acgctgcaa tgcgatccct 780
ggggctcccc gctgccagtg cccagccggc gccgacctgc aggcagacgg gcgctcctgc 840
accgcatccg cgacgcagtc ctgcaacgac ctctgcgagc acttctgcgt tcccaacccc 900
gaccagccgg gctcctactc gtgcatgtgc gagaccggct accggctggc ggccgaccaa 960
caccggtgcg aggacgtgga tgactgcata ctggagccca gtccgtgtcc gcagcgctgt 1020
gtcaacacac aggttggtct cgagtgccac tgctacccta actacgacct ggtggacggc 1080
gagtgtgtgg agcccggtga cccgtgcttc agagccaact gcgagtacca gtgccagccc 1140
ctgaaccaaa ctagtctacct ctgcgtctgc gccgagggct tcgcgcccac tccccacgag 1200
ccgcacaggt gccagatggt ttgcaaccag actgcctgtc cagccgactg cgacccaac 1260
accaggtcta gctgtgagtg ccctgaaggc tacatcctgg acgacggttt catctgcacg 1320
gacatcgacg agtgcgaaaa cggcggcttc tgctccgggg tgtgccacaa cctccccggg 1380
acctgcaact acatctcgga gcccgactcg gcccttcccc gccacattga caccgactgt 1440

```

<210> 9

<211> 516

<212> PRT

<213> human

<400> 9

```

Met Leu Gly Val Leu Val Leu Gly Ala Leu Ala Leu Ala Gly Leu Gly
1      5      10      15
Phe Pro Ala Pro Ala Glu Pro Gln Pro Gly Gly Ser Gln Cys Val Glu
      20      25      30
His Asp Cys Phe Ala Leu Tyr Pro Gly Pro Ala Thr Phe Leu Asn Ala
      35      40      45
Ser Gln Ile Cys Asp Gly Leu Arg Gly His Leu Met Thr Val Arg Ser
      50      55      60
Ser Val Ala Ala Asp Val Ile Ser Leu Leu Leu Asn Gly Asp Gly Gly
65      70      75      80
Val Gly Arg Arg Arg Leu Trp Ile Gly Leu Gln Leu Pro Pro Gly Cys
      85      90      95
Gly Asp Pro Lys Arg Leu Gly Pro Leu Arg Gly Phe Gln Trp Val Thr
      100      105      110
Gly Asp Asn Asn Thr Ser Tyr Ser Arg Trp Ala Arg Leu Asp Leu Asn
      115      120      125
Gly Ala Pro Leu Cys Gly Pro Leu Cys Val Ala Val Ser Ala Ala Glu
      130      135      140
Ala Thr Val Pro Ser Glu Pro Ile Trp Glu Glu Gln Gln Cys Glu Val
145      150      155      160
Lys Ala Asp Gly Phe Leu Cys Glu Phe His Phe Pro Ala Thr Cys Arg
      165      170      175
Pro Leu Ala Val Glu Pro Gly Ala Ala Ala Ala Val Ser Ile Thr
      180      185      190
Tyr Gly Thr Pro Phe Ala Ala Arg Gly Ala Asp Phe Gln Ala Leu Pro
      195      200      205
Val Gly Ser Ser Ala Ala Val Ala Pro Leu Gly Leu Gln Leu Met Cys
210      215      220
Thr Ala Pro Pro Gly Ala Val Gln Gly His Trp Ala Arg Glu Ala Pro
225      230      235      240
Gly Ala Trp Asp Cys Ser Val Glu Asn Gly Gly Cys Glu His Ala Cys
      245      250      255
Asn Ala Ile Pro Gly Ala Pro Arg Cys Gln Cys Pro Ala Gly Ala Ala
260      265      270
Leu Gln Ala Asp Gly Arg Ser Cys Thr Ala Ser Ala Thr Gln Ser Cys
275      280      285

Asn Asp Leu Cys Glu His Phe Cys Val Pro Asn Pro Asp Gln Pro Gly
290      295      300
Ser Tyr Ser Cys Met Cys Glu Thr Gly Tyr Arg Leu Ala Ala Asp Gln
305      310      315      320
His Arg Cys Glu Asp Val Asp Asp Cys Ile Leu Glu Pro Ser Pro Cys
      325      330      335
Pro Gln Arg Cys Val Asn Thr Gln Gly Gly Phe Glu Cys His Cys Tyr
      340      345      350
Pro Asn Tyr Asp Leu Val Asp Gly Glu Cys Val Glu Pro Val Asp Pro
355      360      365
Cys Phe Arg Ala Asn Cys Glu Tyr Gln Cys Gln Pro Leu Asn Gln Thr
370      375      380
Ser Tyr Leu Cys Val Cys Ala Glu Gly Phe Ala Pro Ile Pro His Glu
385      390      395      400
Pro His Arg Cys Gln Met Phe Cys Asn Gln Thr Ala Cys Pro Ala Asp
      405      410      415
Cys Asp Pro Asn Thr Gln Ala Ser Cys Glu Cys Pro Glu Gly Tyr Ile
420      425      430
Leu Asp Asp Gly Phe Ile Cys Thr Asp Ile Asp Glu Cys Glu Asn Gly
435      440      445
Gly Phe Cys Ser Gly Val Cys His Asn Leu Pro Gly Thr Phe Glu Cys
450      455      460
Ile Cys Gly Pro Asp Ser Ala Leu Val Arg His Ile Gly Thr Asp Cys
465      470      475      480
Asp Ser Gly Lys Val Asp Gly Gly Asp Ser Gly Ser Gly Glu Pro Pro
      485      490      495

```

[illegible]

<210> 10
<211> 1548
<212> DNA
<213> human

<400> 10						
atgcttgggg	tcttgggtcct	tggcgcgctg	gccctggccg	gcttgggggt	ccccgcaccc	60
gcagagccgc	agccgggtgg	cagccagtg	gtcgagcacg	actgcttcgc	gctctaccgc	120
ggccccgcga	ccttcctcaa	tgccagtcag	atctgcgacg	gactgcgggg	ccacctaatg	180
acagtgcgct	cctcgggtgg	gtccagatgtc	atttctttgc	tactgaacgc	cgacgcggcg	240
gttgccgcgc	ggcgctcttg	gctgcgctg	gactgtccac	cgggtgcg	gcaccccaag	300
cgcttcgggc	ccctgcgcgg	cttcacagtg	gttacgggag	acaacaacac	cagctatagc	360
aggtgggcac	ggctcgacct	caatggggct	cccctctgcg	gccggtgtg	cgtcgctgtc	420
tccgctgctg	aggccactgt	gcccagcgag	ccgactctgg	aggagcagca	gtcgaagtgt	480
aaggccgctg	gcttcctctg	cgagtctccac	ttccagcca	cctgcaggcc	actggtgtgt	540
gagcccggtg	cccgcgctgc	cgcgctctcg	atcacctacg	gcaccgcgtt	cgcggccgcg	600
ggagcggact	tccaggcgct	gccggtgggc	agctccgcgc	cgggtgctcc	cctcggctta	660
cagctaattgt	gcaccgcgcc	gcccggagcg	gtccaggggc	actgggccag	ggaggcgccg	720
ggcgcttggg	actgcagcgt	ggagaacggc	ggctgcgagc	acgcgtgcaa	tgcgatccct	780
ggggctcccc	gctgccagt	cccagccggc	gccgcctgc	aggcagacgc	gcgctcctgc	840
accgcatccg	cgacgcagtc	ctgcaacgac	ctctgcgagc	acttctcgct	tcccaacccc	900
gaccagccgg	gtcctacttc	gtgcatgtgc	gagacccgct	accggctggc	ggccgaccaa	960
caccgggtgcg	aggacgtgga	tgactgcata	ctggagccca	gtccgtgtcc	gcagcgtgt	1020
gtcaacacac	aggggtggctt	cgagtgccac	tgtacccta	actacgacct	ggtgcagcgc	1080
gagtggtgtg	agccctgtga	cccgctgctt	agagccaact	gcgagtacca	gtgccagccc	1140
ctgaaccaaa	ctagctacct	ctgcgtctgc	gcggagggct	tcgcgcccat	tcccacgag	1200
ccgcacaggt	gccagatgtt	ttgcaaccag	actgcctgtc	acgcgacgtg	cgaccccaac	1260
accagggtcta	gctgtgagtg	ccctgaaggc	tacatcctgg	acgacggttt	catctgcacg	1320
gacatcgacg	agtgcgaaaa	cggcgcttcc	tgtccgggg	tgtgccaaaa	cctccccggt	1380
accttcgagt	gcattctgcg	gcccgaactc	gcccttgtcc	gccacattgc	caccgactgt	1440
gactccggca	aggtggacgg	tggcgacagc	ggctctggcg	agcccccggc	cagcccgacg	1500
cccggtctcca	ccctgactcc	tccggccgtg	gggctcgtgc	attcgggc		1560

<210> 11
<211> 516
<212> PRT
<213> human

<400> 11															
Met	Leu	Gly	Val	Leu	Val	Leu	Gly	Ala	Leu	Ala	Leu	Ala	Gly	Leu	Gly
1				5					10					15	
Phe	Pro	Ala	Pro	Ala	Glu	Pro	Gln	Pro	Gly	Gly	Ser	Gln	Cys	Val	Glu
			20					25					30		
His	Asp	Cys	Phe	Ala	Leu	Tyr	Pro	Gly	Pro	Ala	Thr	Phe	Leu	Asn	Ala
		35					40					45			
Ser	Gln	Ile	Cys	Asp	Gly	Leu	Arg	Gly	His	Leu	Met	Thr	Val	Arg	Ser
	50					55					60				
Ser	Val	Ala	Ala	Asp	Val	Ile	Ser	Leu	Leu	Leu	Asn	Gly	Asp	Gly	Gly
65					70					75					80
Val	Gly	Arg	Arg	Arg	Leu	Trp	Ile	Gly	Leu	Gln	Leu	Pro	Pro	Gly	Cys
				85					90					95	
Gly	Asp	Pro	Lys	Arg	Leu	Gly	Pro	Leu	Arg	Gly	Phe	Gln	Trp	Val	Thr
			100					105					110		
Gly	Asp	Asn	Asn	Thr	Ser	Tyr	Ser	Arg	Trp	Ala	Arg	Leu	Asp	Leu	Asn
		115					120					125			
Gly	Ala	Pro	Leu	Cys	Gly	Pro	Leu	Cys	Val	Ala	Val	Ser	Ala	Ala	Glu
	130					135					140				
Ala	Thr	Val	Pro	Ser	Glu	Pro	Ile	Trp	Glu	Glu	Gln	Gln	Cys	Glu	Val
145					150					155					160
Lys	Ala	Asp	Gly	Phe	Leu	Cys	Glu	Phe	His	Phe	Pro	Ala	Thr	Cys	Arg
				165					170					175	

Pro Leu Ala Val Glu Pro Gly Ala Ala Ala Ala Ala Val Ser Ile Thr
 180 185 190
 Tyr Gly Thr Pro Phe Ala Ala Arg Gly Ala Asp Phe Gln Ala Leu Pro
 195 200 205
 Val Gly Ser Ser Ala Ala Val Ala Pro Leu Gly Leu Gln Leu Met Cys
 210 215 220
 Thr Ala Pro Pro Gly Ala Val Gln Gly His Trp Ala Arg Glu Ala Pro
 225 230 235 240
 Gly Ala Trp Asp Cys Ser Val Glu Asn Gly Gly Cys Glu His Ala Cys
 245 250 255
 Asn Ala Ile Pro Gly Ala Pro Arg Cys Gln Cys Pro Ala Gly Ala Ala
 260 265 270
 Leu Gln Ala Asp Gly Arg Ser Cys Thr Ala Ser Ala Thr Gln Ser Cys
 275 280 285
 Asn Asp Leu Cys Glu His Phe Cys Val Pro Asn Pro Asp Gln Pro Gly
 290 295 300
 Ser Tyr Ser Cys Met Cys Glu Thr Gly Tyr Arg Leu Ala Ala Asp Gln
 305 310 315 320
 His Arg Cys Glu Asp Val Asp Asp Cys Ile Leu Glu Pro Ser Pro Cys
 325 330 335
 Pro Gln Arg Cys Val Asn Thr Gln Gly Gly Phe Glu Cys His Cys Tyr
 340 345 350
 Pro Asn Tyr Asp Leu Val Asp Gly Glu Cys Val Glu Pro Val Asp Pro
 355 360 365
 Cys Phe Arg Ala Asn Cys Glu Tyr Gln Cys Gln Pro Leu Asn Gln Thr
 370 375 380
 Ser Tyr Leu Cys Val Cys Ala Glu Gly Phe Ala Pro Ile Pro His Glu
 385 390 395 400
 Pro His Arg Cys Gln Met Phe Cys Asn Gln Thr Ala Cys Pro Ala Asp
 405 410 415
 Cys Asp Pro Asn Thr Gln Ala Ser Cys Glu Cys Pro Glu Gly Tyr Ile
 420 425 430
 Leu Asp Asp Gly Phe Ile Cys Thr Asp Ile Asp Glu Cys Glu Asn Gly
 435 440 445
 Gly Phe Cys Ser Gly Val Cys His Asn Leu Pro Gly Thr Phe Glu Cys
 450 455 460
 Ile Cys Gly Pro Asp Ser Ala Leu Ala Arg His Ile Gly Thr Asp Cys
 465 470 475 480

 Asp Ser Gly Lys Val Asp Gly Gly Asp Ser Gly Ser Gly Glu Pro Pro
 485 490 495
 Pro Ser Pro Thr Pro Gly Ser Thr Leu Thr Pro Pro Ala Val Gly Leu
 500 505 510
 Val His Ser Gly
 515

<210> 12

<211> 1548

<212> DNA

<213> human

<400> 12

atgcttgggg tectgttct tggcgcgctg gccctggcgg gcctgggggt ccccgacccc 60
 gcagagccgc agccgggtgg cagccagtgc gtcgagcacg actgcttcgc gctctacccg 120
 ggccccgcga ccttcctcaa tgccagttag atctgcgacg gactgcgggg ccacctaata 180
 acagtgcgct cctcgggtgg tgccgatgtc atttccttgc tactgaacgg cgacggcggc 240
 gttggccgcc ggccctctg gatcgccctg cagctgccac ccggctgcgg cgacccaag 300
 cgcctcgggc cctcgcggcg cttccagtgg gttacgggag acaacaacac cagctatagc 360
 aggtgggcac ggctcgacct caatggggct cccctctgcg gcccggtgtg cgtcgtgtgc 420
 tccgtgctg agggcaactg gccagcgag ccgatctggg aggagcagca gtgcgaagtg 480
 aaggccgatg gcttcctctg cgagttccac ttcccagcca cctgcaggcc actggctgtg 540
 gagcccgcg ccgcggtgc gcgcgtctcg atcacctacg gcaccccggt cgcgcccgcc 600
 ggagcggact tccaggcgct gccggtgggc agctccgcgg cgggtggctcc cctcggctta 660
 cagctaatagt gcaccgcgcc gcccgagcgg gtccaggggc actggggccag ggaggcgccg 720
 ggcgcttggg actgcagcgt ggagaacggc ggctgcgagc acgcgtgcaa tgcgatccct 780
 ggggctcccc gctgccagtg cccagccggc gccgccttgc aggcagacgg gcgctcctgc 840
 accgcatccg cgacgcagtc ctgcaacgac ctctgcgagc acttctgcgt tcccaacccc 900
 gaccagccgg gctcctactc gtgcatgtgc gagaccggct accggctggc ggccgaccaa 960
 caccggtgcg aggacgtgga tgactgcata ctggagccca gtccgtgtcc gcagcgctgt 1020
 ctcaacacac aggttcgctt cgaatggcac tgcctaccta actacagcct cgtggacggc 1080

```

ggcaccacac agggggggcc cgggagccac cgcaccccca acccagaccc ggaggacggc 1000
gagtgtgtgg agcccgtgga cccgtgcttc agagccaact gcgagtacca gtgccagccc 1140
ctgaacacaa ctagctacct ctgctgtctgc gccgagggtc tcgcgcccat tccccacgag 1200
ccgcacaggt gccagatggt ttgcaaccag actgcctgtc cagccgactg cgacccaac 1260
accagggcta gctgtgagtg ccctgaaggc tacatcctgg acgacggttt catctgcacg 1320
gacatcgacg agtgcgaaaa cggcgggttc tgctccgggg tgtgccacaa cctccccggt 1380
accttcgagt gcatctgcgg gcccgactcg gcccttgccc gccacattgg caccgactgt 1440
gactccggca aggtggacgg tggcgacagc ggctctggcg agcccccgcc cagcccagcg 1500
cccggtcca ccttgactcc tccggccgtg gggctcgtgc attcgggc 1548

```

<210> 13

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic DNA

<400> 13

aatgtggcgg gcaagggccg a 21

SEQUENCE LISTING

[0144]

<110> Asahi Kasei Pharma corporation

<120> Medicament for therapeutic treatment and/or improvement of sepsis

<130> F112127-WO

<150> US 61/559,864

<151> 2011-11-15

<160> 13

<210> 1

<211> 132

<212> PRT

<213> human

<400> 1

```

Met Leu Gly Val Leu Val Leu Gly Ala Leu Ala Leu Ala Gly Leu Gly
1      5      10      15
Phe Pro Asp Pro Cys Phe Arg Ala Asn Cys Glu Tyr Gln Cys Gln Pro
20      25      30
Leu Asn Gln Thr Ser Tyr Leu Cys Val Cys Ala Glu Gly Phe Ala Pro
35      40      45
Ile Pro His Glu Pro His Arg Cys Gln Met Phe Cys Asn Gln Thr Ala
50      55      60
Cys Pro Ala Asp Cys Asp Pro Asn Thr Gln Ala Ser Cys Glu Cys Pro
65      70      75      80
Glu Gly Tyr Ile Leu Asp Asp Gly Phe Ile Cys Thr Asp Ile Asp Glu
85      90      95
Cys Glu Asn Glv Glv Phe Cys Ser Glv Val Cys His Asn Leu Pro Glv

```

```

      100      105      110
Thr Phe Glu Cys Ile Cys Gly Pro Asp Ser Ala Leu Val Arg His Ile
      115      120      125
Gly Thr Asp Cys
      130

```

<210> 2

<211> 396

<212> DNA

<213> human

<400> 2

```

atgcttgggg tcctggtcct tggcgcgctg gccctggccg gcctgggggt ccccgaccgg 60
tgcttcagag ccaactgcga gtaccagtgc cagcccttga accaaactag ctacctctgc 120
gtctgcgccc agggcttcgc gcccattccc cagagccgc acaggtgcca gatgttttgc 180
aaccagactg cctgtccagc cgactgcgac cccaacaccc aggctagctg tgagtgcctt 240
gaaggctaca tcctggacga cggtttcac tgcacggaca tcgacgagtg cgaaaacggc 300
ggcttctgct ccgggggtgt ccacaacctc cccggtacct tcgagtgcac ctgcggggcc 360
gactcggccc ttgtccgcca cattggcacc gactgt

```

<210> 3

<211> 132

<212> PRT

<213> human

<400> 3

```

Met Leu Gly Val Leu Val Leu Gly Ala Leu Ala Leu Ala Gly Leu Gly
1      5      10      15
Phe Pro Asp Pro Cys Phe Arg Ala Asn Cys Glu Tyr Gln Cys Gln Pro
20      25      30
Leu Asn Gln Thr Ser Tyr Leu Cys Val Cys Ala Glu Gly Phe Ala Pro
35      40      45
Ile Pro His Glu Pro His Arg Cys Gln Met Phe Cys Asn Gln Thr Ala
50      55      60
Cys Pro Ala Asp Cys Asp Pro Asn Thr Gln Ala Ser Cys Glu Cys Pro
65      70      75      80
Glu Gly Tyr Ile Leu Asp Asp Gly Phe Ile Cys Thr Asp Ile Asp Glu
85      90      95
Cys Glu Asn Gly Gly Phe Cys Ser Gly Val Cys His Asn Leu Pro Gly
100      105      110
Thr Phe Glu Cys Ile Cys Gly Pro Asp Ser Ala Leu Ala Arg His Ile
115      120      125
Gly Thr Asp Cys
130

```

<210> 4

<211> 396

<212> DNA

<213> human

<400> 4

```

atgcttgggg tcctggtcct tggcgcgctg gccctggccg gcctgggggt ccccgaccgg 60
tgcttcagag ccaactgcga gtaccagtgc cagcccttga accaaactag ctacctctgc 120
gtctgcgccc agggcttcgc gcccattccc cagagccgc acaggtgcca gatgttttgc 180
aaccagactg cctgtccagc cgactgcgac cccaacaccc aggctagctg tgagtgcctt 240
gaaggctaca tcctggacga cggtttcac tgcacggaca tcgacgagtg cgaaaacggc 300
ggcttctgct ccgggggtgt ccacaacctc cccggtacct tcgagtgcac ctgcggggcc 360
gactcggccc ttgtccgcca cattggcacc gactgt

```

<210> 5

<211> 480

<212> PRT

<213> human

<400> 5

Met Leu Gly Val Leu Val Leu Gly Ala Leu Ala Leu Ala Gly Leu Gly
 1 Phe Pro Ala Pro 5 Ala Glu Pro Gln Pro Gly Gly Ser Gln Cys Val Glu
 20 His Asp Cys 35 Phe Ala Leu Tyr Pro 40 Gly Pro Ala Thr Phe 45 Leu Asn Ala
 Ser Gln Ile Cys Asp Gly 55 Leu Arg Gly His Leu 60 Met Thr Val Arg Ser
 65 Ser Val Ala Ala Asp Val 70 Ile Ser Leu Leu Leu Asn Gly Asp Gly Gly
 Val Gly Arg Arg 85 Leu Trp Ile Gly Leu 90 Gln Leu Pro Pro Gly Cys
 Gly Asp Pro Lys 100 Arg Leu Gly Pro Leu 105 Arg Gly Phe Gln Trp Val Thr
 Gly Asp Asn Asn Thr Ser Tyr Ser 120 Arg Trp Ala Arg Leu 125 Asp Leu Asn
 130 Gly Ala Pro Leu Cys Gly Pro 135 Cys Val Ala Val Ser Ala Ala Glu
 145 Ala Thr Val Pro Ser Glu 150 Pro Ile Trp Glu Glu 155 Gln Gln Cys Glu Val
 Lys Ala Asp Gly Phe 165 Leu Cys Glu Phe His 170 Phe Pro Ala Thr Cys Arg
 Pro Leu Ala Val 180 Glu Pro Gly Ala Ala 185 Ala Ala Val Ser Ile Thr
 Tyr Gly Thr 195 Pro Phe Ala Ala Arg 200 Gly Ala Asp Phe Gln 205 Ala Leu Pro
 Val Gly Ser Ser Ala Ala Val 215 Ala Pro Leu Gly Leu 220 Gln Leu Met Cys
 Thr Ala Pro Pro Gly Ala Val 230 Gln Gly His Trp 235 Ala Arg Glu Ala Pro
 240 Gly Ala Trp Asp Cys 245 Ser Val Glu Asn Gly Gly Cys Glu His Ala Cys
 Asn Ala Ile Pro 260 Gly Ala Pro Arg Cys 265 Gln Cys Pro Ala Gly Ala Ala
 Leu Gln Ala Asp Gly Arg Ser Cys 280 Thr Ala Ser Ala Thr 285 Gln Ser Cys
 Asn Asp 290 Leu Cys Glu His Phe 295 Cys Val Pro Asn Pro 300 Asp Gln Pro Gly
 Ser Tyr Ser Cys Met Cys 310 Glu Thr Gly Tyr Arg 315 Leu Ala Ala Asp Gln
 320 His Arg Cys Glu Asp Val Asp Asp Cys Ile Leu Glu Pro Ser Pro Cys
 335 Pro Gln Arg Cys Val Asn Thr Gln Gly 345 Gly Phe Glu Cys His Cys Tyr
 Pro Asn Tyr Asp Leu Val Asp Gly 360 Glu Cys Val Glu Pro Val Asp Pro
 370 Cys Phe Arg Ala Asn Cys Glu Tyr Gln Cys Gln Pro Leu Asn Gln Thr
 Ser Tyr Leu Cys Val Cys 390 Glu Gly Phe Ala 395 Pro Ile Pro His Glu
 400 Pro His Arg Cys Gln Met Phe Cys Asn Gln Thr Ala Cys Pro Ala Asp
 415 Cys Asp Pro Asn Thr Gln Ala Ser Cys 425 Glu Cys Pro Glu Gly Tyr Ile
 430

Leu Asp Asp Gly Phe Ile Cys Thr Asp Ile Asp Glu Cys Glu Asn Gly
 435
 Gly Phe Cys Ser Gly Val Cys 440 His Asn Leu Pro Gly Thr Phe Glu Cys
 450
 Ile Cys Gly Pro Asp Ser Ala Leu Val Arg His 460 Ile Gly Thr Asp Cys
 465 470 475 480

<210> 6

<211> 1440

<212> DNA

<213> human

<400> 6

atgcttgaggg tcctggtcct tggcgcgctg gccctggcgg gcctgggggtt ccccgacacc 60
 gcagagccgc agccgggtgg cagccagtgc gtcgagcacg actgcttcgc gctctaccgc 120
 ggccccgcga ccttcctcaa tgccagtcag atctgcgacg gactgcgggg ccacctaatg 180
 acagtgcgct cctcggtggc tgccgatgtc atttccttgc tactgaacgg cgacggcggc 240
 gttggccgcc ggcgctcttg gatcggtctg cagctgccac ccggtgcgg cgaccccaag 300
 cgcttcgggc ccttcgctgg cttccagtgg gttacgggag acaacaacac cagctatagc 360
 aggtgggcac ggctcgacct caatggggct cccctctgcg gccggttggt cgtcgctgtc 420
 tccgctgctg agggccactgt gcccagcgag ccgatctggg agggagcagca gtgcgaagtg 480
 aaggccgatg gcttctcttg cgagtccac ttcccagcca cctgcaggcc actggctgtg 540
 gagcccgccg ccgcggtctg cgccgtctcg atcacctacg gcaccccggt cgcggcccg 600
 ggagcggact tccaggcgct gccgggtggc agctccgcgg cggtggctcc cctcggtta 660
 cagctaattg gcaccgcgcc gcccggagcg gtccaggggc actgggacag ggaggcgccg 720
 ggcgcttggt actgcagcgt ggagaacggc ggctgcgagc acgctgcaaa tgcgatccct 780

```

ggggctccc gctgccagt cccagccggc gccgccctgc aggcagacgg gcgctcctgc 840
accgcatccg cgacgcagtc ctgcaacgac ctctgcgagc acttctgcgt tcccaacccc 900
gaccagccgg gctcctactc gtgcatgtgc gagaccggct accggctggc ggccgaccaa 960
caccggtgcg aggcagtggg tgactgcata ctggagccca gtccggtgtcc gcagcgctgt 1020
gtcaacacac aggggtggctt cgagtgccac tgctacccta actacgacct ggtggacggc 1080
gagtgtgtgg agcccgtgga cccgtgcttc agagccaact gcgagtacca gtgccagccc 1140
ctgaacaaaa cttagctacct ctgcgtctgc gccgagggct tcgcgcccac tccccacgag 1200
ccgcacaggt gccagatgtt ttgcaaccag actgcctgtc cagccgactg cgaccccaac 1260
accgaggcta gctgtgagtg ccctgaaggc tacatcctgg acgacggttt catctgcacg 1320
gacatcgacg agtgcgaaaa cggcggcttc tgctccgggg tgtgccacaa cctccccggt 1380
accttcgagt gcatctgcgg gcccgactcg gcccttgctc gccacattgg caccgactgt 1440

```

<210> 7

<211> 480

<212> PRT

<213> human

<400> 7

```

Met Leu Gly Val Leu Val Leu Gly Ala Leu Ala Leu Ala Gly Leu Gly
1      5      10     15
Phe Pro Ala Pro Ala Glu Pro Gln Pro Gly Gly Ser Gln Cys Val Glu
20     25     30
His Asp Cys Phe Ala Leu Tyr Pro Gly Pro Ala Thr Phe Leu Asn Ala
35     40     45
Ser Gln Ile Cys Asp Gly Leu Arg Gly His Leu Met Thr Val Arg Ser
50     55     60
Ser Val Ala Ala Asp Val Ile Ser Leu Leu Leu Asn Gly Asp Gly Gly
65     70     75     80
Val Gly Arg Arg Arg Leu Trp Ile Gly Leu Gln Leu Pro Pro Gly Cys
85     90     95
Gly Asp Pro Lys Arg Leu Gly Pro Leu Arg Gly Phe Gln Trp Val Thr
100    105
Gly Asp Asn Asn Thr Ser Tyr Ser Arg Trp Ala Arg Leu Asp Leu Asn
115    120    125
Gly Ala Pro Leu Cys Gly Pro Leu Cys Val Ala Val Ser Ala Ala Glu
130    135    140
Ala Thr Val Pro Ser Glu Pro Ile Trp Glu Glu Gln Gln Cys Glu Val
145    150    155    160
Lys Ala Asp Gly Phe Leu Cys Glu Phe His Phe Pro Ala Thr Cys Arg
165    170    175
Pro Leu Ala Val Glu Pro Gly Ala Ala Ala Ala Val Ser Ile Thr
180    185    190
Tyr Gly Thr Pro Phe Ala Ala Arg Gly Ala Asp Phe Gln Ala Leu Pro
195    200    205
Val Gly Ser Ser Ala Ala Val Ala Pro Leu Gly Leu Gln Leu Met Cys
210    215    220

```

```

Thr Ala Pro Pro Gly Ala Val Gln Gly His Trp Ala Arg Glu Ala Pro
225    230    235    240
Gly Ala Trp Asp Cys Ser Val Glu Asn Gly Gly Cys Glu His Ala Cys
245    250    255
Asn Ala Ile Pro Gly Ala Pro Arg Cys Gln Cys Pro Ala Gly Ala Ala
260    265    270
Leu Gln Ala Asp Gly Arg Ser Cys Thr Ala Ser Ala Thr Gln Ser Cys
275    280    285
Asn Asp Leu Cys Glu His Phe Cys Val Pro Asn Pro Asp Gln Pro Gly
290    295    300
Ser Tyr Ser Cys Met Cys Glu Thr Gly Tyr Arg Leu Ala Ala Asp Gln
305    310    315    320
His Arg Cys Glu Asp Val Asp Asp Cys Ile Leu Glu Pro Ser Pro Cys
325    330    335
Pro Gln Arg Cys Val Asn Thr Gln Gly Gly Phe Glu Cys His Cys Tyr
340    345    350
Pro Asn Tyr Asp Leu Val Asp Gly Glu Cys Val Glu Pro Val Asp Pro
355    360    365
Cys Phe Arg Ala Asn Cys Glu Tyr Gln Cys Gln Pro Leu Asn Gln Thr
370    375    380
Ser Tyr Leu Cys Val Cys Ala Glu Gly Phe Ala Pro Ile Pro His Glu
385    390    395    400
Pro His Arg Cys Gln Met Phe Cys Asn Gln Thr Ala Cys Pro Ala Asp
405    410    415
Cys Asp Pro Asn Thr Gln Ala Ser Cys Glu Cys Pro Glu Gly Tyr Ile
420    425    430
Leu Asp Asp Gly Phe Ile Cys Thr Asp Ile Asp Glu Cys Glu Asn Gly
435    440    445
Gly Phe Cys Ser Gly Val Cys His Asn Leu Pro Gly Thr Phe Glu Cys
450    455    460
Ile Cys Gly Pro Asp Ser Ala Leu Ala Arg His Ile Gly Thr Asp Cys
465    470    475    480

```

<210> 8

<211> 1440

<212> DNA

<213> human

<400> 8

```

atgcttgggg tcctggtcct tggcgcgctg gccctggccg gcctgggggt ccccgacccc 60
gcagagccgc agccgggtgg cagccagtgc gtcgagcacg actgcttcgc gctctacccc 120
ggccccgcga ccttcctcaa tgccagtcag atctgcgacg gactgcgggg ccacctaatg 180
acagtgcgct cctcgggtggc tgccgatgtc atttccttgc tactgaacgg cgacggcggc 240
gttgggcgcg ggcgctctct gatcggcctg cagctgccac ccggctgcgg cgaccccaag 300
cgctcggggc ccctgcgcgg ctccagtggt gttacgggag acaacaacac cagctatagc 360
aggtagggcag ggctcgacct caatggggct cccctctgcg gcccgttgtg cgtcgttgtc 420
tccgctgctg aggcactgt gcccagcgag ccgatctggg aggagcagca gtgcgaagtg 480
aaggccgatg gcttcctctg cgagttccac ttcccagcca cctgcaggcc actggctgtg 540
gagcccgggc cgcggtctgc cgcgtctctg atcacctacg gcaccccggt cgcggcccg 600
ggagcggact tccagggcgt gccggtgggc agctccgcgg cgggtggctc cctcggctta 660
cagctaagtg gcaccgcgcc gccggagcgg gtccaggggc actgggccag ggaggcggc 720
ggcgttggtg actgcagcgt ggagaacggc ggctgcgagc acgcgtgcaa tgcgatccct 780
ggggctcccc gctgcagtg cccagccggc gccgcctgac aggcagacgg gcgctcctgc 840
accgcatccc cgacgcagtc ctgcaacgac ctctgcgagc acttctgcgt tcccaacccc 900
gaccagccgg gctcctactc gtgcatgtgc gagaccggct accggctggc ggccgaccaa 960
caccggtgctg aggcgtgga tgactgcata ctggagccca gtccgtgtcc gcagcgtgt 1020
gtcaacacac aggggtggct cgagtggcac tgctacccta actacgacct ggtggacggc 1080
gagtggtgtg agccgtgga cccgtgcttc agagccaaact gcgagtacca gtgccagccc 1140
ctgaacaaaa ctagtacct ctgctgtctg gccgagggct tcgcgcccac tccccacgag 1200
ccgcacaggt gccagatgtt ttgcaaccag actgcctgtc cagccgactg cgaccccaac 1260
acccaggcta gctgtgagtg ccctgaaggc tacatcctgg acgacggttt catctgcacg 1320
gacatcgacg agtgcgaaaa cggcggttcc tgctcggggg tgtgccacaa cctccccggg 1380
accttcgagt gcatctgcgg gcccgactcg gcccttgccc gccacattgg caccgactgt 1440

```

<210> 9

<211> 516

<212> PRT

<213> human

<400> 9

```

Met Leu Gly Val Leu Val Leu Gly Ala Leu Ala Leu Ala Gly Leu Gly
1           5           10           15

```

```

Phe Pro Ala Pro Ala Glu Pro Gln Pro Gly Gly Ser Gln Cys Val Glu
20
His Asp Cys Phe Ala Leu Tyr Pro Gly Pro Ala Thr Phe Leu Asn Ala
35
Ser Gln Ile Cys Asp Gly Leu Arg Gly His Leu Met Thr Val Arg Ser
50
Ser Val Ala Ala Asp Val Ile Ser Leu Leu Leu Asn Gly Asp Gly Gly
65
Val Gly Arg Arg Arg Leu Trp Ile Gly Leu Gln Leu Pro Pro Gly Cys
85
Gly Asp Pro Lys Arg Leu Gly Pro Leu Arg Gly Phe Gln Trp Val Thr
100
Gly Asp Asn Thr Ser Tyr Ser Arg Trp Ala Arg Leu Asp Leu Asn
115
Gly Ala Pro Leu Cys Gly Pro Leu Cys Val Ala Val Ser Ala Ala Glu
130
Ala Thr Val Pro Ser Glu Pro Ile Trp Glu Glu Gln Gln Cys Glu Val
145
Lys Ala Asp Gly Phe Leu Cys Glu Phe His Phe Pro Ala Thr Cys Arg
165
Pro Leu Ala Val Glu Pro Gly Ala Ala Ala Ala Val Ser Ile Thr
180
Tyr Gly Thr Pro Phe Ala Ala Arg Gly Ala Asp Phe Gln Ala Leu Pro
195
Val Gly Ser Ser Ala Ala Val Ala Pro Leu Gly Leu Gln Leu Met Cys
210
Thr Ala Pro Pro Gly Ala Val Gln Gly His Trp Ala Arg Glu Ala Pro
225
Gly Ala Trp Asp Cys Ser Val Glu Asn Gly Gly Cys Glu His Ala Cys
245
Asn Ala Ile Pro Gly Ala Pro Arg Cys Gln Cys Pro Ala Gly Ala Ala
260
Leu Gln Ala Asp Gly Arg Ser Cys Thr Ala Ser Ala Thr Gln Ser Cys
275
Asn Asp Leu Cys Glu His Phe Cys Val Pro Asn Pro Asp Gln Pro Gly
290

```

```

Ser Tyr Ser Cys Met Cys Glu Thr Gly Tyr Arg Leu Ala Ala Asp Gln
305 310 315 320
His Arg Cys Glu Asp Val Asp Asp Cys Ile Leu Glu Pro Ser Pro Cys
325 330 335
Pro Gln Arg Cys Val Asn Thr Gln Gly Gly Phe Glu Cys His Cys Tyr
340 345 350
Pro Asn Tyr Asp Leu Val Asp Gly Glu Cys Val Glu Pro Val Asp Pro
355 360 365
Cys Phe Arg Ala Asn Cys Glu Tyr Gln Cys Gln Pro Leu Asn Gln Thr
370 375 380
Ser Tyr Leu Cys Val Cys Ala Glu Gly Phe Ala Pro Ile Pro His Glu
385 390 395 400
Pro His Arg Cys Gln Met Phe Cys Asn Gln Thr Ala Cys Pro Ala Asp
405 410 415
Cys Asp Pro Asn Thr Gln Ala Ser Cys Glu Cys Pro Glu Gly Tyr Ile
420 425 430
Leu Asp Asp Gly Phe Ile Cys Thr Asp Ile Asp Glu Cys Glu Asn Gly
435 440 445
Gly Phe Cys Ser Gly Val Cys His Asn Leu Pro Gly Thr Phe Glu Cys
450 455 460
Ile Cys Gly Pro Asp Ser Ala Leu Val Arg His Ile Gly Thr Asp Cys
465 470 475 480
Asp Ser Gly Lys Val Asp Gly Gly Asp Ser Gly Ser Gly Glu Pro Pro
485 490 495
Pro Ser Pro Thr Pro Gly Ser Thr Leu Thr Pro Pro Ala Val Gly Leu
500 505 510
Val His Ser Gly
515

```

<210> 10
 <211> 1548
 <212> DNA
 <213> human

<400> 10

```

atgcttgaggg tcctgggtcct tggcgcgctg gccctggcgg gcctgggggtt ccccgacacc 60
gcagagccgc agccgggtgg cagccagtgc gtcgagcacg actgcttcgc gctctaccgc 120
ggccccgcga ccttccctcaa tgccagtcag atctgcgacg gactgcgggg ccacctaatg 180
acagtgcgct cctcgggtggc tgccgatgtc atttccttgc tactgaacgg cgacggcggc 240
gttgggccgc ggcgccctctg gacggccttg cagctgccac ccggctgcgg cgaccccaag 300
cgctcggggc cctgcgcggg cttccagtgg gttacgggag acaacaacac cagctatagc 360
aggtggggcac ggctcgacct caatggggct cccctcttgc gccggtgtg cgtcgtgtc 420
tccgctgtct aggccactgt gcccagcgag ccgatctggg aggagcagca gtgcgaagtg 480
aaggccgatg gcttccctctg cgagtccac tccccagcca cctgcaggcc actggctgtg 540
gagcccgggc ccgcggtctg cgccgtctcg atcacctacg gcaccccggt cgcgcccgcc 600
ggagcgggact tccaggcgct gccgggtggg agctccgcgg cggtggctcc cctcggctta 660
cagctaatgt gcaccgcgcc gcccgagcg gtccaggggc actgggcccag ggaggcgccg 720
ggcgcttggg actgcagcgt ggagaacggc ggctgcgagc acgctgtgcaa tgcgatccct 780
ggggctcccc gctgcagtg cccagccggc gccgccctgc aggcagacgg gcgctcctgc 840
accgcatccg cgacgcagtc ctgcaacgac ctctgcgagc acttctgcgt tcccaacccc 900
gaccagccgg gctcctactc gtgcattgtc gagaccggct accggctggc ggccgaccaa 960
ctgaacacaa ctgactacct ctgcgtctgc ctggagccca gtccgtgtcc gcagcgctgt 1020
gtcaacacac aggggtggctt cgagtccac tgctacccta actacgacct ggtggacggc 1080
gagtgtgtgg agcccggtga cccgtgcttc agagccaact gcgagtacca gtgccagccc 1140
ctgaacacaa ctgactacct ctgcgtctgc gccgagggct tcgcgcccat tccccacgag 1200
ccgacagagt gccagatgtt ttgcaaccag actgcctgtc cagccgactg cgaccccaac 1260
acccaggcta cgtgtgagtg cctgaaggc tacatcctgg acgacgggtt catctgcag 1320
gacatcgacg agtgcgaaaa cgcggtcttc tgctccgggg tgtgccacaa cctccccgg 1380
accttcgagt gcatctgcgg gcccgactcg gccctgttcc gccacattgg caccgactgt 1440
gactccggca aggtggacgg tggcgacagc ggctctggcg agccccgcc cagcccgacg 1500
cccggtccca ccttgactcc tccggccgtg gggctcgtgc attcgggc 1548

```

<210> 11
 <211> 516
 <212> PRT
 <213> human

<400> 11

```

Met Leu Gly Val Leu Val Leu Gly Ala Leu Ala Leu Ala Gly Leu Gly
1 5 10 15
Phe Pro Ala Pro Ala Glu Pro Gln Pro Gly Gly Ser Gln Cys Val Glu
20 25 30
His Asp Cys Phe Ala Leu Tyr Pro Gly Pro Ala Thr Phe Leu Asn Ala
35 40 45
Ser Gln Ile Cys Asp Gly Leu Arg Gly His Leu Met Thr Val Arg Ser
50 55 60
Ser Val Ala Ala Asp Val Ile Ser Leu Leu Leu Asn Glv Asp Glv Glv

```

```

65      70      75      80
Val Gly Arg Arg Arg Leu Trp Ile Gly Leu Gln Leu Pro Pro Gly Cys
Gly Asp Pro Lys Arg Leu Gly Pro Leu Arg Gly Phe Gln Trp Val Thr
Gly Asp Asn Asn Thr Ser Tyr Ser Arg Trp Ala Arg Leu Asp Leu Asn
Gly Ala Pro Leu Cys Gly Pro Leu Cys Val Ala Val Ser Ala Ala Glu
Ala Thr Val Pro Ser Glu Pro Ile Trp Glu Glu Gln Gln Cys Glu Val
Lys Ala Asp Gly Phe Leu Cys Glu Phe His Phe Pro Ala Thr Cys Arg
Pro Leu Ala Val Glu Pro Gly Ala Ala Ala Ala Val Ser Ile Thr
Tyr Gly Thr Pro Phe Ala Ala Arg Gly Ala Asp Phe Gln Ala Leu Pro
Val Gly Ser Ser Ala Ala Val Ala Pro Leu Gly Leu Gln Leu Met Cys
Thr Ala Pro Pro Gly Ala Val Gln Gly His Trp Ala Arg Glu Ala Pro
Gly Ala Trp Asp Cys Ser Val Glu Asn Gly Gly Cys Glu His Ala Cys
Asn Ala Ile Pro Gly Ala Pro Arg Cys Gln Cys Pro Ala Gly Ala Ala
Leu Gln Ala Asp Gly Arg Ser Cys Thr Ala Ser Ala Thr Gln Ser Cys
275      280      285

```

```

Asn Asp Leu Cys Glu His Phe Cys Val Pro Asn Pro Asp Gln Pro Gly
290      295      300
Ser Tyr Ser Cys Met Cys Glu Thr Gly Tyr Arg Leu Ala Ala Asp Gln
305      310      315
His Arg Cys Glu Asp Val Asp Asp Cys Ile Leu Glu Pro Ser Pro Cys
320      325      330
Pro Gln Arg Cys Val Asn Thr Gln Gly Gly Phe Glu Cys His Cys Tyr
335      340      345
Pro Asn Tyr Asp Leu Val Asp Gly Glu Cys Val Glu Pro Val Asp Pro
350      355      360
Cys Phe Arg Ala Asn Cys Glu Tyr Gln Cys Gln Pro Leu Asn Gln Thr
365      370      375
Ser Tyr Leu Cys Val Cys Ala Glu Gly Phe Ala Pro Ile Pro His Glu
380      385      390
Pro His Arg Cys Gln Met Phe Cys Asn Gln Thr Ala Cys Pro Ala Asp
395      400      405
Cys Asp Pro Asn Thr Gln Ala Ser Cys Glu Cys Pro Glu Gly Tyr Ile
410      415      420
Leu Asp Asp Gly Phe Ile Cys Thr Asp Ile Asp Glu Cys Glu Asn Gly
425      430      435
Gly Phe Cys Ser Gly Val Cys His Asn Leu Pro Gly Thr Phe Glu Cys
440      445      450
Ile Cys Gly Pro Asp Ser Ala Leu Ala Arg His Ile Gly Thr Asp Cys
455      460      465
Asp Ser Gly Lys Val Asp Gly Gly Asp Ser Gly Ser Gly Glu Pro Pro
470      475      480
Pro Ser Pro Thr Pro Gly Ser Thr Leu Thr Pro Pro Ala Val Gly Leu
485      490      495
Val His Ser Gly
500      505      510
515

```

<210> 12

<211> 1548

<212> DNA

<213> human

<400> 12

```

atgcttgaggg tctctgggtcct tggcgcgcgtg gccctggccg gcctgggggtt ccccgacacc 60
gcagagccgc agccgggtgg cagccagtgc gtcgagcacg actgcttcgc gctctaccgc 120
ggccccgcga ctttctctcaa tgccagtcag atctgcgacg gactgcgggg ccacctaatg 180
acagtgcgct cctcggtggc tgccgatgtc atttcttgc tactgaacgg cgacggcggc 240
gttgggcggc ggcgctctcg gatcgccctg cagctgccac ccgctgcgg cgaccccaag 300
cgcctcgggc cctcgccggt cttccagtgg gttacgggag acaacaacac cagctatagc 360
aggctgggac ggctcgacct caatggggct cccctctgcg gccggtgtgt cgtcgtgtgc 420
tccgctgctg aggccactgt gccagcgag ccgatctggg aggagcagca gtgcgaagtg 480
aaggccgatg gcttctctcg caggttccac ttcccagcca cctgcaggcc actggctgtg 540
gagcccggcg ccgcgggtgc cgcgtctcgc atcacctacg gcaccccggt cgcggcccg 600
ggagcggact tccaggcgct gccgggtggc agctccgcgg cgggtggctcc cctcggttta 660
cagctaatgt gcaccgcgcc gccggagcg gtccagggg actgggccag ggaggcgccg 720
ggcgcttggg actgcagcgt ggagaacggc ggctgcgagc acgcgtgcaa tgcgatccct 780
gggggtcccc gctgccagtg ccagccggc gccgcccctg aggcagacgg gcgctcctgc 840
accgcatccg cgacgcagtc ctgcaacgac cttctgcgag acttctgcgt tcccaacccc 900
gaccagccgg gctcctactc gtgcatgtgc gagaccggct accggtggc ggccgaccaa 960

```

```

caccggtgcy aggacgtgga tgaatgcata ctggagccca gcccgtgtcc gcagcgttgc 1020
gtcaacacac aggggtggctt cgagtgccac tgctacccta actacgacct ggtggacggc 1080
gagtgtgtgg agcccgtgga cccgtgcttc agagccaact gcgagtacca gtgccagccc 1140
ctgaacaaaa ctagctacct ctgcgtctgc gccgagggct tcgcgcccac tccccacgag 1200
ccgcacaggt gccagatggt ttgcaaccag actgcctgtc cagccgactg cgaccccaac 1260
acccagggcta gctgtgagtg ccctgaaggc tacatcctgg acgacgggtt catctgcacg 1320
gacatcgacg agtgcgaaaa cggcgggctt tgctccgggg tgtgccacaa cttccccggg 1380
accttcgagt gcatctgcgg gcccgactcg gcccttgccc gccacattgg caccgactgt 1440
gactccggca aggtggacgg tggcgacagc ggctctggcg agcccccggc cagcccgacg 1500
cccggctcca ccttgactcc tccggccgtg gggctcgtgc attcgggc 1548

```

<210> 13

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Description of Artificial sequence: Synthetic DNA

<400> 13

aatgtggcgg gcaagggccg a 21

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

- [JP646219A](#) [0009] [0023] [0036] [0047] [0047] [0048] [0107] [0120]
- [JP5213998A](#) [0009] [0047] [0048]
- [JP2255699A](#) [0009] [0047]
- [JP3133380A](#) [0009]
- [JP3259084A](#) [0009]
- [JP4210700A](#) [0009]
- [WO9200325A](#) [0009]
- [WO9203149A](#) [0009]
- [WO9315755A](#) [0009]
- [JP3086900A](#) [0009]
- [JP3218399A](#) [0009]
- [WO03061687A](#) [0009]
- [JP11341990A](#) [0043]
- [WO889811A](#) [0044]

- [JP5310787A](#) [0047]
- [JP7155176A](#) [0047]
- [JP5042920A](#) [0056]
- [JP6445398B](#) [0056]
- [JP6205692A](#) [0056]
- [JP9110900A](#) [0056]
- [JP6321805A](#) [0107]
- [US61559864B](#) [0143] [0144]

Non-patent literature cited in the description

- CHEST/101/6American College of Chest Physicians199206001481-1483 [0010]
- Koji Suzuki, Igaku no Ayumi (Progress of Medicine), 1983, vol. 125, 901- [0010]
- K. GOMI et al.Blood, 1990, vol. 75, 1396-1399 [0010]
- M. ZUSHI et al.J. Biol. Chem., 1989, vol. 246, 10351-10353 [0010]
- D.Z. WEN et al.Biochemistry, 1987, vol. 26, 4350-4357 [0010]
- S.M. BATES et al.Br. J. Pharmacol., 2005, vol. 144, 1017-1028 [0010]
- T. KOGARHEUMATOLOGY INTERNATIONAL CLINICAL AND EXPERIMENTAL INVESTIGATIONS, 2011, vol. 31, 1657-1659 [0010]
- Crit. Care Med., 2003, vol. 31, 1250-1256 [0012]
- JAMA, 2003, vol. 290, 2238-247 [0012]
- XIGRISN. Engl. J. Med., 2001, vol. 344, 10699-709 [0014]
- Thrombosis and Haemostasis, 1993, vol. 70, 3418-422 [0023]
- The Journal of Biological Chemistry, 1989, vol. 264, 94872-4876 [0023]
- Blood, 2008, vol. 112, 3361-3670 [0023]
- The Journal of Clinical Investigation, 2005, vol. 115, 51267-1274 [0023]
- Biochemical Experimental Methods, Vol. 23, Methods of Researches on Glycoprotein Sugar ChainsJapan Scientific Societies Press19900000vol. 23, [0043]
- Lecture of New Biochemical ExperimentsSugar I, GlycoproteinTokyo Kagaku Dojin19900000vol. 3, [0043]
- MULLIGAN R.C. et al.Proc. Natl. Acad. Sci. U.S.A., 1981, vol. 78, 2072- [0045]
- HOWLEY P.M. et al.Methods in EmzymologyAcademic Press19830000vol. 101, 387- [0045]
- J. Biol. Chem., 1989, vol. 264, 10351-10353 [0047]
- ZOLLER M.J. et al.Method in EmzymologyAcademic Press19830000vol. 100, 468-500 [0048]
- Tanpakushitsu/Koso no Kiso Jikken Ho (Fundamental Experimental Methods for Proteins and Enzymes)19810000 [0056]
- GOMI K. et al.Blood, 1990, vol. 75, 1396-1399 [0057]
- LEVY M. et al.Crit. Care. Med., vol. 31, 1250-1256 [0068]

- Members of the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference Crit. Care Med., 1992, vol. 20, 864-874 [0068]
- Harrison's Principles of Internal Medicine Medical Science International, Ltd.vol. 124, 828-833 [0069]
- **FUNADA H.**Elucidation and Treatment Strategy for Sepsis Iyaku Journal Co., Ltd.2006000038- [0086]
- Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock 2008 Crit. Care Med., 2008, vol. 36, 1296-327 [0086]
- **MASAO NAKAGAWA**Search report regarding use of criteria of disseminated intravascular coagulation (DIC)Research Study Team of Intractable Disease (Blood Coagulation Abnormality), the Ministry of Health and Welfare, 1999, vol. 1999, 65-72 [0091]
- **KATSUMI DEGUCHI**Tentative plan regarding standards for initiation of early treatment of DICResearch Study Team of Intractable Disease (Blood Coagulation Abnormality), the Ministry of Health and Welfare, Study report 1999, vol. 1999, 73-77 [0091]
- **KATSUMI NAKAGAWA****HAJIME TSUJI**Current diagnosis of DIC-Reports on results of inquiry surveyClinical Blood., 1999, vol. 40, 362-364 [0091]
- Crit. Care Med., 2008, vol. 36, 296-327 [0097]
- Crit. Care Med., 2003, vol. 32, 31250-56 [0097]

P a t e n t k r a v

- 5 **1.** Medikament, som omfatter thrombomodulin som en aktiv bestanddel, til anvendelse i terapeutisk behandling og/eller forbedring af sepsis, hvor medikamentet skal indgives til en patient med alvorlig sepsis ledsaget af en eller flere organdysfunktioner, hvor en værdi ifølge International Normalized Ratio (INR) af en plasmaprøve, der er taget fra patienten, er mere end 1,4.
- 10 **2.** Medikament, som omfatter thrombomodulin som en aktiv bestanddel, til anvendelse til at reducere dødeligheden hos en patient med sepsis, hvor medikamentet skal indgives til en patient med alvorlig sepsis ledsaget af en eller flere organdysfunktioner, hvor en værdi ifølge International Normalized Ratio (INR) af en plasmaprøve, der er taget fra patienten, er mere end 1,4.
- 15 **3.** Medikament til anvendelse ifølge krav 1 eller 2, hvor medikamentet skal indgives til en patient med alvorlig sepsis ledsaget af en eller flere organdysfunktioner, hvor en værdi ifølge International Normalized Ratio (INR) af en plasmaprøve, der er taget fra patienten, er mere end 1,4 og lig med eller mindre end 1,6.
- 20 **4.** Medikament til anvendelse ifølge et af kravene 1 til 3, hvor patienten med alvorlig sepsis er en alvorligt septisk patient, som ikke er en septisk patient med organdysfunktion, der er begrænset til lever eller nyre.
- 25 **5.** Medikament til anvendelse ifølge et af kravene 1 til 4, hvor medikamentet skal indgives til en patient med alvorlig sepsis ledsaget af en eller flere organdysfunktioner udvalgt fra gruppen bestående af leverdysfunktion, nyredysfunktion, åndedrætsorgandysfunktion og kredsløbsorgandysfunktion.
- 30 **6.** Medikament til anvendelse ifølge et af kravene 1 til 4, hvor medikamentet skal indgives til en patient med alvorlig sepsis ledsaget af en eller flere organdysfunktioner udvalgt fra gruppen bestående af åndedrætsorgandysfunktion og kredsløbsorgandysfunktion.

7. Medikament til anvendelse ifølge et af kravene 1 til 6, hvor thrombomodulin er et opløseligt thrombomodulin.
- 5 8. Medikament til anvendelse ifølge et af kravene 1 til 7, hvor thrombomodulin er et peptid, som kan opnå ud fra en transformeret celle fremstillet ved transficering af en værtselle med en DNA, der koder for aminosyresekvensen med SEQ ID NO: 9 eller SEQ ID NO: 11.
- 10 9. Medikament til anvendelse ifølge et af kravene 1 til 8, hvor thrombomodulin er et peptid, indeholdende aminosyresekvensen (i-1); (i-1) aminosyresekvensen af positionerne 19 til 516 aminosyresekvensen med SEQ ID NO: 9 eller 11.
- 15 10. Medikament til anvendelse ifølge et af kravene 1 til 9, hvor thrombomodulin indgives i en dosis på 0,005 til 1 mg/kg inden for 5 minutter ved intravenøs bolusindgivelse.
- 20 11. Medikament til anvendelse ifølge et af kravene 1 til 10, hvor sepsis er alvorlig sepsis med koagulopati.
- 25 12. Medikament til anvendelse ifølge et af kravene 1 til 11, hvor kredsløbsorgan dysfunktionen er chok.
- 30 13. Medikament til anvendelse ifølge et af kravene 1 til 12, hvor organ dysfunktionerne er induceret af sepsis.
14. Medikament til anvendelse ifølge et af kravene 1 til 13, hvor medikamentet skal indgives til en patient med alvorlig sepsis, hvor en patient med en INR-værdi på 1,7 er udelukket.
15. Medikament til anvendelse ifølge et af kravene 1 til 14, hvor nævnte thrombomodulin indgives til patienten intravenøst.

16. Medikament til anvendelse ifølge et af kravene krav 1 til 15, hvor nævnte thrombomodulin indgives til patienten i en dosis på 0,04 til 0,08 mg/kg/dag.