DESCRIPTION

ANTI-LPS O11 ANTIBODY

Technical Field

[0001]

The present invention relates to a substance useful as a therapeutic and/or prophylactic agent for pseudomonal infection, and to a method for the treatment and/or prophylaxis of pseudomonal infection.

Background Art

[0002]

Pseudomonas aeruginosa is a species of typical resident microbiota which exists in the natural environment, and is well known as a pathogen causing opportunistic infections in immune-compromised humans. In addition, Pseudomonas aeruginosa is one of the species that are most problematic from the high isolation rate and mortality rate in healthcare-associated infections. It has been reported that the isolation rate for Pseudomonas aeruginosa is the sixth highest out of all healthcare-associated pathogenic infections and the second highest for patients having pneumonia derived from tracheal intubation, and the mortality rate due to Pseudomonas aeruginosa is 27.6-47.9% in bloodstream infections and 42.1-87% in tracheal intubation (Non-Patent Literatures 1 to 3). Further, since Pseudomonas aeruginosa originally had

a high resistance to disinfectants and antibiotics and there are many strains that have acquired drug resistance, it is often difficult to treat the pseudomonal infections. Antimicrobial agents for Pseudomonas aeruginosa include carbapenems, cephems, penicillins, quinolones, and the like, and the resistance rate against these antimicrobial agents is a high rate such as 25.3%, 11.2%, 17.5%, and 30.7%, respectively (Non-Patent Literature 1). There is therefore a demand for a new anti-pseudomonas aeruginosa drug which is also effective against multidrug-resistant Pseudomonas aeruginosa, especially since there are almost no antimicrobial agents effective against multidrug-resistant bacteria showing a resistance to the above antimicrobial agents.

[0003]

Pseudomonas aeruginosa is a gram-negative bacterium having a lipopolysaccharide (LPS) on the surface layer, and a sugar chain structure called O antigen outside of this LPS. The O-antigen has antigenicity and because of its diversity, it has been used as a classification method for bacterial strains (Non-Patent Literatures 4 and 5). In general 20 species of the LPS O antigen serotype of Pseudomonas aeruginosa are known, and in recent years, it has been reported that the strains having O11 antigen (O11 strains) occupy a high ratio in the multidrug-resistant Pseudomonas aeruginosa. In Japan there are reports that the isolation rates of O11 stains in the

multidrug-resistant Pseudomonas aeruginosa were 99% or about 60%, and in Europe such as France, Belgium, and Czech Republic, there are also reports that a number of multidrug-resistant Pseudomonas aeruginosa strains were O11 strains (Non-Patent Literatures 6 to 11).

[0004]

Antibodies are known to provide long-term protection against infection in vivo. Many antibodies against the LPS O antigens of Pseudomonas aeruginosa have been reported to exhibit a therapeutic efficacy by their opsonophagocytic killing activities on infections in an animal infection model (Non-Patent Literatures 12 to 14). From this, such antibodies against the LPS O11 antigens of Pseudomonas aeruginosa are expected to be a new drug against multidrug-resistant Pseudomonas aeruginosa, and there are already some reports on anti-LPS O11 antibodies (Patent Literatures 1 and 2).

[0005]

The present invention relates to a therapeutic and diagnostic antibody that targets the Pseudomonas aeruginosa O11 strain. The therapeutic and diagnostic antibody is desired to have a high activity, and its opsonophagocytic killing activity is important. Further, antibodies are known to have high antigen specificity, and those having a high coverage ratio capable of broadly covering the O11 strains are desired as a therapeutic and diagnostic antibodies. Since the present antibodies are

antibodies to the LPS O11 antigens and show a stronger opsonophagocytic killing activity and a higher coverage ratio compared to conventional antibodies, they are more useful.

Citation List

Patent Literature

[0006]

Patent Literature 1: WO 2006/084758

Patent Literature 2: WO 2011/102551

Non-Patent Literature

[0007]

Non-Patent Literature 1: Infect. Control Hosp. Epidemiol., 29, 996-1011 (2008)

Non-Patent Literature 2: DATAMONITOR, DMHC2281, Nosocomial infections (2007)

Non-Patent Literature 3: Chest, 139, 909-919 (2011)

Non-Patent Literature 4: Crit. Rev. Microbiol., 17, 273-304 (1990)

Non-Patent Literature 5: J. Endotoxin Res., 12, 324-335 (2006)

Non-Patent Literature 6: J. Clin. Microbiol., 45, 979-989 (2007)

Non-Patent Literature 7: Inter. J. Antimicrob. Agents, 39, 518-521 (2012)

Non-Patent Literature 8: J. Clin. Microbiol., 49, 2578-2583 (2011)

Non-Patent Literature 9: J. Antimicrob. Chemother., 65, 866-871 (2010)

Non-Patent Literature 10: Research in Microbiology, 161, 234-242 (2010)

Non-Patent Literature 11: PLoS ONE, 6, e25617 (2011)

Non-Patent Literature 12: Infect. Immun., 57, 174-179 (1989)

Non-Patent Literature 13: FEMS Microbiol. Immunol., 2, 263-268 (1990)

Non-Patent Literature 14: Infect. Immun., 66, 4137-4142 (1998)

Summary of the Invention

Technical Problem

[0008]

The purpose of the present invention is to provide a substance having superior antimicrobial activity against Pseudomonas aeruginosa, and to provide a therapeutic and/or prophylactic agent for pseudomonal infection.

Solution to the Problem

[0009]

The present inventors made intensive studies in order to solve the above problem and as a result, they have succeeded in obtaining a novel antibody which specifically binds to the

LPS O11 antigen of Pseudomonas aeruginosa and which has therapeutic and/or prophylactic effects on pseudomonal infection. The present invention has been completed based on these findings.

That is, the present invention encompasses the following inventions.

(1) An antibody, or an antigen-binding fragment thereof, that recognizes LPS of Pseudomonas aeruginosa, binds to O11 antigen and does not bind to O1, O2, O3, O4, O5, O6, O7, O8, O9, O10, O13, O14, O15, O16, O17, O18, O19, O20 antigens, and O antigen-deficient strain, and has a coverage ratio of 85% or more for the O11 clinical isolates,.

(2) The antibody or an antigen-binding fragment thereof according to (1), wherein the coverage ratio for the O11 clinical isolates is 95% or more.

(3) An antibody, or an antigen-binding fragment thereof, that recognizes LPS of Pseudomonas aeruginosa, binds to the O11 antigen, and does not bind to O1, O2, O3, O4, O5, O6, O7, O8, O9, O10, O13, O14, O15, O16, O17, O18, O19, O20 antigens and O antigen-deficient strain, and exhibits 50% minimum growth inhibitory concentration of opsonophagocytic killing activity against Pseudomonas aeruginosa which is identified by ATCC 29260 is 12 ng/mL or less.

(4) The antibody or an antigen-binding fragment thereof according to (3), wherein the 50% minimum growth inhibitory

concentration of opsonophagocytic killing activity against Pseudomonas aeruginosa identified by ATCC 29260 is 4.1 ng/mL or less.

(5) An antibody, or an antigen-binding fragment thereof, characterized in that it cross-competes with at least one of the antibodies selected from the group consisting of an antibody containing a heavy chain variable region sequence comprising amino acid residues 20 to 140 of the amino acid sequence shown in SEQ ID NO: 8 and a light chain variable region sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 4; an antibody containing a heavy chain variable region sequence comprising amino acid residues 20 to 144 of the amino acid sequence shown in SEQ ID NO: 16 and a light chain variable region sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 12; and an antibody containing a heavy chain variable region sequence comprising amino acid residues 20 to 140 of the amino acid sequence shown in SEQ ID NO: 24 and a light chain variable region sequence comprising 21 to 129 amino acid residues of the amino acid sequence shown in SEQ ID NO: 12.

(6) The antibody or an antigen-binding fragment thereof according to (5), characterized in that it binds to an epitope to which at least one antibody binds, wherein the antibody is selected from the group consisting of an antibody containing a heavy chain variable region sequence comprising amino acid

residues 20 to 140 of the amino acid sequence shown in SEQ ID NO: 8 and a light chain variable region sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 4; an antibody containing a heavy chain variable region sequence comprising amino acid residues 20 to 144 of the amino acid sequence shown in SEQ ID NO: 16 and a light chain variable region sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 12; and an antibody containing a heavy chain variable region sequence comprising amino acid residues 20 to 140 of the amino acid sequence shown in SEQ ID NO: 24 and a light chain variable region sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 12.

(7) The antibody or an antigen-binding fragment thereof according to any one of (1) to (6), characterized in that:

the heavy chain sequence contains a variable region having CDRH1, CDRH2, and CDRH3, and the CDRH1 comprises the amino acid sequence shown in SEQ ID NO: 9, the CDRH2 comprises the amino acid sequence shown in SEQ ID NO: 10, and the CDRH3 comprises the amino acid sequence shown in SEQ ID NO: 11, and

the light chain sequence contains a variable region having CDRL1, CDRL2, and CDRL3, and the CDRL1 comprises the amino acid sequence shown in SEQ ID NO: 5, the CDRL2 comprises the amino acid sequence shown in SEQ ID NO: 6, and the CDRL3 comprises the amino acid sequence shown in SEQ ID NO: 7.

(8) The antibody or an antigen-binding fragment thereof according to (7), characterized by containing a heavy chain variable region sequence comprising amino acid residues 20 to 140 of the amino acid sequence shown in SEQ ID NO: 8 and a light chain variable region sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 4.

(9) The antibody or an antigen-binding fragment thereof according to any one of (1) to (6), characterized in that:

the heavy chain sequence contains a variable region having CDRH1, CDRH2, and CDRH3, and the CDRH1 comprises the amino acid sequence shown in SEQ ID NO: 17, the CDRH2 comprises the amino acid sequence shown in SEQ ID NO: 18, and the CDRH3 comprises the amino acid sequence shown in SEQ ID NO: 19, and

the light chain sequence contains a variable region having CDRL1, CDRL2, and CDRL3, and the CDRL1 comprises the amino acid sequence shown in SEQ ID NO: 13, the CDRL2 comprises the amino acid sequence shown in SEQ ID NO: 14, and the CDRL3 comprises the amino acid sequence shown in SEQ ID NO: 15.

(10) The antibody or an antigen-binding fragment thereof according to (9), characterized by containing a heavy chain variable region sequence comprising amino acid residues 20 to 144 of the amino acid sequence shown in SEQ ID NO: 16 and a light chain variable region sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 12.

(11) The antibody or an antigen-binding fragment of the

antibody according to any one of (1) to (6), characterized in that:

the heavy chain sequence contains a variable region having CDRH1, CDRH2, and CDRH3, and the CDRH1 comprises the amino acid sequence shown in SEQ ID NO: 25, the CDRH2 comprises the amino acid sequence shown in SEQ ID NO: 26, and the CDRH3 comprises the amino acid sequence shown in SEQ ID NO: 27, and

the light chain sequence contains a variable region having CDRL1, CDRL2, and CDRL3, and the CDRL1 comprises the amino acid sequence shown in SEQ ID NO: 13, the CDRL2 comprises the amino acid sequence shown in SEQ ID NO: 14, and the CDRL3 comprises the amino acid sequence shown in SEQ ID NO: 15.

(12) The antibody or an antigen-binding fragment thereof according to (11), characterized by containing a heavy chain variable region sequence comprising amino acid residues 20 to 140 of the amino acid sequence shown in SEQ ID NO: 24 and a light chain variable region sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 12.

(13) An antigen-binding fragment of the antibody according to any one of (1) to (12), characterized by being selected from the group consisting of Fab, F(ab')2, Fab' and Fv.

(14) The antibody according to any one of (1) to (12), characterized by being scFv.

(15) The antibody or an antigen-binding fragment thereof according to (1) to (12), characterized by being a chimeric

antibody.

(16) The antibody or an antigen-binding fragment thereof according to (1) to (12), characterized by being a humanized antibody.

(17) The antibody according to any one of (1) to (16), wherein the heavy chain contains a constant region of a human immunoglobulin G1 heavy chain, and the light chain contains a constant region of a human immunoglobulin κ light chain.

(18) An antibody, or an antigen-binding fragment thereof, which has a binding activity to the LPS O11 antigen of Pseudomonas aeruginosa characterized in that the antibody contains:

(a) a heavy chain variable region sequence selected from the group consisting of the following amino acid sequences:

a1) an amino acid sequence comprising amino acid residues 20 to 144 of the amino acid sequence shown in SEQ ID NO: 57;

a2) an amino acid sequence comprising amino acid residues 20 to 144 of the amino acid sequence shown in SEQ ID NO: 59;

a3) an amino acid sequence comprising amino acid residues 20 to 144 of the amino acid sequence shown in SEQ ID NO: 61;

a4) an amino acid sequence comprising amino acid residues 20 to 144 of the amino acid sequence shown in SEQ ID NO: 63;

a5) an amino acid sequence comprising amino acid residues 20 to 144 of the amino acid sequence shown in SEQ ID NO: 65;

a6) an amino acid sequence having a homology of at least 95% with any one of the amino acid sequences selected from a1)

to a5);

a7) an amino acid sequence having a homology of at least 99% with any one of the amino acid sequences selected from a1) to a5); and

a8) an amino acid sequence including substitution, deletion, or addition of one to several amino acid residues in any one of the amino acid sequences selected from a1) to a5); and

(b) a light chain variable region sequence selected from the group consisting of the following amino acid sequences:

b1) an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 67;

b2) an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 69;

b3) an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 71;

b4) an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 73;

b5) an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 75;

b6) an amino acid sequence having a homology of at least 95% with any one of the amino acid sequences selected from b1) to b5);

b7) an amino acid sequence having a homology of at least 99% with any one of the amino acid sequences selected from b1)

to b5); and

b8) an amino acid sequence including substitution, deletion, or addition of one to several amino acid residues in any one of the amino acid sequences selected from b1) to b5).

(19) The antibody or an antigen-binding fragment thereof according to (18), characterized by containing a heavy chain variable region sequence comprising amino acid residues 20 to 144 of the amino acid sequence shown in SEQ ID NO: 59 and a light chain variable region sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 69.

(20) The antibody or an antigen-binding fragment thereof according to (18), characterized by containing a heavy chain variable region sequence comprising amino acid residues 20 to 144 of the amino acid sequence shown in SEQ ID NO: 61 and a light chain variable region sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 71.

(21) An antibody or an antigen-binding fragment thereof having a binding activity to the LPS O11 antigen of Pseudomonas aeruginosa, characterized by containing:

(a) a heavy chain variable region sequence selected from the group consisting of the following amino acid sequences:

a1) an amino acid sequence comprising amino acid residues 20 to 140 of the amino acid sequence shown in SEQ ID NO: 77;

a2) an amino acid sequence comprising amino acid residues 20 to 140 of the amino acid sequence shown in SEQ ID NO: 79;

a3) an amino acid sequence comprising amino acid residues 20 to 140 of the amino acid sequence shown in SEQ ID NO: 81;

a4) an amino acid sequence comprising amino acid residues 20 to 140 of the amino acid sequence shown in SEQ ID NO: 83;

a5) an amino acid sequence comprising amino acid residues 20 to 140 of the amino acid sequence shown in SEQ ID NO: 85;

a6) an amino acid sequence having a homology of at least 95% with any one of the amino acid sequences selected from a1) to a5);

a7) an amino acid sequence having a homology of at least 99% with any one of the amino acid sequences selected from a1) to a5); and

a8) an amino acid sequence including substitution, deletion, or addition of one to several amino acid residues in any one of the amino acid sequences selected from a1) to a5); and

(b) a light chain variable region sequence selected from the group consisting of the following amino acid sequences:

b1) an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 67;

b2) an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 69;

b3) an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 71;

b4) an amino acid sequence comprising amino acid residues

21 to 129 of the amino acid sequence shown in SEQ ID NO: 73;

b5) an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 75;

b6) an amino acid sequence having a homology of at least 95% with any one of the amino acid sequences selected from b1) to b5);

b7) an amino acid sequence having a homology of at least 99% with any one of the amino acid sequences selected from b1) to b5); and

b8) an amino acid sequence including substitution, deletion, or addition of one to several amino acid residues in any one of the amino acid sequences selected from b1) to b5).

(22) The antibody or an antigen-binding fragment thereof according to (21), characterized by containing a heavy chain variable region sequence comprising amino acid residues 20 to 140 of the amino acid sequence shown in SEQ ID NO: 79 and a light chain variable region sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 69.

(23) The antibody or an antigen-binding fragment thereof according to (21), characterized by containing a heavy chain variable region sequence comprising amino acid residues 20 to 140 of the amino acid sequence shown in SEQ ID NO: 81 and a light chain variable region sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 71.

(24) The antibody according to any one of (18) to (23),

comprising a heavy chain including deletion of one to several amino acids from the carboxyl terminus.

(25) A pharmaceutical composition comprising at least one of the antibody or the antigen-binding fragment thereof according to (1) to (24).

(26) The pharmaceutical composition according to (25), characterized by being a therapeutic and/or prophylactic agent for pseudomonal infection.

(27) A pharmaceutical composition for the treatment and/or prophylaxis of pseudomonal infection, characterized by comprising at least one of the antibody or antigen-binding fragment thereof according to any one of (1) to (24) and at least one selected from the group consisting of penicillin, cephem, carbapenem, monobactam, quinolone, aminoglycoside, polymyxin, rifampicin, and macrolide antimicrobial agents.

(28) The pharmaceutical composition according to (26) or (27), characterized in that the pseudomonal infection is a systemic infectious disease caused by a multidrug-resistant Pseudomonas aeruginosa.

(29) The pharmaceutical composition according to (26) or (27), characterized in that the pseudomonal infection is:

bloodstream infection, sepsis, meningitis, endocarditis, otitis media, sinusitis, pneumonia, lung abscess, empyema, chronic respiratory tract infection, peritonitis, post-operative infection, cholecystitis, cholangitis, eyelid

tumor, dacryocystitis, conjunctivitis, corneal ulcer, corneal abscess, panophthalmitis, orbital infection, urinary tract infection, catheter infection, perianal abscess, secondary infection of burn wounds, decubitus infection, cystic fibrosis disease, lymphangitis, lymphadenitis, osteomyelitis, arthritis, tonsillitis, liver abscess, skin and soft tissue infections, intrauterine infection, uterine adnexitis, parametritis, perimandibular phlegmon, or jaw inflammation.

(30) The pharmaceutical composition according to (26) or (27), characterized in that the pseudomonal infection is pneumonia.

(31) A method for the treatment and/or prophylaxis of pseudomonal infection, characterized by administering at least one of the antibody, or the antigen-binding fragment thereof, according to (1) to (24), or the pharmaceutical composition according to (26) or (27).

(32) A method for the treatment and/or prophylaxis of pseudomonal infection, characterized by simultaneously or sequentially administering at least one of the antibodies or the antigen-binding fragment thereof according to any one of (1) to (24), or the pharmaceutical composition according to (26), and at least one selected from the group consisting of penicillin, cephem, carbapenem, monobactam, quinolone, aminoglycoside, polymyxin, rifampicin, and macrolide antimicrobial agents.

(33) The method for the treatment and/or prophylaxis of

pseudomonal infection according to (31) or (32), characterized in that the pseudomonal infection is a systemic infectious disease caused by a multidrug-resistant Pseudomonas aeruginosa.

(34) The method for the treatment and/or prophylaxis of pseudomonal infection according to (33), characterized in that the pseudomonal infection is bloodstream infection, sepsis, meningitis, endocarditis, otitis media, sinusitis, pneumonia, lung abscess, empyema, chronic respiratory tract infection, peritonitis, post-operative infection, cholecystitis, cholangitis, eyelid tumor, dacryocystitis, conjunctivitis, corneal ulcer, corneal abscess, panophthalmitis, orbital infection, urinary tract infection, catheter infection, perianal abscess, secondary infection of burn wounds, decubitus infection, cystic fibrosis disease, lymphangitis, lymphadenitis, osteomyelitis, arthritis, tonsillitis, liver abscess, skin and soft tissue infections, intrauterine infection, uterine adnexitis, parametritis, perimandibular phlegmon, or jaw inflammation.

(35) The method for the treatment and/or prophylaxis of pseudomonal infection according to (33), characterized in that the pseudomonal infection is pneumonia.

(36) A diagnostic agent for pseudomonal infection, characterized by containing at least one of the antibodies or the antigen-binding fragments thereof according to (1) to (24).

(37) A detection kit for Pseudomonas aeruginosa,

characterized by containing at least one of the antibodies or the antigen-binding fragments thereof according to (1) to (24).

(38) A polynucleotide encoding any one of the antibodies according to (1) to (24).

(39) The polynucleotide according to (38), characterized by containing:

(a) a polynucleotide selected from the group consisting of the following nucleotide sequences:

a1) a nucleotide sequence comprising nucleotides 58 to 432 of the nucleotide sequence shown in SEQ ID NO: 56;

a2) a nucleotide sequence comprising nucleotides 58 to 432 of the nucleotide sequence shown in SEQ ID NO: 58;

a3) a nucleotide sequence comprising nucleotides 58 to 432 of the nucleotide sequence shown in SEQ ID NO: 60;

a4) a nucleotide sequence comprising nucleotides 58 to 432 of the nucleotide sequence shown in SEQ ID NO: 62;

a5) a nucleotide sequence comprising nucleotides 58 to 432 of the nucleotide sequence shown in SEQ ID NO: 64;

a6) a nucleotide sequence having a homology of at least 95% with any one of the nucleotide sequences selected from a1) to a5);

a7) a nucleotide sequence having a homology of at least 99% with any one of the nucleotide sequences selected from a1) to a5);

a8) a nucleotide sequence of a polynucleotide which

hybridizes to a polynucleotide comprising a nucleotide sequence complementary to any one of the nucleotide sequences selected from a1) to a5) under stringent conditions; and

a9) a nucleotide sequence including substitution, deletion, or addition of one to several amino acid residues in any one of the nucleotide sequences selected from a1) to a5); and

(b) a polynucleotide selected from the group consisting of the following nucleotide sequences:

b1) a nucleotide sequence comprising nucleotides 61 to 387 of the nucleotide sequence shown in SEQ ID NO: 66;

b2) a nucleotide sequence comprising nucleotides 61 to 387 of the nucleotide sequence shown in SEQ ID NO: 68;

b3) a nucleotide sequence comprising nucleotides 61 to 387 of the nucleotide sequence shown in SEQ ID NO: 70;

b4) a nucleotide sequence comprising nucleotides 61 to 387 of the nucleotide sequence shown in SEQ ID NO: 72;

b5) a nucleotide sequence comprising nucleotides 61 to 387 of the nucleotide sequence shown in SEQ ID NO: 74;

b6) a nucleotide sequence having a homology of at least 95% with any one of the nucleotide sequences selected from b1) to b5);

b7) a nucleotide sequence having a homology of at least 99% with any one of the nucleotide sequences selected from b1) to b5);

b8) a nucleotide sequence of a polynucleotide which hybridizes to a polynucleotide comprising a nucleotide sequence complementary to any one of the nucleotide sequences selected from b1) to b5) under stringent conditions; and

b9) a nucleotide sequence including substitution, deletion, or addition of one to several nucleotides in any one of the nucleotide sequences selected from b1) to b5).

(40) The polynucleotide according to (39), characterized by containing a polynucleotide comprising nucleotides 58 to 432 of the nucleotide sequence shown in SEQ ID NO: 58 and a polynucleotide comprising nucleotides 61 to 387 of the nucleotide sequence shown in SEQ ID NO: 68.

(41) The polynucleotide according to (39), characterized by containing a polynucleotide comprising nucleotides 58 to 432 of the nucleotide sequence shown in SEQ ID NO: 60 and a polynucleotide comprising nucleotides 61 to 387 of the nucleotide sequence shown in SEQ ID NO: 70.

(42) The polynucleotide according to (38), characterized by containing:

(a) a polynucleotide selected from the group consisting of the following nucleotide sequences:

a1) a nucleotide sequence comprising nucleotides 58 to 420 of the nucleotide sequence shown in SEQ ID NO: 76;

a2) a nucleotide sequence comprising nucleotides 58 to 420 of the nucleotide sequence shown in SEQ ID NO: 78;

a3) a nucleotide sequence comprising nucleotides 58 to 420 of the nucleotide sequence shown in SEQ ID NO: 80;

a4) a nucleotide sequence comprising nucleotides 58 to 420 of the nucleotide sequence shown in SEQ ID NO: 82;

a5) a nucleotide sequence comprising nucleotides 58 to 420 of the nucleotide sequence shown in SEQ ID NO: 84;

a6) a nucleotide sequence having a homology of at least 95% with any one of the nucleotide sequences selected from a1) to a5);

a7) a nucleotide sequence having a homology of at least 99% with any one of the nucleotide sequences selected from a1) to a5);

a8) a nucleotide sequence of a polynucleotide which hybridizes to a polynucleotide comprising a nucleotide sequence complementary to any one of the nucleotide sequences selected from a1) to a5) under stringent conditions; and

a9) a nucleotide sequence including substitution, deletion, or addition of one to several amino acid residues in any one of the nucleotide sequences selected from a1) to a5); and

(b) a polynucleotide selected from the group consisting of the following nucleotide sequences:

b1) a nucleotide sequence comprising nucleotides 61 to 387 of the nucleotide sequence shown in SEQ ID NO: 66;

b2) a nucleotide sequence comprising nucleotides 61 to

387 of the nucleotide sequence shown in SEQ ID NO: 68;

b3) a nucleotide sequence comprising nucleotides 61 to 387 of the nucleotide sequence shown in SEQ ID NO: 70;

b4) a nucleotide sequence comprising nucleotides 61 to 387 of the nucleotide sequence shown in SEQ ID NO: 72;

b5) a nucleotide sequence comprising nucleotides 61 to 387 of the nucleotide sequence shown in SEQ ID NO: 74;

b6) a nucleotide sequence having a homology of at least 95% with any one of the nucleotide sequences selected from b1) to b5);

b7) a nucleotide sequence having a homology of at least 99% with any one of the nucleotide sequences selected from b1) to b5);

b8) a nucleotide sequence of a polynucleotide which hybridizes to a polynucleotide comprising a nucleotide sequence complementary to any one of the nucleotide sequences selected from b1) to b5) under stringent conditions; and

b9) a nucleotide sequence including substitution, deletion, or addition of one to several nucleotides in any one of the nucleotide sequences selected from b1) to b5).

(43) The polynucleotide according to (42), characterized by containing a polynucleotide comprising nucleotides 58 to 420 of the nucleotide sequence shown in SEQ ID NO: 78 and a polynucleotide comprising nucleotides 61 to 387 of the nucleotide sequence shown in SEQ ID NO: 68.

(44) The polynucleotide according to (42), characterized by containing a polynucleotide comprising nucleotides 58 to 420 of the nucleotide sequence shown in SEQ ID NO: 80 and a polynucleotide comprising nucleotides 61 to 387 of the nucleotide sequence shown in SEQ ID NO: 70.

(45) A vector containing any one of the polynucleotides according to (38) to (44).

(46) A transformed host cell containing any one of the polynucleotides according to (38) to (44).

(47) A transformed host cell containing the vector according to (45).

(48) A method for producing the antibody according to any one of (1) to (24), containing the steps of culturing the host cell according to (46) or (47) and purifying the antibody from the resulting cultured product.

Advantageous Effects of the Invention

[0010]

According to the present invention, it is possible to obtain a therapeutic and/or prophylactic agent for a pseudomonal infection that is a systemic infectious disease caused by Pseudomonas aeruginosa including a multidrug-resistant Pseudomonas aeruginosa.

Brief Description of Drawings

[0011]

Fig. 1 shows the amino acid sequence of each CDR sequence of No.76 antibody.

Fig. 2 shows the amino acid sequence of each CDR sequence of #1G5 antibody.

Fig. 3 shows the amino acid sequence of each CDR sequence of #4C12 antibody.

Fig. 4 is a graph showing the binding specificity to Pseudomonas aeruginosa O11 strains.

Fig. 5 is a graph showing the therapeutic efficacy (intravenous administration) on a mouse model of lung infection by Pseudomonas aeruginosa ATCC 29260.

Fig. 6 is a graph showing the therapeutic efficacy (mixed administration of bacteria and antibodies) on a mouse model of lung infection by Pseudomonas aeruginosa clinical isolate No. 12.

Fig. 7 is a graph showing the therapeutic efficacy (mixed administration of bacteria and antibodies) on a mouse model of lung infection by Pseudomonas aeruginosa clinical isolate No. 31.

Fig. 8 is a graph showing the therapeutic efficacy (intravenous administration) on a mouse model of lung infection by Pseudomonas aeruginosa clinical isolate No. 12.

Fig. 9 shows the nucleotide sequence and the amino acid sequence of h#1G5-H1.

Fig. 10 shows the nucleotide sequence and the amino acid sequence of h#1G5-H2.

Fig. 11 shows the nucleotide sequence and the amino acid sequence of h#1G5-H3.

Fig. 12 shows the nucleotide sequence and the amino acid sequence of h#1G5-H4.

Fig. 13 shows the nucleotide sequence and the amino acid sequence of h#1G5-H5.

Fig. 14 shows the nucleotide sequence and the amino acid sequence of h#1G5-L1.

Fig. 15 shows the nucleotide sequence and the amino acid sequence of h#1G5-L2.

Fig. 16 shows the nucleotide sequence and the amino acid sequence of h#1G5-L3.

Fig. 17 shows the nucleotide sequence and the amino acid sequence of h#1G5-L4.

Fig. 18 shows the nucleotide sequence and the amino acid sequence of h#1G5-L5.

Fig. 19 shows the nucleotide sequence and the amino acid sequence of h#4C13K-H1.

Fig. 20 shows the nucleotide sequence and the amino acid sequence of h#4C13K-H2.

Fig. 21 shows the nucleotide sequence and the amino acid sequence of h#4C13K-H3.

Fig. 22 shows the nucleotide sequence and the amino acid

sequence of h#4C13K-H4.

Fig. 23 shows the nucleotide sequence and the amino acid sequence of h#4C13K-H5.

Fig. 24 shows the amino acid sequence of each CDR sequence of #4C13 antibody.

Fig. 25 is a graph showing the binding specificity to Pseudomonas aeruginosa O11 strains.

Fig. 26 is a graph showing the binding activity to clinical isolates of Pseudomonas aeruginosa O11.

Fig. 27 is a graph showing the therapeutic efficacy (intravenous administration) on a mouse model of lung infection by Pseudomonas aeruginosa ATCC 29260.

Fig. 28 is a graph showing the competitive test with No.76 mIgG2a.

Description of Embodiments

[0012]

1. Definition

The term “gene” as used herein includes not only DNA, but also mRNA, cDNA, and cRNA.

[0013]

The term “polynucleotide” as used herein is used with the same meaning as a “nucleic acid” and also includes DNA, RNA, probes, oligonucleotides, and primers.

[0014]

The terms “polypeptide” and “protein” as used herein are used without distinction.

[0015]

The term “RNA fraction” as used herein refers to a fraction containing RNA.

[0016]

The term “cell” as used herein includes cells in an animal individual and also cultured cells.

[0017]

The term “LPS” as used herein is used with the same meaning as a “lipopolysaccharide”.

[0018]

The term “serotype” as used herein refers to any known serotype of Pseudomonas aeruginosa.

[0019]

The term “multidrug-resistant” as used herein means “resistant to all of the three series of carbapenems (imipenem or meropenem), quinolones (ciprofloxacin or levofloxacin), and aminoglycosides (tobramycin or amikacin)”. The sensitivity of each drug was determined based on the breakpoints of Clinical and Laboratory Standards Institute (CLSI).

[0020]

The term “O11 clinical isolate” as used herein means a positive strain by a commercially available immune serum for grouping Pseudomonas aeruginosa specific to the O11 antigen or

by PCR using O11 antigen-specific primers.

[0021]

The term "healthcare-associated infection" is also known as nosocomial infection or hospital-acquired infection, and means a newly acquired disease separate from an underlying disease in patients in a medical institution or in medical care at home, and an infection in health care workers affected in a medical institution or in medical care at home.

[0022]

The term "antigen-binding fragment of an antibody" as used herein is also called as "functional fragment of an antibody" and refers to a partial fragment of an antibody having an antigen binding activity and includes Fab, F(ab')2, Fv, scFv, diabody, linear antibodies, polyspecific antibodies formed from antibody fragments, and the like. The term also encompasses Fab' which is a monovalent fragment in a variable region of an antibody obtained by treating F(ab')2 under reducing conditions. However, the term is not limited to these molecules as long as the fragment has a binding affinity for an antigen. Further, these antigen-binding fragments include not only a fragment obtained by treating a full-length molecule of an antibody protein with an appropriate enzyme, but also a protein produced in an appropriate host cell using a genetically modified antibody gene.

[0023]

The term “epitope” as used herein refers to a partial structure of the LPS O11 antigen to which a specific anti-LPS O11 antibody binds. Since the O11 antigen is a repeating structure composed of three sugars (Microbiol. Mol. Biol. Rev. 63,523-553 (1999)), it is possible to determine the epitope by synthesizing sugar chains of different lengths and examining the reactivity of the antibodies to such sugars. Further, the epitope which is a partial tertiary structure of the LPS O11 antigen that binds to a specific anti-LPS O11 antibody can be determined by specifying the structure of the LPS O11 antigen adjacent to the antibody by X-ray crystallography. If the second anti-LPS O11 antibody binds to the binding partial sugar chains of the first anti-LPS O11 antibody, the first antibody and the second antibody can be determined to have a common epitope. In addition, by confirming that the second anti-LPS O11 antibody cross-competes for binding to the LPS O11 antigen of the first anti-LPS O11 antibody (i.e., the second antibody interferes with the binding between the first antibody and the LPS O11 antigen), the first antibody and the second antibody can be determined to have a common epitope, even if specific sequence or structure of the epitope is not determined. Furthermore, if the first antibody and the second antibody bind to a common epitope and the first antibody has a special effect such as neutralizing activity to the antigen, the second antibody can be expected to also have the same activity.

[0024]

It is known that each heavy and light chain of an antibody molecule has three complementarity determining regions (CDRs). The complementarity determining region is also called the hypervariable domain and is present in a variable region of each heavy and light chain of an antibody. Such a region is a site which has unusually high variability in its primary structure, and there are three separate CDRs in the primary structure of each heavy and light polypeptide chain. In this description, as for the complementarity determining regions of an antibody, the complementarity determining regions of the heavy chain are represented by CDRH1, CDRH2, and CDRH3 from the amino terminus of the amino acid sequence of the heavy chain, and the complementarity determining regions of the light chain are represented by CDRL1, CDRL2, and CDRL3 from the amino terminus of the amino acid sequence of the light chain. These regions are proximate to one another in the tertiary structure and determine the specificity for an antigen to which the antibody binds.

[0025]

The phrase "hybridization is performed under stringent conditions" as used herein refers to hybridization that is performed under conditions under which identification can be achieved by performing hybridization at 68°C in a commercially available hybridization solution, ExpressHyb Hybridization

Solution (manufactured by Clontech, Inc.) or performing hybridization at 68°C in the presence of 0.7 to 1.0 M NaCl using a filter having DNA immobilized thereon, followed by washing at 68°C using 0.1 to 2-fold SSC solution (1-fold SSC solution is composed of 150 mM NaCl and 15 mM sodium citrate) or under conditions equivalent thereto.

[0026]

As used herein, the term "several" in the description of "one to several" and "one or several" refers to from 2 to 10. Such term is preferably 10 or less, more preferably 5 or 6 or less, and furthermore preferably 2 or 3.

2. LPS O11

"LPS" to which an antibody of the present invention binds is a component of Gram-negative bacteria cell wall outer membrane and is a substance (glycolipid) composed of lipids and polysaccharides. A sugar chain portion is composed of a portion called a core polysaccharide (or core oligosaccharide) and a portion called O-antigen (O-side chain polysaccharides).

[0027]

For different serotypes of Pseudomonas aeruginosa, there is typing by IATS (International Antigenic Typing System), and a grouping by the Serotyping Committee of Pseudomonas Aeruginosa (Japan Pseudomonas Aeruginosa Society, JPAS). Table 1 shows the correspondence relation of typing according to IATS and grouping by JPAS. Serotypes of Pseudomonas aeruginosa can be

determined by using commercially available immune serums for grouping Pseudomonas aeruginosa.

[0028]

[Table 1]

IATS	JPAS
O1	I
O2	B
O3	A
O4	F
O5	B
O6	G
O7	C
O8	C
O9	D
O10	H
O11	E
O12	L
O13	K
O14	K
O15	J
O16	B
O17	N
O18	-
O19	-
O20	B

[0029]

In recent years, the ratio of the strains having the O11 antigen (O11 strains) in multidrug-resistant Pseudomonas aeruginosa has been reported to be high. Pseudomonas aeruginosa of the LPS O11 serotype includes, for example, ATCC accession numbers 26290, 33358, etc.

3. Production of Anti-LPS O11 Antibody

An antibody against the LPS O11 antigen of Pseudomonas aeruginosa according to the present invention can be obtained, for example, by the method described in WO 2009/091048, WO

2011/027808, or WO 2012/133572. That is, a plasma cell and/or a plasmablast that specifically binds to a target antigen is selected by immunizing a non-human animal to the target antigen and collecting lymph fluid, lymphoid tissue, blood cell sample, or bone marrow derived cells from the non-human animal once immunization has been established. The antibody genes against the target antigen are collected from the plasma cells and/or plasmablasts obtained and the base sequence is identified, thereby to be able to obtain the antibody or a fragment of the antibody based on the base sequence of the identified gene. Moreover, by obtaining the plasma cells and/or plasmablasts similarly from blood of infected human patients, it is possible to obtain an antibody or antibody fragment. An antibody applicable to human disease can be obtained by testing the obtained antibody on the binding to the LPS O11 antigen. The monoclonal antibodies obtained in this way include No.76, #1G5, #4C12, #4C13, and #4C13K. The heavy chains of #4C12, #4C13, and #4C13K are identical with one another. #4C13 is an antibody wherein the amino acid No. 75 cysteine in the light chain of #4C12 is substituted by tyrosine, and #4C13K is an antibody wherein the light chain of #4C12 is substituted by the light chain of #1G5.

[0030]

Further, a monoclonal antibody can be obtained by fusing antibody-producing cells which produce an antibody against an

LPS O11 antigen with myeloma cells to establish a hybridoma according to a known method (for example, Kohler and Milstein, Nature, (1975) 256, pp. 495-497; Kennet, R. ed., Monoclonal Antibodies, pp. 365-367, Plenum Press, N.Y. (1980)). A specific example of such a method is described in WO 2009/48072 (published on Apr. 16, 2009) and WO 2010/117011 (published on Oct. 14, 2010). However, the method of obtaining monoclonal antibodies corresponds to a field which has been well established, and is not limited to the above specific example.

[0031]

The antibody of the present invention includes not only the above-mentioned monoclonal antibody against the LPS O11 antigen but also a recombinant antibody obtained by artificial modification for the purpose of decreasing heterologous antigenicity to human such as a chimeric antibody, a humanized antibody and a human antibody. These antibodies can be produced using a known method.

[0032]

An example of a chimeric antibody is an antibody in which antibody variable and constant regions are derived from different species, for example, a chimeric antibody in which a mouse- or rat-derived antibody variable region is joined to a human-derived constant region (see Proc. Natl. Acad. Sci. USA, 81, 6851-6855, (1984)). One example of a chimeric antibody derived from No.76 is an antibody containing a heavy chain having an amino acid

sequence comprising amino acid residues 20 to 470 of SEQ ID NO: 33 in the Sequence Listing and a light chain having an amino acid sequence comprising amino acid residues 21 to 234 of SEQ ID NO: 31; one example of a chimeric antibody derived from #1G5 is an antibody containing a heavy chain having an amino acid sequence comprising amino acid residues 20 to 474 of SEQ ID NO: 37 in the Sequence Listing and a light chain having an amino acid sequence comprising amino acid residues 21 to 234 of SEQ ID NO: 35; and one example of a chimeric antibody derived from #4C13 is an antibody containing a heavy chain having an amino acid sequence comprising amino acid residues 20 to 470 of SEQ ID NO: 41 in the Sequence Listing and a light chain having an amino acid sequence comprising amino acid residues 21 to 234 of SEQ ID NO: 39.

[0033]

An example of a humanized antibody is an antibody obtained by integrating only the CDRs into a human-derived antibody (see Nature (1986) 321, pp. 522-525), and an antibody obtained by grafting a part of the amino acid residues of the framework as well as the CDR sequence to a human antibody by a CDR-grafting method (WO 90/07861).

[0034]

Humanized antibodies derived from the antibody No.76 are included in the antibodies of the present invention as long as the humanized antibodies carry all six CDR sequences of No.76

and have a binding activity to the LPS O11 antigen of Pseudomonas aeruginosa. In addition, the heavy chain variable region of No.76 antibody carries CDRH1 (NYWIN) comprising the amino acid sequence shown in SEQ ID NO: 9, CDRH2 (DIYPGTSTTNYNEKFKN) comprising the amino acid sequence shown in SEQ ID NO: 10, and CDRH3 (IYYDYDGYYFDY) comprising the amino acid sequence shown in SEQ ID NO: 11. Further, the light chain variable region of No.76 antibody carries CDRL1 (KASENVGTSVS) comprising the amino acid sequence shown in SEQ ID NO: 5, CDRL2 (GASNRYT) comprising the amino acid sequence shown in SEQ ID NO: 6, and CDRL3 (GQSYSYPYT) comprising the amino acid sequence shown in SEQ ID NO: 7. The amino acid sequences of these CDRs are also shown in Fig. 1.

[0035]

Humanized antibodies derived from the #1G5 antibody are included in the antibodies of the present invention as long as the humanized antibodies carry all six CDR sequences of #1G5 and have a binding activity to the LPS O11 antigen of Pseudomonas aeruginosa. In addition, the heavy chain variable region of the #1G5 antibody carries CDRH1 (SYWIN) comprising the amino acid sequence shown in SEQ ID NO: 17, CDRH2 (NIYPGSSSINYNEKFKS) comprising the amino acid sequence shown in SEQ ID NO: 18, and CDRH3 (TIYNYGSSGYNYAMDY) comprising the amino acid sequence shown in SEQ ID NO: 19. Moreover, the light chain variable region of the #1G5 antibody carries CDRL1 (KASENVGNSVS) comprising the amino acid sequence shown in SEQ ID NO: 13, CDRL2 (GASNRYT)

comprising the amino acid sequence shown in SEQ ID NO: 14, and CDRL3 (GQSYSYPYT) comprising the amino acid sequence shown in SEQ ID NO: 15. The amino acid sequences of these CDRs are also shown in Fig. 2.

[0036]

Humanized antibodies derived from the #4C12 antibody are included in the antibodies of the present invention as long as the humanized antibodies carry all six CDR sequences of #4C12 and have a binding activity to the LPS O11 antigen of Pseudomonas aeruginosa. In addition, the heavy chain variable region of the #4C12 antibody carries CDRH1 (TYWIN) comprising the amino acid sequence shown in SEQ ID NO: 25, CDRH2 (NIYPGTRSSNYNEKFKN) comprising the amino acid sequence shown in SEQ ID NO: 26, and CDRH3 (VYYDHVGYYFDY) comprising the amino acid sequence shown in SEQ ID NO: 27. Moreover, the light chain variable region of the #4C12 antibody carries CDRL1 (KASENVGVSVS) comprising the amino acid sequence shown in SEQ ID NO: 21, CDRL2 (GASNRCT) comprising the amino acid sequence shown in SEQ ID NO: 22, and CDRL3 (GQSYSYPYT) comprising the amino acid sequence shown in SEQ ID NO: 23. The amino acid sequences of these CDRs are also shown in Fig. 3.

[0037]

Humanized antibodies derived from the #4C13 antibody are included in the antibodies of the present invention as long as the humanized antibodies carry all six CDR sequences of #4C13

and have a binding activity to the LPS O11 antigen of Pseudomonas aeruginosa. In addition, the heavy chain variable region of the #4C13 antibody carries CDRH1 (TYWIN) comprising the amino acid sequence shown in SEQ ID NO: 86, CDRH2 (NIYPGTRSSNYNEKFKN) comprising the amino acid sequence shown in SEQ ID NO: 87, and CDRH3 (VYYDHVGYYFDY) comprising the amino acid sequence shown in SEQ ID NO: 88. Moreover, the light chain variable region of the #4C13 antibody carries CDRL1 (KASENVGVSVS) comprising the amino acid sequence shown in SEQ ID NO: 123, CDRL2 (GASNRYT) comprising the amino acid sequence shown in SEQ ID NO: 124, and CDRL3 (GQSYSYPYT) comprising the amino acid sequence shown in SEQ ID NO: 125. The amino acid sequences of these CDRs are also shown in Fig. 24.

Humanized antibodies derived from the #4C13K antibody are included in the antibodies of the present invention as long as the humanized antibodies carry all six CDR sequences of #4C13K and have a binding activity to the LPS O11 antigen of Pseudomonas aeruginosa. In addition, the heavy chain variable region of the #4C13K antibody carries CDRH1 (TYWIN) comprising the amino acid sequence shown in SEQ ID NO: 86, CDRH2 (NIYPGTRSSNYNEKFKN) comprising the amino acid sequence shown in SEQ ID NO: 87, and CDRH3 (VYYDHVGYYFDY) comprising the amino acid sequence shown in SEQ ID NO: 88. Moreover, the light chain variable region of the #4C13K antibody is completely common with that of the #1G5 antibody, and carries CDRL1 (KASENVGNSVS) comprising the

amino acid sequence shown in SEQ ID NO: 13, CDRL2 (GASNRYT) comprising the amino acid sequence shown in SEQ ID NO: 14, and CDRL3 (GQSYSYPYT) comprising the amino acid sequence shown in SEQ ID NO: 15. The amino acid sequences of these CDRs are also shown in Fig. 24 and Fig. 2.

[0038]

In addition, CDR-modified humanized antibodies obtained by substituting 1 to 3 amino acid residues in each CDR with other amino acid residues are also included in the antibodies of the present invention as long as such humanized antibodies have a binding activity to the LPS O11 antigen of Pseudomonas aeruginosa.

[0039]

Examples of humanized antibodies derived from the #1G5 antibody include an arbitrary combination of a heavy chain containing a heavy chain variable region comprising an amino acid sequence comprising amino acid residues 20 to 144 of the amino acid sequence shown in SEQ ID NO: 57 in the Sequence Listing, an amino acid sequence comprising amino acid residues 20 to 144 of the amino acid sequence shown in SEQ ID NO: 59, an amino acid sequence comprising amino acid residues 20 to 144 of the amino acid sequence shown in SEQ ID NO: 61, an amino acid sequence comprising amino acid residues 20 to 144 of the amino acid sequence shown in SEQ ID NO: 63, or an amino acid sequence comprising amino acid residues 20 to 144 of the amino acid sequence shown

in SEQ ID NO: 65 and a light chain containing a light chain variable region comprising an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 67, an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 69, an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 71, an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 73, or an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 75.

[0040]

A preferred combination includes an antibody comprising a heavy chain containing a heavy chain variable region comprising an amino acid sequence comprising amino acid residues 20 to 144 of the amino acid sequence shown in SEQ ID NO: 57 and a light chain containing a light chain variable region comprising an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 67, an antibody comprising a heavy chain containing a heavy chain variable region comprising an amino acid sequence comprising amino acid residues 20 to 144 of the amino acid sequence shown in SEQ ID NO: 59 and a light chain containing a light chain variable region comprising an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 69, an antibody

comprising a heavy chain containing a heavy chain variable region comprising an amino acid sequence comprising amino acid residues 20 to 144 of the amino acid sequence shown in SEQ ID NO: 61 and a light chain containing a light chain variable region comprising an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 71, an antibody comprising a heavy chain containing a heavy chain variable region comprising an amino acid sequence comprising amino acid residues 20 to 144 of the amino acid sequence shown in SEQ ID NO: 63 and a light chain containing a light chain variable region comprising an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 73, or an antibody comprising a heavy chain containing a heavy chain variable region comprising an amino acid sequence comprising amino acid residues 20 to 144 of the amino acid sequence shown in SEQ ID NO: 65 and a light chain containing a light chain variable region comprising an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 75.

[0041]

A more preferred combination includes an antibody comprising a heavy chain containing a heavy chain variable region comprising an amino acid sequence comprising amino acid residues 20 to 144 of the amino acid sequence shown in SEQ ID NO: 59 and a light chain containing a light chain variable region comprising an amino acid sequence comprising amino acid residues 21 to 129

of the amino acid sequence shown in SEQ ID NO: 69, or an antibody comprising a heavy chain containing a heavy chain variable region comprising an amino acid sequence comprising amino acid residues 20 to 144 of the amino acid sequence shown in SEQ ID NO: 61 and a light chain containing a light chain variable region comprising an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 71.

[0042]

Examples of humanized antibodies derived from the #4C13K antibody include an arbitrary combination of a heavy chain containing a heavy chain variable region comprising an amino acid sequence comprising amino acid residues 20 to 140 of the amino acid sequence shown in SEQ ID NO: 77, an amino acid sequence comprising amino acid residues 20 to 140 of the amino acid sequence shown in SEQ ID NO: 79, an amino acid sequence comprising amino acid residues 20 to 140 of the amino acid sequence shown in SEQ ID NO: 81, an amino acid sequence comprising amino acid residues 20 to 140 of the amino acid sequence shown in SEQ ID NO: 83, or an amino acid sequence comprising amino acid residues 20 to 140 of the amino acid sequence shown in SEQ ID NO: 85 and a light chain containing a light chain variable region comprising an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 67, an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 69, an amino acid sequence

comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 71, an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 73, or an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 75.

[0043]

A preferred combination includes an antibody comprising a heavy chain containing a heavy chain variable region comprising an amino acid sequence comprising amino acid residues 20 to 140 of the amino acid sequence shown in SEQ ID NO: 77 and a light chain containing a light chain variable region comprising an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 67, an antibody comprising a heavy chain containing a heavy chain variable region comprising an amino acid sequence comprising amino acid residues 20 to 140 of the amino acid sequence shown in SEQ ID NO: 79 and a light chain containing a light chain variable region comprising an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 69, an antibody comprising a heavy chain containing a heavy chain variable region comprising an amino acid sequence comprising amino acid residues 20 to 140 of the amino acid sequence shown in SEQ ID NO: 81 and a light chain containing a light chain variable region comprising an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 71, an antibody

comprising a heavy chain containing a heavy chain variable region comprising an amino acid sequence comprising amino acid residues 20 to 140 of the amino acid sequence shown in SEQ ID NO: 83 and a light chain containing a light chain variable region comprising an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 73, or an antibody comprising a heavy chain containing a heavy chain variable region comprising an amino acid sequence comprising amino acid residues 20 to 140 of the amino acid sequence shown in SEQ ID NO: 85 and a light chain containing a light chain variable region comprising an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 75.

[0044]

A more preferred combination includes an antibody comprising a heavy chain containing a heavy chain variable region comprising an amino acid sequence comprising amino acid residues 20 to 140 of the amino acid sequence shown in SEQ ID NO: 79 and a light chain containing a light chain variable region comprising an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 69, or an antibody comprising a heavy chain containing a heavy chain variable region comprising an amino acid sequence comprising amino acid residues 20 to 140 of the amino acid sequence shown in SEQ ID NO: 81 and a light chain containing a light chain variable region comprising an amino acid sequence comprising amino acid residues 21 to 129

of the amino acid sequence shown in SEQ ID NO: 71.

[0045]

Further, the antibody of the present invention includes a human antibody. An anti-LPS O11 human antibody refers to a human antibody having only a gene sequence of an antibody derived from a human chromosome. An anti-LPS O11 human antibody can be obtained by a method using a human antibody-producing mouse having a human chromosome fragment containing heavy and light chain genes of a human antibody (see Tomizuka, K. et al., Nature Genetics (1997) 16, pp. 133-143; Kuroiwa, Y. et al., Nucl. Acids Res. (1998) 26, pp. 3447-3448; Yoshida, H. et al., Animal Cell Technology: Basic and Applied Aspects vol. 10, pp. 69-73 (Kitagawa, Y., Matsuda, T. and Iijima, S. eds.), Kluwer Academic Publishers, 1999; Tomizuka, K. et al., Proc. Natl. Acad. Sci. USA (2000) 97, pp. 722-727, etc).

[0046]

Such a human antibody-producing mouse can be created specifically as follows. A genetically modified animal in which endogenous immunoglobulin heavy and light chain gene loci have been disrupted, and instead, human immunoglobulin heavy and light chain gene loci have been introduced via a yeast artificial chromosome (YAC) vector or the like, is created by producing a knockout animal and a transgenic animal and mating these animals each other.

[0047]

Further, according to a genetic engineering technique, by using cDNAs encoding such a heavy chain and a light chain of a human antibody, respectively, and preferably a vector containing the cDNAs, eukaryotic cells are transformed, and a transformant which produces a recombinant human monoclonal antibody is cultured, and thus, the antibody can also be obtained from the culture supernatant. Here, as the host, for example, eukaryotic cells, preferably mammalian cells such as CHO cells, lymphocytes or myeloma cells can be used.

[0048]

In addition, a method of obtaining a phage display-derived human antibody screened from a human antibody library (see Wormstone, I. M. et al., Investigative Ophthalmology & Visual Science (2002) 43(7), pp. 2301-2308; Carmen, S. et al., Briefings in Functional Genomics and Proteomics (2002), 1(2), pp. 189-203; Siriwardena, D. et al., Ophthalmology (2002) 109(3), pp. 427-431, etc.) is also known.

[0049]

For example, a phage display method in which a variable region of a human antibody is expressed on the surface of a phage as a single-chain antibody (scFv) and a phage which binds to an antigen is selected (Nature Biotechnology (2005), 23(9), pp. 1105-1116) can be used. By analyzing the gene of the phage selected based on the binding to an antigen, a DNA sequence encoding the variable region of a human antibody which binds

to the antigen can be determined. If the DNA sequence of an scFv which binds to the antigen is determined, a human antibody can be obtained by preparing an expression vector having the sequence and introducing the vector into an appropriate host to express it (WO 92/01047, WO 92/20791, WO 93/06213, WO 93/11236, WO 93/19172, WO 95/01438, WO 95/15388, Annu. Rev. Immunol. (1994) 12, pp. 433-455, Nature Biotechnology (2005) 23(9), pp. 1105-1116).

[0050]

The above antibody is evaluated on its binding activity to an antigen, opsonophagocytic killing activity, complement-dependent killing activity and/or agglutination activity, thereby to be able to select a suitable antibody. The binding activity to antigens can be evaluated by whole cell ELISA method shown in Example 3, which makes it possible to determine the range of serotypes of Pseudomonas aeruginosa to which the antibody exhibit a binding activity. The opsonophagocytic killing activity can be evaluated by the viable cell count measurement shown in Example 1 for percentage or amount of bacteria that are phagocytosed by phagocytic cells or by treatment of the bacteria with a fluorescent label, followed by quantification using a flow cytometry, etc. The complement-dependent killing activity can be evaluated by a method such as viable cell count measurement for the amount of bacteria to be sterilized by the complement and the antibody. Also, the

agglutination activity can be evaluated as an agglutination titer per the amount of IgG by detecting agglutination ability of serially diluted antibody to bacteria. Further, antimicrobial activity against systemic infection and lung infection can be assessed by the method described in Example 4 and the like.

[0051]

As one example of another index for use in the comparison of the properties of antibodies, the stability of antibodies can be exemplified. The differential scanning calorimetry (DSC) is a method capable of quickly and accurately measuring a thermal denaturation midpoint temperature (Tm) to be used as a favorable index of the relative conformational stability of proteins. By measuring the Tm values using DSC and comparing the values, the difference in thermal stability can be compared. It is known that the storage stability of antibodies shows some correlation with the thermal stability of antibodies (Lori Burton, et. al., Pharmaceutical Development and Technology (2007) 12, pp. 265-273), and a preferred antibody can be selected by using thermal stability as an index. Examples of other indices for selecting antibodies include the following factors: the yield in an appropriate host cell is high and the agglutinating property in an aqueous solution is low. For example, an antibody which shows the highest yield does not always show the highest thermal stability, and therefore, it is necessary to select an antibody most suitable for the administration to humans by making a

comprehensive evaluation based on the above-described indices.

[0052]

Further, a method in which the full-length heavy and light chain sequences of an antibody are connected using an appropriate linker to obtain a single-chain immunoglobulin is also known (Lee, H-S, et. al., Molecular Immunology (1999) 36, pp. 61-71; Shirrmann, T. et. al., mAbs (2010), 2(1) pp. 1-4). By dimerizing such a single-chain immunoglobulin, the resulting dimer can have a structure and an activity similar to those of an antibody which is a tetramer itself. Further, the antibody of the present invention may be an antibody which has a single heavy chain variable region and does not have a light chain sequence. Such an antibody is called a single domain antibody (sdAb) or a nanobody, and in fact, such an antibody is observed in camels and llamas and has been reported to have an antigen-binding affinity (Muyldemans S. et. al., Protein Eng. (1994) 7(9), 1129-35, Hamers-Casterman C. et. al., Nature (1993) 363(6428), 446-8). The above-described antibodies can also be interpreted as a type of antigen-binding fragment of the antibodies of the present invention.

[0053]

Further, by controlling the modification of sugar chain that is bound to the antibody of the present invention, it is possible to enhance an opsonophagocytic killing activity. The technique for controlling the sugar chain modification of

antibodies is known from WO 99/54342, WO 2000/61739, WO 2002/31140, etc. However, such techniques are not limited thereto.

[0054]

In the case where an antibody is produced by first isolating an antibody gene and then introducing the gene into an appropriate host, a combination of an appropriate host and an appropriate expression vector can be used. Specific examples of the antibody gene include a combination of a gene encoding a heavy chain sequence of an antibody described in this specification and a gene encoding a light chain sequence thereof. When a host cell is transformed, it is possible to insert the heavy chain sequence gene and the light chain sequence gene into the same expression vector, and also into different expression vectors separately. In the case where eukaryotic cells are used as the host, animal cells, plant cells, and eukaryotic microorganisms can be used. As the animal cells, (1) mammalian cells, for example, dihydrofolate reductase-deficient strains (Urlaub, G. and Chasin, L. A., Proc. Natl. Acad. Sci. USA (1980) 77, pp. 4126-4220) of simian COS cells (Gluzman, Y., Cell, (1981) 23, pp. 175-182, ATCC CRL-1650), murine fibroblasts NIH3T3 (ATCC No. CRL-1658), and Chinese hamster ovarian cells (CHO cells; ATCC: CCL-61) can be exemplified. Further, in the case where prokaryotic cells are used, for example, Escherichia coli and Bacillus subtilis can be exemplified. By introducing a target antibody gene into

these cells through transformation and culturing the thus transformed cells in vitro, the antibody can be obtained. In the above-described culture method, the yield may sometimes vary depending on the sequence of the antibody, and therefore, it is possible to select an antibody which is easily produced as a medicine by using the yield as an index among the antibodies having comparable binding activity.

[0055]

There is no limitation on isotype of the antibody of the present invention, and examples thereof include IgG (IgG1, IgG2, IgG3, IgG4), IgM, IgA (IgA1, IgA2), IgD, and IgE, and preferred examples thereof include IgG and IgM, and further more preferred examples thereof include IgG1 and IgG3.

[0056]

Further, the antibody of the present invention may be an antigen-binding fragment of the antibody having an antigen-binding site of the antibody or a modified fragment thereof. The fragment of the antibody can be obtained by treating the antibody with a protease such as papain or pepsin, or modifying the antibody gene according to a genetic engineering technique and expressing the modified gene in suitable cultured cells. Among these antibody fragments, a fragment having all or part of the functions of the full-length molecule of the antibody can be called an antigen-binding fragment of the antibody. The functions of the antibody generally include an antigen-binding

activity, neutralizing the activity of an antigen, enhancing the activity of an antigen, an opsonophagocytic killing activity, and a complement-dependent killing activity. The function of the antigen-binding fragment of the antibody according to the present invention is to bind to the LPS O11 antigen of Pseudomonas aeruginosa, preferably the opsonophagocytic killing activity to the LPS O11 antigen of Pseudomonas aeruginosa strains.

[0057]

Examples of the fragment of the antibody include Fab, F(ab')2, Fv, single-chain Fv (scFv) in which Fv molecules of the heavy chain and the light chain are connected via an appropriate linker, a diabody (diabodies), a linear antibody, and a polyspecific antibody composed of the antibody fragments. Further, Fab' which is a monovalent fragment in a variable region of an antibody obtained by treating F(ab')2 under reducing conditions is also included in the fragment of the antibody.

[0058]

Further, the antibody of the present invention may be a polyspecific antibody with specificity for at least two different antigens. In general, such a molecule binds to two antigens (that is, bispecific antibody), however, the term "polyspecific antibody" as used herein includes an antibody having specificity to two or more (for example, three) antigens.

[0059]

The polyspecific antibody of the present invention may

be a full-length antibody or a fragment of such an antibody (for example, a F(ab')2 bispecific antibody). The bispecific antibody can be produced by connecting the heavy and light chains (HL pairs) of two types of antibodies, or can also be produced by fusing hybridomas which produce different monoclonal antibodies to prepare bispecific antibody-producing fused cells (Millstein et al., Nature (1983) 305, pp. 537-539).

[0060]

The antibody of the present invention may be a single-chain antibody (also referred to as scFv). The single-chain antibody can be obtained by connecting the heavy chain variable region and the light chain variable region of the antibody via a polypeptide linker (Pluckthun, The Pharmacology of Monoclonal Antibodies, 113 (edited by Rosenberg and Moore), Springer Verlag, New York, pp. 269-315 (1994), Nature Biotechnology (2005), 23, pp. 1126-1136). Further, a BiscFv fragment produced by connecting two scFv molecules via a polypeptide linker can also be used as the bispecific antibody.

[0061]

A method of producing a single-chain antibody is well known in this technical field (see, for example, US Patent Nos. 4,946,778, 5,260,203, 5,091,513, 5,455,030, etc). In this scFv, the heavy chain variable region and the light chain variable region are connected via a linker that does not form a conjugate, preferably via a polypeptide linker (Huston, J. S. et al., Proc.

Natl. Acad. Sci. USA (1988), 85, pp. 5879-5883). In the scFv, the heavy chain variable region and the light chain variable region may be derived from the same antibody or different antibodies. As the polypeptide linker to be used for connecting the variable regions, any single-chain peptide composed of 12 to 19 residues is used, for example.

[0062]

DNA encoding scFv can be obtained by performing amplification using a DNA encoding the entire amino acid sequence or a desired partial amino acid sequence of a DNA selected from a DNA encoding the heavy chain or the heavy chain variable region of the above-described antibody and a DNA encoding the light chain or the light chain variable region thereof as a template by a PCR method using a primer pair that defines both ends thereof, and further performing amplification by combining a DNA encoding a polypeptide linker portion and a primer pair that defines both ends thereof so as to connect the both ends to the heavy chain and the light chain, respectively.

[0063]

Further, once DNA encoding a scFv is produced, an expression vector containing the same and a host transformed by the expression vector can be obtained according to common procedures. Further, by using the resulting host, scFv can be obtained according to a common procedure. An antibody fragment thereof can be produced in a host by obtaining a gene and expressing

the gene in the same manner as described above.

[0064]

The antibody of the present invention may be polymerized to increase its affinity for an antigen. The antibody to be polymerized may be one type of antibody or a plurality of antibodies which recognize a plurality of epitopes of the same antigen. A method of polymerization of the antibody includes binding of the IgG CH3 domain to two scFv molecules, binding to streptavidin, introduction of a helix-turn-helix motif, and the like.

[0065]

The antibody of the present invention may be a polyclonal antibody which is a mixture of plural types of anti-LPS O11 antibodies having different amino acid sequences. One example of a polyclonal antibody includes a mixture of plural types of antibodies having different CDRs. Such polyclonal a antibodies can be obtained by culturing a mixture of cells which produce different antibodies and then purifying the antibodies from the resulting culture (see WO 2004/061104).

[0066]

The antibody of the present invention may be an antibody having an identity of 80% to 99% as compared to the heavy and/or light chains of the antibodies described above. By combining sequences having a high homology to the heavy chain amino acid sequence and the light chain amino acid sequence, it is possible

to select an antibody having an antigen-binding affinity, opsonophagocytic killing activity, and/or complement-dependent killing activity equivalent to those of each antibody. Such a homology is generally a homology of 80% or more, preferably a homology of 90% or more, more preferably a homology of 95% or more, most preferably a homology of 99% or more. Further, by combining an amino acid sequence wherein one to several amino acid residues are substituted, deleted or added in the heavy chain and/or light chain amino acid sequence, it is also possible to select an antibody having various activities equivalent to those of each of the above-described antibodies. The number of the amino acid residues to be substituted, deleted or added is generally 10 amino acid residues or less, preferably 5 to 6 amino acid residues or less, more preferably 2 or 3 amino acid residues or less, most preferably 1 amino acid residue.

[0067]

It is known that a lysine residue at the carboxyl terminus of the heavy chain of an antibody produced in a cultured mammalian cell is deleted (Journal of Chromatography A, 705: 129-134 (1995)), and it is also known that two amino acid residues (glycine and lysine) at the carboxyl terminus of the heavy chain of an antibody produced in a cultured mammalian cell are deleted and a proline residue located at the carboxyl terminus is newly amidated (Analytical Biochemistry, 360: 75-83 (2007)). However, such deletion and modification of the heavy chain sequence does

not affect the antigen-binding affinity and the effector function (activation of a complement or opsonophagocytic killing activity, etc.) of the antibody. Therefore, in the present invention, an antibody subjected to such modification is also included, and a deletion variant in which one or two amino acids have been deleted at the carboxyl terminus of the heavy chain, and a variant obtained by amidation of the deletion variant (for example, a heavy chain in which the carboxyl terminal proline residue has been amidated), and the like can be exemplified. The type of deletion variant having a deletion at the carboxyl terminus of the heavy chain of the antibody according to the present invention is not limited to the above variants as long as the antigen-binding affinity and the effector function are conserved. The two heavy chains constituting the antibody according to the present invention may be of one type selected from the group consisting of a full-length heavy chain and the above-described deletion variant, or may be of two types in combination selected therefrom. The ratio of the amount of each deletion variant can be affected by the type of cultured mammalian cells which produce the antibody according to the present invention and the culture conditions, however, a case where one amino acid residue at the carboxyl terminus has been deleted in both of the two heavy chains contained as main components in the antibody according to the present invention can be exemplified.

[0068]

The homology between two amino acid sequences can be determined using default parameters of Blast algorithm version 2.2.2 (Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schaffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25: 3389-3402). The Blast algorithm can be used also through the Internet by accessing the site www.ncbi.nlm.nih.gov/blast. Incidentally, two types of percentage values of identity (or identities) and positivity (or positivities) are calculated by the Blast algorithm. The former is a value when amino acid residues match each other in two amino acid sequences for which a degree of homology is to be determined, and the latter is a value obtained by also considering amino acid residues having a similar chemical structure. In this specification, the value of the identity when amino acid residues match each other is used as the homology value.

[0069]

As a modified antibody, an antibody bound to any of various types of molecules such as polyethylene glycol (PEG) can also be used.

[0070]

Further, the antibody of the present invention may be in the form of a conjugate formed between any of these antibodies and another medicinal agent (immunoconjugate). Examples of

such an antibody include one in which the antibody is conjugated to a radioactive material or a compound having a pharmacological action (Nature Biotechnology (2005) 23, pp. 1137-1146).

[0071]

The obtained antibody can be purified to homogeneity. The separation and purification of the antibody can be performed by employing a conventional protein separation and purification method. For example, the antibody can be separated and purified by appropriately selecting and combining column chromatography, filter filtration, ultrafiltration, salt precipitation, dialysis, preparative polyacrylamide gel electrophoresis, isoelectric focusing electrophoresis, and the like (Strategies for Protein Purification and Characterization: A Laboratory Course Manual, Daniel R. Marshak et al. eds., Cold Spring Harbor Laboratory Press (1996); Antibodies: A Laboratory Manual. Ed Harlow and David Lane, Cold Spring Harbor Laboratory (1988)), but the method is not limited thereto.

[0072]

Examples of such chromatography include affinity chromatography, ion exchange chromatography, hydrophobic chromatography, gel filtration chromatography, reverse phase chromatography, adsorption chromatography, and the like.

[0073]

Such chromatography can be performed by employing liquid chromatography such as HPLC, FPLC, and the like.

[0074]

A column to be used in affinity chromatography includes a Protein A column and a Protein G column.

[0075]

For example, a column using a Protein A column includes Hyper D, POROS, Sepharose F. F. (Pharmacia) and the like.

[0076]

Further, by using a carrier having an antigen immobilized thereon, an antibody can also be purified utilizing the binding property of the antibody to the antigen.

4. Medicament Containing Anti-LPS O11 Antibody

Since the anti-LPS O11 antibody obtained by the method described in the above section "3. Production of anti-LPS O11 antibody" exhibits opsonophagocytic killing action (OPK) and/or complement dependent killing (CDK) action, it therefore can be used as a therapeutic and/or prophylactic agent for pseudomonal infection.

[0077]

Examples of pseudomonal infection with which an anti-LPS O11 antibody is capable of treatment and/or prophylaxis include systemic infectious disease caused by pseudomonal infection, preferably caused by multidrug-resistant pseudomonal infection. The systemic pseudomonal infection caused by pseudomonal infection includes, for example, bloodstream infection, sepsis, meningitis, endocarditis, otitis media, sinusitis, pneumonia,

lung abscess, empyema, chronic respiratory tract infection, peritonitis, post-operative infection, cholecystitis, cholangitis, eyelid tumor, dacryocystitis, conjunctivitis, corneal ulcer, corneal abscess, panophthalmitis, orbital infection, urinary tract infection, catheter infection, perianal abscess, secondary infection of burn wounds, decubitus infection, cystic fibrosis disease, lymphangitis, lymphadenitis, osteomyelitis, arthritis, tonsillitis, liver abscess, skin and soft tissue infections, intrauterine infection, uterine adnexitis, parametritis, perimandibular phlegmon, or jaw inflammation, preferably pneumonia, but is not limited thereto as long as such pseudomonal infection is a systemic infectious disease caused by pseudomonal infection.

[0078]

Examples of anti-LPS O11 antibodies as a medicament for the above diseases include chimeric antibody or humanized antibody produced from #1G5 antibody or #4C13K antibody according to the method described in "3. Production of Anti-LPS O11 Antibody". Furthermore, chimeric antibodies, humanized antibodies, and human antibodies having the same epitope as the #1G5 antibody and/or #4C13K antibody can also be used as a medicament. It is possible to confirm that some anti-LPS O11 antibodies have the same epitope as the #1G5 antibody and/or #4C13K antibody by observing whether or not these antibodies commonly bind to specific sugar chain of the LPS O11 antigen.

Also, if some anti-LPS O11 antibodies compete with the #1G5 antibody and/or #4C13K antibody in binding to the LPS O11 antigen, these antibodies can be determined to have common epitopes.

[0079]

The in vitro antimicrobial activity of the anti-LPS O11 antibodies can be confirmed by measuring the opsonophagocytic killing activity or complement dependent killing activity against, for example, Pseudomonas aeruginosa ATCC 29260. The ATCC 29260 strain is one of the most famous O11 strains and was obtained from ATCC. This strain has been deposited at ATCC by P. V. Liu of University of Louisville (J. Infect. Dis, 128:506-513 (1973)). This strain is available from the Institute Pasteur as CIP 102967, or even from The National Institute of Public Health as CNCTC 5997.

[0080]

The therapeutic or prophylactic effect of the anti-LPS O11 antibody on pseudomonal infection in vivo using experimental animals can be confirmed by intravenously administering the anti-LPS O11 antibody in a mouse or rat model of lung infection, systemic infection, thigh infection, or burn wound infection, and measuring the change of the viable cell count of the infected sites or the survival rate.

[0081]

The thus obtained anti-LPS O11 antibody is useful as a medicament, in particular as a pharmaceutical composition for

treatment or prophylaxis of pseudomonal infections such as blood stream infection, pneumonia, chronic respiratory tract infections, septicemia, peritonitis, skin soft tissue infection, secondary infections of burn wounds, or as an antibody for immunological diagnosis of such diseases.

[0082]

The anti-LPS O11 antibody of the present invention is an antibody that recognizes the LPS of Pseudomonas aeruginosa, and binds to the O11 antigen, but does not bind to O1, O2, O3, O4, O5, O6, O7, O8, O9, O10, O13, O14, O15, O16, O17, O18, O19, O20 antigens and O-antigen-deficient strain.

[0083]

The anti-LPS O11 antibody of the present invention has a coverage ratio for O11 clinical isolates, namely a ratio of the strains to which an antibody can bind being 85% or more, preferably 90% or more, more preferably 95% or more, and most preferably 100%.

[0084]

Further, the anti-LPS O11 antibody of the present invention shows that the 50% minimum growth inhibitory concentration of opsonophagocytic killing activity against Pseudomonas aeruginosa identified by ATCC 29260 is 12 ng/mL or less, preferably 4.1 ng/mL or less, more preferably 1.4 ng/mL or less.

[0085]

As one example, the anti-LPS O11 antibody can be

administered alone or in combination with at least one other anti-infective agent for the treatment or prophylaxis of Pseudomonas aeruginosa infectious diseases. As one example, the anti-LPS O11 antibody can be administered in combination with a therapeutically effective amount of a therapeutic agent. Examples of such anti-infective agent that can be administered in combination with the anti-LPS O11 antibody include, but not limited to, penicillin, cephem, carbapenem, monobactam, quinolone, aminoglycoside, polymyxin, rifampicin, macrolide antimicrobial agents, etc.

[0086]

The penicillin antimicrobial agent includes piperacillin, piperacillin/tazobactam, ticarcillin, ticarcillin/clavulanic acid, etc., preferably piperacillin or piperacillin/tazobactam.

[0087]

The cephem antimicrobial agent includes cefotaxime, ceftizoxime, ceftriaxone, ceftazidime, cefepime, cefozopran, etc., preferably ceftazidime or cefepime.

[0088]

The carbapenem antimicrobial agent includes imipenem, panipenem, meropenem, doripenem, etc., preferably meropenem or doripenem.

[0089]

The monobactam antimicrobial agent includes aztreonam,

carumonam, etc., preferably aztreonam.

[0090]

The quinolone antimicrobial agent includes ciprofloxacin, levofloxacin, ofloxacin, moxifloxacin, gatifloxacin, sitafloxacin, etc., preferably ciprofloxacin or levofloxacin.

[0091]

The aminoglycoside antimicrobial agent includes gentamicin, tobramycin, amikacin, arbekacin, etc., preferably tobramycin or amikacin.

[0092]

The polymyxin antimicrobial agent includes polymyxin, colistin, etc.

[0093]

The macrolide antimicrobial agent includes erythromycin, clarithromycin, azithromycin, etc., preferably azithromycin.

[0094]

Depending on the state of pseudomonal infection or the intended degree of treatment and/or prophylaxis, two or three, or more types of therapeutic agents can be administered, and these other therapeutic agents can be administered simultaneously by encapsulating them in the same preparation. These other therapeutic agents and the anti-LPS O11 antibody can be administered simultaneously by encapsulating them in the same preparation. Also, the anti-LPS O11 antibody and these other therapeutic agents can be administered simultaneously by

encapsulating them in separate preparations. Further, these other therapeutic agents and the anti-LPS O11 antibody can be separately administered one after another. In other words, after administration of these other therapeutic agents, a therapeutic agent containing an anti-LPS O11 antibody or an antigen-binding fragment thereof as an active ingredient may be administered, or after administration of a therapeutic agent containing an anti-LPS O11 antibody or an antigen-binding fragment thereof as an active ingredient, other therapeutic agent may be administered. In the case of administration in gene therapy, a gene of a proteinaceous therapeutic agent for infectious disease and a gene of the anti-LPS O11 antibody can be inserted downstream of the same promoter region or different promoter regions, and can be introduced into the same vector or different vectors.

[0095]

By conjugating a therapeutic agent for infectious disease to the anti-LPS O11 antibody or a fragment thereof, a targeted drug conjugate as described in M. C. Garnet "Targeted drug conjugates: principles and progress", Advanced Drug Delivery Reviews, (2001) 53, 171-216 can be produced. For achieving this purpose, other than the antibody molecule, any antibody fragment can be applied as long as it does not completely lose the ability to recognize the LPS O11 antigen, and examples thereof include fragments such as Fab, F(ab')2, Fv, and the like. In the present

invention, the antibody, fragment and the like can be used in the same manner. The conjugation mode between the anti-LPS O11 antibody or a fragment thereof and a therapeutic agent for infectious disease can be any of various forms described in M. C. Garnet "Targeted drug conjugates: principles and progress", Advanced Drug Delivery Reviews, (2001) 53, 171-216, G. T. Hermanson "Bioconjugate Techniques" Academic Press, California (1996), Putnam and J. Kopecek "Polymer Conjugates with Anticancer Activity" Advances in Polymer Science (1995) 122, 55-123 and the like. That is, a conjugate form in which the LPS O11 antibody and a therapeutic agent for infectious disease are conjugated to each other chemically and directly or via a spacer such as an oligopeptide, and a conjugate form via an appropriate drug carrier can be exemplified. Examples of a drug carrier include a liposome and a water-soluble polymer. More specific examples of a conjugate formed via such a drug carrier include a conjugate form in which the antibody and a therapeutic agent for infectious disease are incorporated in a liposome, and the liposome and the antibody are conjugated to each other, and a conjugate form in which a therapeutic agent for infectious disease is conjugated to a water-soluble polymer (a compound having a molecular weight of about 1000 to 100000) chemically and directly or via a spacer such as an oligopeptide and the antibody is conjugated to the water-soluble polymer. Conjugation of the antibody (or a fragment thereof) to a therapeutic agent for infectious disease

or a drug carrier such as a liposome or a water-soluble polymer can be effected by a method well known to those skilled in the art such as the method described in G. T. Hermanson "Bioconjugate Techniques" Academic Press, California (1996), Putnam and J. Kopecek "Polymer Conjugates with Anticancer Activity" Advances in Polymer Science (1995) 122, 55-123 or the like. Incorporation of a therapeutic agent for infectious disease in a liposome can be effected by a method well known to those skilled in the art such as the method described in D. D. Lasic "Liposomes: From Physics to Applications" Elsevier Science Publishers B. V., Amsterdam (1993) or the like. Conjugation of a therapeutic agent for infectious disease to a water-soluble polymer can be effected by a method well known to those skilled in the art such as the method described in D. Putnam and J. Kopecek "Polymer Conjugates with Anticancer Activity" Advances in Polymer Science (1995) 122, 55-123 or the like. A conjugate between the antibody (or a fragment thereof) and a proteinaceous therapeutic agent for infectious disease (or a fragment thereof) can be produced by a method well known to those skilled in the art through genetic engineering other than the above-mentioned method.

[0096]

The present invention also provides a pharmaceutical composition containing a therapeutically and/or prophylactically effective amount of the anti-LPS O11 antibody and a pharmaceutically acceptable diluent, carrier,

solubilizing agent, emulsifier, preservative and/or adjuvant.

[0097]

The present invention also provides a pharmaceutical composition containing a therapeutically and/or prophylactically effective amount of the anti-LPS O11 antibody, a therapeutically and/or prophylactically effective amount of at least one therapeutic agent for infectious disease, and a pharmaceutically acceptable diluent, carrier, solubilizing agent, emulsifier, preservative and/or adjuvant.

[0098]

A substance to be used in a pharmaceutical formulation acceptable in a pharmaceutical composition according to the present invention is preferably non-toxic to a person to whom the pharmaceutical composition is to be administered, in terms of the dose and concentration.

[0099]

The pharmaceutical composition of the present invention can contain a substance for pharmaceutical formulation which is capable of changing or maintaining the pH, osmotic pressure, viscosity, transparency, color, isotonicity, aseptic condition, stability, solubility, release rate, absorption rate, or permeability. Examples of the substance for pharmaceutical formulation include, but are not limited to, amino acids such as glycine, alanine, glutamine, asparagine, arginine and lysine; antimicrobial agents; antioxidants such as ascorbic acid, sodium

sulfate and sodium hydrogen sulfite; buffers such as phosphate, citrate, borate buffers, bicarbonate and Tris-Hcl solutions; fillers such as mannitol and glycine; chelating agents such as ethylenediamine tetraacetate (EDTA); complexing agents such as caffeine, polyvinylpyrrolidine, β-cyclodextrin and hydroxypropyl-β-cyclodextrin; fillers such as glucose, mannose and dextrin; other carbohydrates such as monosaccharide and disaccharides; coloring agents; flavors; diluents; emulsifiers; hydrophilic polymers such as polyvinylpyrrolidine; preservatives such as low molecular weight polypeptides, salt forming counter ions, benzalkonium chloride, benzoate, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid, and hydrogen peroxide; solvents such as glycerin, propylene glycol and polyethylene glycol; sugar alcohols such as mannitol and sorbitol; suspending agents; surfactants such as sorbitan ester, polysorbates including polysorbate 20 and polysorbate 80, triton, tromethamine, lecithin and cholesterol; stability enhancing agents such as sucrose and sorbitol; elasticity enhancing agents such as sodium chloride, potassium chloride and mannitol/sorbitol; transport agents; excipients; and/or pharmaceutical adjuvants. The addition amount of these substances for pharmaceutical formulation is preferably from 0.01 to 100 times, particularly preferably from 0.1 to 10 times the weight of the anti-LPS O11 antibody. Those skilled in the

art can appropriately determine a preferred composition of the pharmaceutical composition in a pharmaceutical formulation depending on the disease to be treated, the route of administration to be applied, or the like.

[0100]

The excipient or carrier in the pharmaceutical composition may be in the form of a liquid or a solid. An appropriate excipient or carrier may be injectable water, physiological saline, an artificial cerebral spinal fluid or other substance commonly used for parenteral administration. Further, neutral physiological saline or physiological saline containing serum albumin can also be used as a carrier. The pharmaceutical composition may contain a Tris buffer of pH 7.0 to 8.5, an acetate buffer of pH 4.0 to 5.5, or a citrate buffer of pH 3.0 to 6.2, which may be supplemented with sorbitol or another compound. Examples of the pharmaceutical composition of the present invention include a pharmaceutical composition containing the anti-LPS O11 antibody, and a pharmaceutical composition containing the anti-LPS O11 antibody and at least one therapeutic agent for infectious disease. The pharmaceutical composition of the present invention is prepared in the form of a lyophilized product or a liquid as a medicinal agent having a selected composition and a required purity. The pharmaceutical composition containing the anti-LPS O11 antibody and the pharmaceutical composition containing the anti-LPS O11 antibody

and at least one therapeutic agent for infectious disease can also be formed into a lyophilized product using an appropriate excipient such as sucrose.

[0101]

The pharmaceutical composition of the present invention can be prepared for parenteral administration or for gastrointestinal absorption via oral administration route. The composition and concentration of a pharmaceutical formulation can be determined depending on the administration method. To the extent that the affinity of the anti-LPS O11 antibody contained in the pharmaceutical composition of the present invention for the LPS O11 antigen is higher, that is, as the dissociation constant (Kd value) for the LPS O11 antigen is lower, the anti-LPS O11 antibody can exhibit its drug efficacy even at a lower dose for humans, and therefore, the dose of the pharmaceutical composition of the present invention for humans can also be determined based on this result. As for the dose, in the case where a human anti-LPS O11 antibody is administered to humans, the antibody may be administered at a dose of from about 0.1 to 100 mg/kg once per 1 to 180 days.

[0102]

Examples of the dosage form of the pharmaceutical composition of the present invention are injections including infusions, suppositories, transnasal agents, sublingual agents and percutaneous absorption agents.

[0103]

The antibody of the present invention binds to LPS which is exposed on the cell surface of Pseudomonas aeruginosa, and thus can also be used as a diagnostic agent for pseudomonal infection.

[0104]

When the antibody of the present invention is formulated as a diagnostic agent, this diagnostic agent can be obtained in any dosage form by adopting any means suitable for its purpose. For example, ascites, a culture solution containing an antibody of interest, or a purified antibody is measured for the antibody titer and appropriately diluted with PBS (phosphate buffer containing a saline) or the like; thereafter, a preservative such as 0.1% sodium azide is added thereto. Alternatively, the antibody of the present invention adsorbed to latex or the like is determined for the antibody titer and appropriately diluted, and a preservative is added thereto for use. As the latex in this case, appropriate resin materials, for example, latex such as polystyrene, polyvinyl toluene, and polybutadiene, can be exemplified.

[0105]

According to the present invention, a diagnostic method for pseudomonal infection using the antibody of the present invention is provided. The diagnostic method of the present invention can be carried out by collecting a biological sample

such as sputum, lung lavage fluid, pus, tear, blood, or urine from mammals including a human at a risk of pseudomonal infection, subsequently bringing the collected sample into contact with the antibody of the present invention, and determining whether or not an antigen-antibody reaction occurs.

[0106]

According to the present invention, a kit is provided for detecting the presence of Pseudomonas aeruginosa, the kit comprising at least the antibody of the present invention.

[0107]

The antibody of the present invention may be labeled. This detection kit is capable of detecting the presence of Pseudomonas aeruginosa by detecting the antigen-antibody reaction.

[0108]

Therefore, the detection kit of the present invention can further comprise various reagents for carrying out the antigen-antibody reaction, for example, a secondary antibody, a chromogenic reagent, a buffer, instructions, and/or an instrument, etc., which are used in an ELISA method or the like, if desired.

Examples

[0109]

Hereinafter, the invention will be more specifically described with reference to Examples; however, the invention

is not limited thereto. Note that the respective operations regarding gene manipulation in the following Examples were performed according to the methods described "Molecular Cloning" (written by Sambrook, J., Fritsch, E. F. and Maniatis, T., published by Cold Spring Harbor Laboratory Press in 1989), or in the case of using commercially available reagents or kits, performed according to the protocols attached thereto unless otherwise stated.

[0110]

Example 1 Screening of Anti-LPS O11 Antibody

[0111]

1)-1 Immunity

Pseudomonas aeruginosa ATCC 29260 cultured in Mueller Hinton Agar (MHA, Becton, Dickinson and Company) was suspended in saline, and ICR mice (Charles River) were immunized by administering the suspension to the gastrocnemius muscle 5 times every 10 to 11 days. The knee lymph nodes were harvested on day 6 after the final immunization, and the cells were individually separated by a cell strainer (Becton, Dickinson and Company), washed with RPMI 1640 (Invitrogen), suspended in Cell Banker (Juji Field Inc.), and stored at -80°C.

[0112]

1)-2 Obtaining a Monoclonal Antibody

A monoclonal antibody was obtained according to the methods described in WO 2009/091048, WO 2011/027808, WO 2012/133572,

BMC Biotechnology, 11, 39 (2011), and BMC Biotechnology 11, 75 (2011).

[0113]

1)-2-1 Isolation of an Antibody-Producing Cell

The cells collected from the lymph nodes of the immunized mice were suspended with 0.5% BSA/2 mM EDTA/PBS, and allophycocyanin-labeled anti-mouse CD138 antibody (Becton, Dickinson and Company) was added thereto. The suspension was incubated at 4°C for 15 minutes, and then the cells were washed in RPMI 1640, added with ER-Tracker Blue-White DPX (Invitrogen), and allowed to react for 5 minutes under light shielding conditions. The cells were suspended in PBS and the double positive cells were individually separated using a FACS Aria cell sorter (Becton, Dickinson and Company).

[0114]1)-2-2 Synthesis of cDNA

The cells individually separated by the cell sorter were dissolved in a cell lysate (50 mM Tris-HCl (pH 7.5), 250 mM LiCl, 5 mM EDTA (pH 8), 0.5% lithium dodecyl sulfate (LiDS), 2.5 mM dithiothreitol (DTT)) containing magnetic beads (Dynabeads mRNA DIRECT Kit, Invitrogen) bound to oligo dT25, so that mRNA was bound to magnetic beads. Then the magnetic beads were washed once with mRNA washing solution A (10 mM Tris-HCl (pH 7.5), 0.15 M LiCl, 1 mM EDTA, 0.1% LiDS, 0.1% Triton X-100) and cDNA synthesis solution (50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 5 mM DTT, 0.5 mM dNTP, 0.2% Triton X-100, and 48 units RNase inhibitor

(Invitrogen)), respectively, and synthesis of cDNA was performed in a cDNA synthesis solution supplemented with 480 units SuperScript III Reverse Transcriptase (Invitrogen). The beads were washed with 3′ tailing reaction solution (50 mM potassium phosphate, 4 mM $MgCl_2$, 0.5 mM dGTP, 0.2% Triton X-100, 48 units RNase inhibitor (Invitrogen)), and the 3′ tailing reaction was carried out in a reaction solution to which 480 units Terminal Transferase, recombinant (Roche) was added.

[0115]

1)-2-3 Amplification of Mouse Heavy and Light Chain Variable Region Gene Fragments

After washing the magnetic beads in TE solution (10 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.1% Triton X-100), amplification of the mouse immunoglobulin heavy and light chain genes was carried out using the 5′-RACE PCR method. First, the magnetic beads were transferred to a PCR reaction solution (0.2 μM primer, 0.2 mM dNTP, 0.1 units PrimeSTAR HS DNA Polymerase (TAKARA)), and 35 cycles of reactions for 30 seconds at 94°C and for 90 seconds at 68°C were conducted (1st PCR). Then the 1st PCR products were diluted 10-fold, and the variable regions of mouse immunoglobulin heavy and light chain genes were separately amplified respectively (2nd PCR) using the diluted 1st PCR products as a template under the same conditions as in the 1st PCR. Each primer set is as follows.

1st PCR Primer Set

5′-cggtaccgcgggcccgggatccccccccccccccdn-3′ (AP3dC-S)(SEQ ID NO: 89)

5′-accytgcatttgaactccttgcc-3′ (mIgγRT1 1111-AS) (SEQ ID NO: 90)

5′-actgccatcaatcttccacttgaca-3′ (mIgκ 1st 589-AS) (SEQ ID NO: 91)

2nd PCR primer set (heavy chain)

5′-cttcgaattctgcagtcgacggtaccgcgggcccggga-3′ (MCS-AP3-S) (SEQ ID NO: 92)

5′-ctggacagggatccagagttcca-3′ (mIgγ 3rd 656T-AS) (SEQ ID NO: 93)

2nd PCR primer set (light chain)

5′-cttcgaattctgcagtcgacggtaccgcgggcccggga-3′ (MCS-AP3-S) (SEQ ID NO: 92)

5′-actgaggcacctccagatgttaact-3′ (mIgκ 3rd 525-AS) (SEQ ID NO: 94)

[0116]

1)-2-4 Preparation of Mouse IgG2a or Human IgG1 Constant Region Gene Joining Double-Stranded DNA fragment and Mouse Light Chain Constant Region Gene Joining Double-Stranded DNA fragment

Mouse IgG2a or human IgG1 constant region gene joining double-stranded DNA fragment and mouse light chain constant region gene joining double-stranded DNA fragment were obtained by amplification by PCR reaction with each primer set using plasmids pJON mIgG2a, pJON mIgG2a-hIgG1, and pJON mIgκ shown in SEQ ID NOs: 1-3, respectively, as a template, followed by

treatment with DpnI (Roche). Twenty-five cycles of PCR reaction using 0.4 ng/ml pJON as a template were run in a reaction solution containing 0.2 mM dNTP, 0.2 μM primer, and 20 units PrimeSTAR HS DNA Polymerase at 94°C for 40 seconds, 65°C for 40 seconds, and 72°C for 30 seconds.

Primer set for pJON mIgG2a and pJON mIgG2a-hIgG1

5′-gcctggtcaagggctatttccctgag-3′ (mIgG joint PCR-S) (SEQ ID NO: 95)

5′-ggggggggggggggggggatcccgg-3′ (polyG-AS) (SEQ ID NO: 96)

Primer set forpJON mIgκ

5′-ctgtatccatcttcccaccatccagt-3′ (mIgκ joint PCR-S) (SEQ ID NO: 97)

5′-ggggggggggggggggggatcccgg-3′ (polyG-AS) (SEQ ID NO: 96)

1)-2-5 Preparation of Mouse or Human Chimeric Immunoglobulin Linearized Expression Vector

16 units of Terminal Transferase, recombinant (Roche), was added to the 2nd PCR product and the mixture was allowed to react at 37°C for 30 minutes and then heated at 94°C for 5 minutes to stop the enzymatic reaction. To the 3′ end polynucleotide addition mouse heavy chain variable region gene solution prepared above were added mouse IgG2a or human IgG1 constant region gene joining double-stranded DNA fragment, 0.2 μM primer, and 0.2 mM dNTP. With use of 0.1 units of PrimeSTAR HS DNA polymerase, 5 cycles of reactions of 30 seconds at 94°C and 4 minutes at 70°C were carried out, followed by 30 cycles

of reactions of 30 seconds at 94°C, 30 seconds at 60°C, and 1 minute at 72°C, thereby to prepare a mouse heavy chain or human chimeric heavy chain gene expression unit. Similarly, to a mouse light chain variable region gene solution were added mouse light chain constant region gene joining double-stranded DNA fragments and a reaction was conducted to prepare a mouse light chain gene expression unit. The primers used were as follows.

Primer set for joining

5′-agagaaaccgtctatcagggcgatggc-3′ (miniCMV f1-S) (SEQ ID NO: 98)

5′-agagacccttgacgttggagtccacg-3′ (miniCMV f1-AS) (SEQ ID NO: 99)

1)-2-6 Expression of Antibody Gene

The full length of mouse heavy chain or human chimeric heavy chain and mouse light chain gene expression unit amplified in the above experiments was genetically introduced into HEK 293T cells using Lipofectamine 2000 (Invitrogen) and the cells were cultured at 37°C for 4 days under 5% CO_2 to obtain an antibody culture supernatant.

[0117]

1)-3 Screening of Monoclonal Antibody

1)-3-1 Measurement of Antibody Concentration

Antibody concentration was measured using Mouse IgG2a ELISA Quantitation Set (Bethyl Laboratories) or Human IgG ELISA Quantitation Set (Bethyl Laboratories). This method was

carried out in accordance with the accompanying manual. In other words, Affinity purified Goat anti-Mouse IgG2a Coating Antibody or Affinity purified Goat anti-Human IgG-Fc Coating Antibody was diluted 101-fold with 0.05 M carbonate-bicarbonate buffer (pH 9.6). The diluted solution of 100 µl was added to a well and immobilized at room temperature for 1 hour. After washing the well three times with 150 µl of Tris buffered saline (TBS; Tween 20-added TBS was TBST) supplemented with 0.05% Tween 20, 200 µl of 1% BSA/TBS was added to the well and blocking was carried out for 0.5 to 2 hours. Each antibody culture supernatant or purified antibody (if necessary, diluted in 1% BSA/TBST) was directly added in an amount of 100 µl to the well and allowed to react at room temperature for 1 hour. After washing the well three times with TBST, 50,000-fold diluted HRP Conjugated Goat anti-Mouse IgG2a Detection Antibody or 100,000-fold diluted HRP Conjugated Goat anti-Human IgG-Fc Detection Antibody was added in an amount of 100 µl/well, and allowed to react for 1 hour. The well was washed three times with TBST, and SuperSignal ELISA Pico Chemiluminescent Substrate (Thermo Fisher Scientific K.K.) was added thereto, and luminescence was measured with ARVO 1420 multilabel counter (Perkin Elmer) immediately after the addition. A calibration curve was obtained by using the standard attached to the kit, thereby to calculate the concentration of antibody.

[0118]

1)-3-2 Measurement of Opsonophagocytic Killing Activity

As a buffer for measurement, 0.1% gelatin/1% FBS/Hank's balanced salt solution (opsonization buffer) was prepared. HL60 as effector cells were cultured in 10% FBS-containing RPMI 1640 (Invitrogen) supplemented with 0.8% N,N-dimethylformamide for 5 or 6 days to prepare neutrophil-like, differentiated HL60 in an opsonization buffer. As the target bacteria, Pseudomonas aeruginosa ATCC 29260 cultured on MHA was prepared by suspending it in the opsonization buffer. As a complement, Baby Rabbit Serum (Cederlane) was prepared by dissolving it with ice-cold distilled water. Bacterial suspension (final concentration of 2×10^4 cells/ml) was mixed with antibody culture supernatant or purified antibody, allowed to stand at 4°C for 30 minutes, and the prepared neutrophil-like, differentiated HL60 cells (final concentration of 4×10^6 cells/ml) and a complement (final concentration 10%) were added thereto, followed by incubation with gentle agitation at 37°C for 1 hour under 5% CO_2. After the incubation, each sample was spread on MHA, and colonies were counted on the next day. Compared with the control to which the antibody was not added, a sample that showed 50% growth inhibitory effect was determined to have an activity.

[0119]

1)-3-3 Determination of CDR Sequence

Using the mouse heavy and light chain variable region gene product obtained in 1)-2-3 as a template, CDR sequence was confirmed with the following sequence primers. Sequence

analysis was performed using a gene sequence analyzer of Applied Biosystems 3730xl DNA Analyzer (Life technologies). Based on the obtained sequences, CDR sequence was determined according to Abysis database (http://www.bioinf.org.uk/abs/#cdrid) by Andrew C. R. Martin et al.

Sequence primer (for heavy chain)

5′-acaccgctggacagggatccagag-3′ (mIgG sequence) (SEQ ID NO: 100)

Sequence primer (for light chain)

5′-gtagaagttgttcaagaagcacac-3′ (mIgκ sequence) (SEQ ID NO: 101)

[0120]

1)-3-4 Selection of Monoclonal Antibodies

From the above screening, antibody culture supernatant samples No.76, #1G5, and #4C12 each having a strong opsonophagocytic killing activity and a unique CDR sequence were found. The No.76 is an antibody prepared by using pJON mIgG2a and pJON mIgκ, and the #1G5 and #4C12 are antibodies prepared by using pJON mIgG2a-hIgG1 and pJON mIgκ, respectively. The amino acid sequences of each heavy and light chain variable region and CDR are shown in SEQ ID NOs: 4 to 27. Although the light chain of #4C12 shown in SEQ ID NO: 20 in the Sequence Listing includes a cysteine residue at amino acid number 75, a light chain in which the cysteine residue at the position of 75 in #4C12 was replaced with tyrosine was named "#4C13". Also, a combination of the heavy chain of #4C12 and the light chain of #1G5 was named "#4C13K". The #4C13K was obtained by expressing

the human chimeric heavy chain gene expression unit of #4C12 prepared in 1)-2-5 and the mouse light chain gene expression unit of #1G5, in HEK293T according to the method of 1)-2-6. The #1G5 and #4C13K were purified by Protein G (GE Healthcare), and were named as "c#1G5M" and "c#4C13KM", respectively. The antibody concentration was calculated according to the method of 1)-3-1.

[0121]

Example 2

Preparation of Human Chimeric Anti-LPS O11 Antibody and Meiji 1640, Meiji 1656, Human IgG1 Type 1BO11 (1BO11 hIgG1), Mouse IgG2a type No.76 (No.76 mIgG2a)

[0122]

2)-1 Construction of Chimeric and Humanized Antibody Light Chain Expression Vector pCMA-LK

An approximately 5.4 kb fragment obtained by digesting a plasmid pcDNA3.3-TOPO/LacZ and (INVITROGEN Corp.) with the restriction enzymes XbaI and PmeI and a DNA fragment comprising a DNA sequence encoding a human light chain secretory signal shown in SEQ ID NO: 28 and a human light chain constant region were ligated to each other by using In-Fusion Advantage PCR Cloning Kit (Clontech Inc.), to prepare pcDNA3.3/LK.

[0123]

Using the pcDNA3.3/LK as a template, a PCR reaction was performed with the following primer set and the resulting

approximately 3.8 kb fragment was phosphorylated and subjected to self-ligation, thereby to construct a chimeric and humanized antibody light chain expression vector pCMA-LK having a signal sequence, a cloning site, and a human light chain constant region downstream of the CMV promoter.

Primer set

5′-tataccgtcgacctctagctagagcttggc-3′ (3.3-F1) (SEQ ID NO: 102)

5′-gctatggcagggcctgccgccccgacgttg-3′ (3.3-R1) (SEQ ID NO: 103)

2)-2 Construction of Chimeric and Humanized Antibody IgG1 Type Heavy Chain Expression Vector pCMA-G1

A DNA fragment obtained by removing the light chain secretory signal and the human light chain constant region by digestion of the pCMA-LK with XbaI and PmeI, and a DNA fragment comprising a DNA sequence encoding a human heavy chain signal sequence shown in SEQ ID NO: 29 and amino acids of a human IgG1 constant region were ligated to each other by using In-Fusion Advantage PCR Cloning Kit (Clontech Inc.), thereby to construct a chimeric and humanized antibody IgG1-type heavy chain expression vector pCMA-G1 having a signal sequence, a cloning site, and a human IgG1 heavy chain constant region downstream of the CMV promoter.

[0124]

2)-3 Preparation of Human Chimeric Anti-LPS O11 Antibody

2)-3-1 Construction of Human Chimeric No.76 Light Chain Expression Vector

By using the cDNA containing the light chain variable region of No.76 obtained in Example 1)-2-3 as a template and also using KOD-Plus-(TOYOBO, Co., Ltd.) and the following primer set, a DNA fragment comprising the cDNA encoding the light chain variable region was amplified, and a chimeric and humanized antibody light chain expression vector (pCMA-LK) was inserted at the site cleaved with the restriction enzyme BsiWI using an In-Fusion HD PCR cloning kit (Clontech, Inc.), and thus, a human chimeric No.76 light chain expression vector was constructed. The thus obtained expression vector was named "pCMA-LK/No.76". The nucleotide sequence of the human chimeric No.76 light chain was shown in SEQ ID NO: 30 in the Sequence Listing, and the amino acid sequence thereof was shown in SEQ ID NO: 31.

Primer set for No.76 light chain

5'-atctccggcgcgtacggcaacattgtaatgacccaatctcccaaatc-3' (76L-F)(SEQ ID NO: 104)

5'-ggaggggcggccacagcccgttttatttccagcttggtcctccc-3' (76L-R) (SEQ ID NO: 105)

[0125]

2)-3-2 Construction of Human Chimeric No.76 Heavy Chain Expression Vector

By using, as a template, the cDNA containing the heavy chain variable region of No.76 obtained in Example 1)-2-3 and also using KOD-Plus- (TOYOBO, Co., Ltd.) and the following primer set, a DNA fragment comprising the cDNA encoding the heavy chain

variable region was amplified, and a chimeric and humanized antibody IgG1-type heavy chain expression vector (pCMA-G1) was inserted at the site cleaved with the restriction enzyme BlpI using an In-Fusion HD PCR cloning kit (Clontech, Inc.), and thus, a human chimeric No.76 heavy chain expression vector was constructed. The thus obtained expression vector was named "pCMA-G1/No.76". The nucleotide sequence of the human chimeric No.76 heavy chain was shown in SEQ ID NO: 32 in the Sequence Listing, and the amino acid sequence thereof was shown in SEQ ID NO: 33.

Primer set for human chimeric No.76 heavy chain

5'-ccagatgggtgctgagccaggtccaactgcagcagcctggtgctgag-3' (76H-F)(SEQ ID NO: 106)

5'-cttggtggaggctgagctgactgtgagagtggtgccttggccccag-3' (76H-R)(SEQ ID NO: 107)

[0126]

2)-3-3 Construction of Human Chimeric #1G5 Light Chain Expression Vector

A DNA fragment comprising a DNA sequence encoding a human chimeric #1G5 light chain shown in SEQ ID NO: 34 in the Sequence Listing was synthesized (GENEART Inc., Artificial Gene Synthesis Service). By using the synthesized DNA fragment as a template and also using KOD-Plus-(TOYOBO, Co., Ltd.) and the following primer set, a DNA fragment comprising a DNA sequence encoding the human chimeric #1G5 light chain was amplified, and a chimeric

and humanized antibody light chain expression vector pCMA-LK was inserted at the site cleaved with the restriction enzymes XbaI and PmeI using an In-Fusion HD PCR cloning kit (Clontech, Inc.), and thus, a human chimeric #1G5 light chain expression vector was constructed. The thus obtained expression vector was named "pCMA-LK/1G5". The amino acid sequence of the human chimeric #1G5 light chain was shown in SEQ ID NO: 35 in the Sequence Listing.

Primer set for human chimeric #1G5 light chain

5'-ccagcctccggactctagagccacc-3' (CM-inf-F) (SEQ ID NO: 108)

5'-agttagcctcccccgtttaaactc-3' (CM-inf-R) (SEQ ID NO: 109)

[0127]

2)-3-4 Construction of Human Chimeric #1G5 Heavy Chain Expression Vector

A DNA fragment containing a DNA sequence encoding a human chimeric #1G5 heavy chain variable region represented by nucleotide numbers 36 to 449 of the human chimeric #1G5 heavy chain nucleotide sequence of SEQ ID NO: 36 in the Sequence Listing was synthesized (GENEART Inc., Artificial Gene Synthesis Service). By using the synthesized DNA fragment as a template and also using KOD-Plus- (TOYOBO, Co., Ltd.) and the following primer set, a DNA fragment comprising a DNA sequence encoding the human chimeric #1G5 heavy chain variable region was amplified, and a chimeric and humanized antibody IgG1-type heavy chain expression vector pCMA-G1 was inserted at the site cleaved with

the restriction enzyme BlpI using an In-Fusion HD PCR cloning kit (Clontech, Inc.), and thus, a human chimeric #1G5 heavy chain expression vector was constructed. The thus obtained expression vector was named "pCMA-G1/1G5". The amino acid sequence of the human chimeric #1G5 heavy chain was shown in SEQ ID NO: 37 in the Sequence Listing.

Primer set for human chimeric #1G5 heavy chain

5'-agctcccagatgggtgctgagc-3' (EG-Inf-F) (SEQ ID NO: 110)

5'-gggcccttggtggaggctgagc-3' (EG1-Inf-R) (SEQ ID NO: 111)

[0128]

2)-3-5 Construction of Human Chimeric #4C13 Light Chain Expression Vector

A DNA fragment containing a DNA sequence encoding a human chimeric #4C13 light chain shown in SEQ ID NO: 38 in the Sequence Listing was synthesized (GENEART Inc., Artificial Gene Synthesis Service). By using the synthesized DNA fragment as a template and also using KOD-Plus- (TOYOBO, Co., Ltd.) and the following primer set, a DNA fragment comprising a DNA sequence encoding the human chimeric #4C13 light chain was amplified, and a chimeric and humanized antibody light chain expression vector pCMA-LK was inserted at the site cleaved with the restriction enzymes XbaI and PmeI using an In-Fusion HD PCR cloning kit (Clontech, Inc.), and thus, a human chimeric #4C13 light chain expression vector was constructed. The thus obtained expression vector was named "pCMA-LK/4C13". The amino acid sequence of the human

chimeric #4C13 light chain was shown in SEQ ID NO: 39 in the Sequence Listing.

Primer set for human chimeric #4C13 light chain

5′-ccagcctccggactctagagccacc-3′ (CM-inf-F) (SEQ ID NO: 108)

5′-agttagcctcccccgtttaaactc-3′ (CM-inf-R) (SEQ ID NO: 109)

[0129]

2)-3-6 Construction of Human Chimeric #4C13 Heavy Chain Expression Vector

A DNA fragment containing a DNA sequence encoding a human chimeric #4C13 heavy chain variable region represented by nucleotide numbers 36 to 437 of the human chimeric #4C13 heavy chain nucleotide sequence of SEQ ID NO: 40 in the Sequence Listing was synthesized (GENEART Inc., Artificial Gene Synthesis Service). By using the synthesized DNA fragment as a template and also using KOD-Plus- (TOYOBO, Co., Ltd.) and the following primer set, a DNA fragment comprising a DNA sequence encoding the human chimeric #4C13 heavy chain variable region was amplified, and a chimeric and humanized antibody IgG1-type heavy chain expression vector pCMA-G1 was inserted at the site cleaved with the restriction enzyme BlpI using an In-Fusion HD PCR cloning kit (Clontech, Inc.) to thereby construct a human chimeric #4C13 heavy chain expression vector. The thus obtained expression vector was named "pCMA-G1/4C13". The amino acid sequence of the human chimeric #4C13 heavy chain was shown in SEQ ID NO: 41 in the Sequence Listing.

Primer set for human chimeric #4C13 heavy chain

5′-agctcccagatgggtgctgagc-3' (EG-Inf-F) (SEQ ID NO: 110)

5′'gggcccttggtggaggctgagc-3' (EG1-Inf-R) (SEQ ID NO: 111)

2)-3-7 Preparation of Human Chimeric Anti-LPS O11 Antibody

2)-3-7-1 Production of Human Chimeric Anti-LPS O11 Antibody

FreeStyle 293-F cells (Invitrogen Inc.) were passaged and cultured according to the manual.

[0130]

FreeStyle 293-F cells (Invitrogen, Inc.) of 2×10^9 in logarithmic growth phase were seeded in 3 L of Fernbach Erlenmeyer Flask (CORNING Co.), diluted with FreeStyle 293 expression medium (INVITROGEN Corp.) to 1.0×10^6 cells/ml, and cultured with shaking at 37°C for one hour at 90 rpm in an 8% CO_2 incubator. Polyethyleneimine (Polyscience, Inc., #24765) of 3.6 mg was dissolved in 20 ml of Opti-Pro SFM medium (INVITROGEN, Inc.), and then a light chain expression vector (0.8 mg) and a heavy chain expression vector (0.4 mg) prepared with use of PureLink HiPure Plasmid kit (Invitrogen Corp.) were suspended in 20 ml of Opti-Pro SFM (Invitrogen Corp). To the polyethyleneimine/ Opti-Pro SFM mixed liquid (20 ml) was added 20 ml of the expression vector/Opti-Pro SFM mixed liquid, and the resulting liquid was gently agitated and allowed to stand for further 5 minutes. After that, the liquid was added to the FreeStyle 293-F cells. The shaking culture was performed at 90 rpm and at 37°C for 7 days in an 8% CO_2 incubator, and the culture supernatant was filtered

with Disposable Capsule Filter (ADVANTEC #CCS-045-E1H).

[0131]

A human chimeric No.76 acquired by a combination of pCMA-LK/No.76 and pCMA-G1/No.76 was named "cNo.76"; a human chimeric #1G5 acquired by a combination of pCMA-LK/1G5 and pCMA-G1/1G5 was named "c#1G5"; and a human chimeric #4C13 acquired by a combination of pCMA-LK/4C13 and pCMA-G1/ 4C13 was named "c#4C13".

[0132]

2)-3-7-2 Purification of Human Chimeric Anti-LPS O11 Antibody

The antibody from the culture supernatant obtained in 2)-3-7-1 was purified by one-step process of rProtein A affinity chromatography (at 4-6°C). After purification by the rProtein A affinity chromatography, buffer replacement process was performed at 4-6°C. First, the culture supernatant was applied to a column filled with MabSelect SuRe (manufactured by GE Healthcare Bioscience Co.) equilibrated with PBS. After all of the culture liquid was placed in the column, the column was washed with two or more times the column volumes of PBS. Then the column was eluted with 2M arginine hydrochloride solution (pH 4.0) to collect fractions containing the antibody. The fractions were dialyzed (Thermo Scientific, Inc., Slide-A-Lyzer Dialysis Cassette) to replace the solution with HBSor (25 mM histidine/5% sorbitol, pH 6.0). The solution was concentrated with Centrifugal UF Filter Device VIVASPIN20 (fractionation

molecular weight UF10K, Sartorius, Inc., at 4°C) to adjust to 5 mg/ml or more of IgG concentration. Finally, the solution was filtered through a Minisart-Plus filter (Sartorius Inc.) and served as a purified sample.

2)-4 Preparation of Meiji 1640 and Meiji 1656, human IgG1 type 1BO11 (1BO11 hIgG1), mouse IgG2a type No.76 (No.76 mIgG2a)

2)-4-1 Preparation of Meiji 1640

The Meiji 1640 was prepared based on the amino acid sequences of the light chain and the heavy chain as described in WO 2011/102551.

[0133]

2)-4-1-1 Construction of Meiji 1640 Light Chain Expression Vector

A DNA fragment containing a DNA sequence encoding a Meiji 1640 light chain variable region represented by nucleotide numbers 38 to 408 of the Meiji 1640 light chain nucleotide sequence of SEQ ID NO: 42 in the Sequence Listing was synthesized (GENEART Inc., Artificial Gene Synthesis Service). By using the synthesized DNA fragment as a template and also using KOD-Plus- (TOYOBO, Co., Ltd.) and the following primer set, a DNA fragment comprising a DNA sequence encoding the Meiji 1640 light chain variable region was amplified, and a chimeric and humanized antibody light chain expression vector pCMA-LK was inserted at the site cleaved with the restriction enzyme BsiWI using an In-Fusion HD PCR cloning kit (Clontech, Inc.) to thereby construct a Meiji 1640 light chain expression vector. The thus

obtained expression vector was named "pCMA-LK/Meiji 1640". The amino acid sequence of the Meiji 1640 light chain was shown in SEQ ID NO: 43 in the Sequence Listing.

Primer set for Meiji 1640 light chain

5'-ctgtggatctccggcgcgtacggc-3' (CM-LKF) (SEQ ID NO: 112)

5'-ggaggggcggccaccgtacg-3' (KCL-Inf-R) (SEQ ID NO: 113)

[0134]

2)-4-1-2 Construction of Meiji 1640 Heavy Chain Expression Vector

A DNA fragment containing a DNA sequence encoding a Meiji 1640 heavy chain variable region represented by nucleotide numbers 36 to 458 of the Meiji 1640 heavy chain nucleotide sequence of SEQ ID NO: 44 in the Sequence Listing was synthesized (GENEART Inc., Artificial Gene Synthesis Service). By using the synthesized DNA fragment as a template and also using KOD-Plus- (TOYOBO, Co., Ltd.) and the following primer set, a DNA fragment comprising a DNA sequence encoding the Meiji 1640 heavy chain variable region was amplified, and a chimeric and humanized antibody IgG1 type heavy chain expression vector pCMA-G1 was inserted at the site cleaved with the restriction enzyme BlpI using an In-Fusion HD PCR cloning kit (Clontech, Inc.) to thereby construct a Meiji 1640 heavy chain expression vector. The thus obtained expression vector was named "pCMA-G1/Meiji 1640". The amino acid sequence of the Meiji 1640 heavy chain was shown in SEQ ID NO: 45 in the Sequence Listing.

Primer set for Meiji 1640 heavy chain

5′-agctcccagatgggtgctgagc-3′ (EG-Inf-F) (SEQ ID NO: 110)

5′-gggcccttggtggaggctgagc-3′ (EG1-Inf-R) (SEQ ID NO: 111)

2)-4-2 Preparation of Meiji 1656

The Meiji 1656 was prepared based on the amino acid sequences of the light chain and the heavy chain as described in WO 2011/102551.

[0135]

2)-4-2-1 Construction of Meiji 1656 Light Chain Expression Vector

A DNA fragment containing a DNA sequence encoding a Meiji 1656 light chain variable region represented by nucleotide numbers 38 to 402 of the Meiji 1656 light chain nucleotide sequence of SEQ ID NO: 46 in the Sequence Listing was synthesized (GENEART Inc., Artificial Gene Synthesis Service). By using the synthesized DNA fragment as a template and also using KOD-Plus- (TOYOBO, Co., Ltd.) and the following primer set, a DNA fragment comprising a DNA sequence encoding the Meiji 1656 light chain variable region was amplified, and a chimeric and humanized antibody light chain expression vector pCMA-LK was inserted at the site cleaved with the restriction enzyme BsiWI using an In-Fusion HD PCR cloning kit (Clontech, Inc.) to thereby construct a Meiji 1640 light chain expression vector. The thus obtained expression vector was named "pCMA-LK/Meiji 1656". The amino acid sequence of the Meiji 1656 light chain was shown in SEQ ID NO: 47 in the Sequence Listing.

Primer set for Meiji 1656 light chain

5′-ctgtggatctccggcgcgtacggc-3′ (CM-LKF) (SEQ ID NO: 112)

5′-ggaggggcggccaccgtacg-3′ (KCL-Inf-R) (SEQ ID NO: 113)

[0136]

2)-4-2-2 Construction of Meiji 1656 Heavy Chain Expression Vector

A DNA fragment containing a DNA sequence encoding a Meiji 1656 heavy chain variable region represented by nucleotide numbers 36 to 467 of the Meiji 1656 heavy chain nucleotide sequence of SEQ ID NO: 48 in the Sequence Listing was synthesized (GENEART Inc., Artificial Gene Synthesis Service). By using the synthesized DNA fragment as a template and also using KOD-Plus- (TOYOBO, Co., Ltd.) and the following primer set, a DNA fragment comprising a DNA sequence encoding the Meiji 1656 heavy chain variable region was amplified, and a chimeric and humanized antibody IgG1 type heavy chain expression vector pCMA-G1 was inserted at the site cleaved with the restriction enzyme BlpI using an In-Fusion HD PCR cloning kit (Clontech, Inc.) to thereby construct a Meiji 1656 heavy chain expression vector. The thus obtained expression vector was named "pCMA-G1/Meiji 1656". The amino acid sequence of the Meiji 1656 heavy chain was shown in SEQ ID NO: 49 in the Sequence Listing.

Primer set for Meiji 1656 heavy chain

5′-agctcccagatgggtgctgagc-3′ (EG-Inf-F) (SEQ ID NO: 110)

5′-gggcccttggtggaggctgagc-3′ (EG1-Inf-R) (SEQ ID NO: 111)

2)-4-3 Preparation of Human IgG1 Type 1BO11 (1BO11 hIgG1)

The 1BO11 hIgG1 was prepared based on the amino acid

sequences of the light chain and the heavy chain as described in Figs. 1 and 2 of WO 2006/084758.

[0137]

2)-4-3-1 Construction of 1BO11 hIgG1 Light Chain Expression Vector

A DNA fragment encoding a mouse IgG2b type chimeric 1BO11 light chain shown in SEQ ID NO: 50 in the Sequence Listing was synthesized (MBL Co., Ltd., Artificial Gene Synthesis Service). By using the synthesized DNA fragment as a template and also using KOD-Plus- (TOYOBO, Co., Ltd.) and the following primer set, a DNA fragment comprising a cDNA encoding the light chain variable region was amplified, and a chimeric and humanized antibody light chain expression vector pCMA-LK was inserted at the site cleaved with the restriction enzyme BsiWI, with use of an In-Fusion HD PCR cloning kit (Clontech, Inc.) to thereby construct a 1BO11 hIgG1 light chain expression vector. The thus obtained expression vector was named "pCMA-LK/1BO11". The nucleotide sequence of the 1BO11 hIgG1 light chain was shown in SEQ ID NO: 51 in the Sequence Listing and the amino acid sequence of the 1BO11 hIgG1 light chain was shown in SEQ ID NO: 52.

Primer set for 1BO11 hIgG1 light chain

5'-atctccggcgcgtacggcgacgtggtgatgacccagagccctctgtcc-3'

(h1BO-LF)(SEQ ID NO: 114)

5'-gggcggccaccgtacgcttgatctccaccttggtgcctccgccg-3'

(h1BO-LR)(SEQ ID NO: 115)

[0138]

2)-4-3-2 Construction of 1BO11 hIgG1 Heavy Chain Expression Vector

A DNA fragment encoding a mouse IgG2b type chimeric 1BO11 heavy chain shown in SEQ ID NO: 53 in the Sequence Listing was synthesized (MBL Co., Ltd., Artificial Gene Synthesis Service). By using the synthesized DNA fragment as a template and also using KOD-Plus- (TOYOBO, Co., Ltd.) and the following primer set, a DNA fragment comprising a cDNA encoding the heavy chain variable region was amplified, and a chimeric and humanized antibody IgG1 type heavy chain expression vector pCMA-G1 was inserted at the site cleaved with the restriction enzyme BlpI using an In-Fusion HD PCR cloning kit (Clontech, Inc.) to thereby construct a 1BO11 hIgG1 heavy chain expression vector. The thus obtained expression vector was named "pCMA-G1/1BO11". The nucleotide sequence of the 1BO11 hIgG1 heavy chain was shown in SEQ ID NO: 54 in the Sequence Listing, and the amino acid sequence of the 1BO11 hIgG1 heavy chain was shown in SEQ ID NO: 55.

Primer set for 1BO11 hIgG1 heavy chain

5'-ccagatgggtgctgagcgaggagcaagtggtggagagcgg-3' (h1BO-HF)

(SEQ ID NO: 116)

5'-cttggtggaggctgagctcacggtcaccatggtgccttgtc-3' (h1BO-HR)

(SEQ ID NO: 117)

[0139]

2)-4-4 Preparation of Mouse IgG2a Type No.76 (No.76 mIgG2a)

2)-4-4-1 Preparation of No.76 mIgG2a Light Chain Expression Vector

A DNA fragment containing a sequence encoding No.76 mIgG2a light chain shown in SEQ ID NO: 118 was synthesized (GENEART Inc., Strings DNA Fragments). The synthesized DNA was inserted at the site where the chimeric and humanized antibody light chain expression vector pCMA-LK was digested with the restriction enzymes XbaI and PmeI to remove the κ chain secretion signal and human κ chain constant region, using an In-Fusion HD PCR cloning kit (Clontech, Inc.) to thereby construct a No.76 mIgG2a light chain expression vector. The thus obtained expression vector was named "pCMA/No.76 mIgG2aL". The amino acid sequence of the No.76 mIgG2a light chain was shown in SEQ ID NO: 119.

2)-4-4-2 Preparation of No.76 mIgG2a Heavy Chain Expression Vector

A DNA fragment containing a sequence encoding No.76 mIgG2a heavy chain shown in SEQ ID NO: 120 was synthesized (GENEART Inc., Artificial Gene Synthesis Service). By using the synthesized DNA fragment as a template and also using KOD-Plus-(TOYOBO, Co., Ltd.) and the following primer set, a DNA fragment comprising a sequence encoding the No.76 mIgG2a heavy chain was amplified, and inserted at the site where the chimeric and humanized antibody light chain expression vector pCMA-LK was digested with the restriction enzymes XbaI and PmeI to remove

the κ chain secretion signal and human κ chain constant region, with use of an In-Fusion HD PCR cloning kit (Clontech, Inc.) to thereby construct a No.76 mIgG2a heavy chain expression vector. The thus obtained expression vector was named "pCMA/No.76 mIgG2aH". The amino acid sequence of the No.76 mIgG2a heavy chain was shown in SEQ ID NO: 121.

Primer set for No.76 mIgG2a heavy chain

5'-ccagcctccggactctagagccacc-3' (CM-inf-F) (SEQ ID NO: 108)

5'-agttagcctcccccgtttaaactc-3' (CM-inf-R) (SEQ ID NO: 109)

2)-4-5 Production and Purification of Meiji 1640, Meiji 1656, 1BO11 hIgG1, and No.76 mIgG2a

2)-4-5-1 Production of Meiji 1640, Meiji 1656, 1BO11 hIgG1, and No.76 mIgG2a

These antibodies were produced in the same manner as in Example 2)-3-7-1.

[0140]

Meiji 1640 was obtained by a combination of the pCMA-LK /Meiji 1640 and the pCMA-G1/Meiji 1640; Meiji 1656 was obtained by a combination of the pCMA-LK/Meiji 1656 and the pCMA-G1/Meiji 1656; 1BO11 hIgG1 was obtained by a combination of the pCMA-LK/1BO11 and the pCMA-G1/1BO11; and the No.76 mIgG2a was obtained by a combination of the pCMA/No.76 mIgG2aL and the pCMA/No.76 mIgG2aH.

2)-4-5-2 Purification of Meiji 1640, Meiji 1656, 1BO11 hIgG1, and No.76 mIgG2a

These antibodies were purified from the culture supernatant obtained in 2)-4-5-1 in the same manner as in Example 2)-3-7-2.

[0141]Example 3 In Vitro Activity of Anti-LPS O11 Antibody

3)-1 Opsonophagocytic Killing activity against Pseudomonas aeruginosa ATCC 29260

Opsonophagocytic killing activity of each of cNo.76, c#1G5M, c#1G5, c#4C13KM, c#4C13, Meiji 1640, Meiji 1656, and 1BO11 hIgG1 against Pseudomonas aeruginosa ATCC 29260 was measured in accordance with the method of 1)-3-2. The opsonophagocytic killing activity was expressed in terms of 50% minimum growth inhibitory concentration. The results are shown in Table 2.

[0142]

[Table 2]

Strain No.	Opsonophagocytic killing activity (ng/mL)							
	cNo.76	c#1G5M	c#1G5	c#4C13KM	c#4C13	Meiji1640	Meiji1656	1BO11 hIgG1
ATCC 29260	4.1	1.4	4.1	1.4	4.1	110	37	1000

[0143]

The cNo.76, c#1G5M, c#1G5, c#4C13KM, and c#4C13 exhibit almost the same level of opsonophagocytic killing activity, and they showed 27-81 times the opsonophagocytic killing activity of Meiji 1640, 9-27 times the opsonophagocytic killing activity of Meiji 1656, and 243-729 times the opsonophagocytic killing activity of 1BO11 hIgG1.

[0144]

3)-2 Binding Specificity to Pseudomonas aeruginosa O11 Strains

The binding specificity of cNo.76, c#1G5, and c#4C13 to Pseudomonas aeruginosa carrying various O antigens was confirmed by the whole cell ELISA. The strains and O antigens used are shown in Table 3.

[0145]

[Table 3]

Strain No	O-antigen
ATCC 33348	O1
ATCC 33349	O2
ATCC 33350	O3
ATCC 33351	O4
ATCC 33352	O5
ATCC 33354	O6
ATCC 33353	O7
ATCC 33355	O8
ATCC 33356	O9
ATCC 33357	O10
ATCC 33358	O11
ATCC 33360	O13
ATCC 33361	O14
ATCC 33362	O15
ATCC 33363	O16
ATCC 33364	O17
ATCC 43390	O18
ATCC 43731	O19
ATCC 43732	O20
ATCC 21636	M (untypable)
ATCC 29260	O11 (positive control)
ATCC 25922	E.coli (negative control)

[0146]

The bacteria cultured on MHA was suspended in saline to adjust to 0.5-0.6 of OD_{600} and immobilized for one hour. After blocking with 5% skim milk (Becton, Dickinson and Company)/TBST

for 2 hours, 0.1 μg/ml of cNo.76, c#1G5, and c#4C13 was added as the primary antibody to the suspension. The suspension was allowed to react for 1 hour, followed by reaction with Anti-Human IgG, HRP-Linked WholeAb Sheep (GE Healthcare) as the secondary antibody for 1 hour, after which SuperSignal ELISA Pico Chemiluminescent Substrate (Thermo Fisher Scientific K.K.) was added, and light emission was measured immediately after the addition with ARVO 1420 multi-label counter (Perkin Elmer). Between each step, two or three times washing with TBST were performed. Pseudomonas aeruginosa ATCC 29260 was used as a positive control, and E. coli ATCC 25922 was used as a negative control. The cNo.76, c#1G5, and c#4C13 showed a high binding activity to the O11 strains. On the other hand, since the cNo.76, c#1G5, and c#4C13 showed no binding activity to other O-antigen-carrying strains, they were found to specifically recognize the antigen O11 (Fig. 4).

[0147]

3)-3 Binding Activity to Pseudomonas aeruginosa O11 Clinical Isolates

The binding activity of the antibodies to 31 clinical isolates of Pseudomonas aeruginosa O11 held by Daiichi Sankyo Co., Ltd. and to ATCC 29260 as a positive control was measured by the whole cell ELISA. The O-antigen of Pseudomonas aeruginosa was determined by mixing 20 μl of immune serum for serotyping Pseudomonas aeruginosa (Denka Seiken Co., Ltd.) and 20 μl of

bacterial solution and observing the presence or absence of agglutination caused by the antigen-antibody reaction. The Whole cell ELISA was performed in the same manner as in 3)-2, and 0.1 μg/ml of cNo.76, c#1G5M, c#4C13KM, Meiji 1640, and Meiji 1656, and 6.4 μg/ml of 1BO11 hIgG1 were prepared as the primary antibody and allowed to react for 1 hour. Information of the bacterial strains used is shown in Table 4 and the measurement results are shown in Table 5.

[0148]

[Table 4]

Strain No.	Isolation year	Isolation country	Isolation part
1	2010	Japan	Respiratory tract
2	2010	Japan	Respiratory tract
3	2010	Japan	Respiratory tract
4	2010	Japan	Respiratory tract
5	2010	Japan	Respiratory tract
6	2010	Japan	Respiratory tract
7	2010	Japan	Respiratory tract
8	2010	Japan	Respiratory tract
9	2010	Japan	Urine
10	2010	Japan	Urine
11	2009	U.S.	Respiratory tract
12	2009	U.S.	Blood
13	2009	U.S.	Respiratory tract
14	2009	U.S.	Respiratory tract
15	2009	U.S.	Blood
16	2009	U.S.	Respiratory tract
17	2009	U.S.	Respiratory tract
18	2009	U.S.	Blood
19	2009	U.S.	Blood
20	2009	U.S.	Blood
21	2009	Germany	Blood
22	2009	Spain	Respiratory tract
23	2009	Spain	Respiratory tract
24	2009	France	Blood
25	2009	Italy	Respiratory tract

26	2009	Italy	Respiratory tract
27	2009	France	Respiratory tract
28	2009	Spain	Blood
29	2010	Sweden	Blood
30	2003	Spain	Respiratory tract
31	2007	Japan	Respiratory tract

[0149]

[Table 5]

Strain No.	cNo.76	c#1G5M	c#4C13KM	Meiji1640	Meiji1656	1BO11 hIgG1
ATCC 29260	+	+	+	+	+	+
1	+	+	+	+	+	+
2	+	+	+	+	+	+
3	+	+	+	+	+	-
4	+	+	+	+	+	+
5	+	+	+	+	+	+
6	+	+	+	+	+	+
7	+	+	+	+	+	+
8	+	+	+	+	+	+
9	+	+	+	-	-	-
10	+	+	+	+	+	+
11	+	+	+	-	-	-
12	+	+	+	-	-	-
13	+	+	+	+	+	+
14	+	+	+	+	+	+
15	+	+	+	-	+	-
16	+	+	+	+	+	+
17	+	+	+	+	+	+
18	+	+	+	-	-	-
19	+	+	+	-	+	-
20	+	+	+	-	+	-
21	+	+	+	+	+	+
22	+	+	+	+	+	+
23	+	+	+	+	+	+
24	+	+	+	+	+	+
25	+	+	+	+	+	+
26	+	+	+	+	+	+
27	+	+	+	+	+	+
28	+	+	+	-	-	-
29	+	+	+	-	-	-
30	+	+	+	+	+	+
31	+	+	+	+	+	+

+: positive, −: negative

[0150]

When the binding activity of the antibody against Pseudomonas aeruginosa O11 clinical isolates is 4 or more times stronger than the binding activity against E. coli, such an antibody was judged to have a binding activity against Pseudomonas aeruginosa O11 clinical isolates. The cNo.76, c#1G5M, and c#4C13KM showed the binding activity against all strains, and Meiji 1640 showed the binding activity against only 22 strains, Meiji 1656 showed the binding activity against only 25 strains, and 1BO11 hIgG1 showed the binding activity against only 21 strains. The coverage ratios of cNo.76, c#1G5M, c#4C13KM, Meiji 1640, Meiji 1656, and 1BO11 hIgG1 for O11 clinical isolates are 100%, 100%, 100%, 71%, 81%, and 68%, respectively.

[0151]

3)-4 Opsonophagocytic Killing Activity against Pseudomonas aeruginosa O11 Clinical Isolates

Opsonophagocytic killing activity against No. 9, No. 12, No. 18, No. 24, No. 26, and No. 31 strains among the O11 strains used in 3)-3 was measured. The method was carried out in the same manner as in 1)-3-2, and the minimum concentration at which the 50% minimum growth inhibitory concentration is observed was determined. The results are shown in Table 6.

[0152]

[Table 6]

Strain	Opsonophagocytic killing activity (ng/mL)

No.	cNo.76	c#1G5 /c#1G5M	c#4C13 /c#4C13KM	Meiji 1640	Meiji 1656	1BO11 hIgG1
9	4.1	4.1	1.4	>3000	>3000	>27000
12	4.1	4.1	12	>3000	>3000	>27000
18	4.1	1.4	1.4	>3000	>3000	>27000
24	4.1	1.4	1.4	110	37	1000
26	4.1	1.4	1.4	110	37	1000
31	4.1	4.1	4.1	330	110	3000

[0153]

The cNo.76, c#1G5 or c#1G5M, c#4C13 or c#4C13KM showed the activity against all the strains. On the other hand, Meiji 1640, Meiji 1656, and 1BO11 hIgG1 did not show opsonophagocytic killing activity against No. 9, 12, and 18 strains to which binding activity was not observed. These results revealed that the cNo.76, c#1G5 or c#1G5M, c#4C13 or c#4C13KM showed a higher coverage ratio against the O11 strains in vitro in comparison with the Meiji 1640, Meiji 1656, and 1BO11 hIgG1.

[0154]

Example 4 In Vivo Activity of Anti-LPS O11 Antibody

4)-1 Therapeutic Efficacy on Mouse Model of Lung Infection by Pseudomonas aeruginosa ATCC 29260 (Intravenous Administration)

Pseudomonas aeruginosa ATCC 29260 which had been cultured on MHA was prepared to OD_{600} = 0.10, and 10-fold dilution series of 10^0 - 10^9 were prepared with Mueller Hinton Broth (Becton, Dickinson and Company) and cultured overnight at 35°C. Using a bacterial suspension of OD_{600} = 0.10 or the highest dilution of greater than OD_{600} = 0.10 among the dilution series, the suspension was prepared to OD_{600} = 0.10, diluted four-fold, and

the diluted suspension was served as an inoculum. ICR mice (Charles River) were intranasally inoculated with the inoculum of 50 µl, and 3 or 10 mg/kg of cNo.76, Meiji 1640, and Meiji 1656 was intravenously administered 1 hour after the inoculation (n = 3). After 24 hours of the inoculation, the viable cell count in lung was measured to determine the therapeutic efficacy. Compared with the control group without administration of the antibody, a good therapeutic efficacy was confirmed in the cNo.76 administration group, and such therapeutic efficacy was equivalent to or greater than the effect of each of Meiji 1640 and Meiji 1656 (Fig. 5).

[0155]

4)-2 Therapeutic Efficacy on Mouse Model of Lung Infection by Pseudomonas aeruginosa Clinical Isolates

4)-2-1 Therapeutic Efficacy on Mouse Model of Lung Infection by Pseudomonas aeruginosa Clinical Isolate No. 12 (Mixed Administration of Bacteria and Antibody)

Pseudomonas aeruginosa clinical isolate No. 12 cultured on MHA was suspended in saline and adjusted to OD_{600} = 2.0 (10-fold diluted suspension was adjusted to prepare a bacterial suspension of OD_{600} = 0.2). The cNo.76, Meiji 1640, or Meiji 1656 of 20 µg/ml or 100 µg/ml were mixed in a ratio of 1 : 9 so that the binding between bacteria and antibodies surely occurs, and ICR mice (Charles River)(n = 3) were intranasally inoculated with 50 µl of the mixture. At 24 hours after the inoculation, the

viable cell count in the lung was measured to determine the therapeutic effect. In addition, the therapeutic efficacy of levofloxacin (LVFX) as a control was also examined (the minimum inhibitory concentration (MIC) of LVFX against No. 12 strain was >128 μg/ ml). In other words, the bacterial suspension and saline were mixed in a ratio of 1 : 9, and 50 μl of the mixture was inoculated into mice and LVFX of 240 mg/kg was subcutaneously administered to the inoculated mice immediately after the inoculation. Compared with the control group without administration of the antibody, a good therapeutic efficacy was confirmed in the cNo.76 administration group, but was not observed in the Meiji 1640, Meiji 1656, or LVFX administration group (Fig. 6).

[0156]

4)-2-2 Therapeutic Efficacy on Mouse Model of Lung Infection by Pseudomonas aeruginosa Clinical Isolate No. 31 (Mixed Administration of Bacteria and Antibody)

Pseudomonas aeruginosa clinical isolate No. 31 cultured on MHA was suspended in saline and adjusted to OD_{600} = 5.0 (10-fold diluted suspension is adjusted to prepare a bacterial suspension of OD_{600} = 0.5). The bacterial suspension and cNo.76, Meiji 1640, or Meiji 1656 of 20 μg/ml or 100 μg/ml were mixed in a ratio of 1:9 so that the binding between bacteria and antibodies surely occurs, and ICR mice (Charles River)(n = 3) were intranasally inoculated with 50 μl of the mixture. At 24 hours after the

inoculation, the viable cell count in the lung was measured to determine the therapeutic efficacy. In addition, the therapeutic efficacy of LVFX as a control was also examined (the MIC of LVFX against strain No. 31 was 32 μg/ml). In other words, the bacterial suspension and saline were mixed in a ratio of 1 : 9, and 50 μl of the mixture was inoculated into mice and LVFX of 240 mg/kg was subcutaneously administered to the inoculated mice immediately after the inoculation. Compared with the control group without administration of the antibody, a good therapeutic efficacy was confirmed in the cNo.76, Meiji 1640, and Meiji 1656 administration groups, but was not observed in the LVFX administration group (Fig. 7).

[0157]

4)-2-3 Therapeutic Efficacy on Mouse Model of Lung Infection by Pseudomonas aeruginosa clinical isolate No. 12 (Intravenous Administration)

Pseudomonas aeruginosa clinical isolate No. 12 which had been cultured on MHA was suspended in saline to adjust it to OD_{600} = 0.20 and this was served as an inoculum. ICR mice (Charles River)(n = 3) were intranasally inoculated with the inoculum (50 μl), and 2 mg/kg or 10 mg/kg of cNo.76, Meiji 1640, or Meiji 1656 was intravenously administered immediately after the inoculation. At 24 hours after the inoculation, the viable cell count in the lung was measured to determine the therapeutic effect. In addition, the therapeutic efficacy of LVFX as a control was

also examined by subcutaneous administration at a dose of 240 mg/kg. The cNo.76 showed a good therapeutic efficacy even in the intravenous administration, but therapeutic efficacy was not observed in the Meiji 1640, Meiji 1656, or LVFX administration group (Fig. 8). It was suggested that the cNo.76 is effective even against multidrug-resistant Pseudomonas aeruginosa against which existing drugs such as LVFX were not effective. In addition, a characteristic high coverage ratio in vitro shown in Example 3 of the cNo.76 was also demonstrated in vivo.

[0158]

Example 5 Designing of Humanized #1G5 and #4C13K

5)-1 Designing of Humanized Version of #1G5

5)-1-1 Molecular Modeling of #1G5 Variable Region

The molecular modeling of the #1G5 variable regions was performed according to a method generally known as homology modeling (Methods in Enzymology, 203, 121-153, (1991)). The primary sequences (three-dimensional structures derived from the X-ray crystal structures are available) of the variable regions of human immunoglobulin registered in Protein Data Bank (Nuc. Acid Res. 35, D301-D303 (2007)) were compared with the #1G5 variable regions determined above. As a result, 1UYW was selected as a sequence having the highest sequence homology with the #1G5 light chain variable region among the antibodies which similarly have a deletion in the framework. Further, 3GI9 was selected as a sequence having the highest sequence homology with

the #1G5 heavy chain variable region. The three-dimensional structure of a framework region was prepared based on a "framework model" by combining the coordinates of 1UYW corresponding to the #1G5 light chain with the coordinates of 3GI9 corresponding to the #1G5 heavy chain. Then, representative conformations for each CDR are incorporated into the framework model.

[0159]

Finally, in order to obtain a possible molecular model of the #1G5 variable region in terms of energy, an energy calculation was performed for excluding disadvantageous interatomic contact. The above procedure was carried out using commercially available protein conformational analysis program Discovery Studio (Accelrys, Inc).

[0160]

5)-1-2 Designing of Amino Acid Sequence of Humanized #1G5

A humanized #1G5 antibody was constructed according to the method generally known as CDR grafting (Proc. Natl. Acad. Sci. USA 86, 10029-10033 (1989)). An acceptor antibody was selected based on the amino acid homology within the framework region. The sequence of the framework region of #1G5 was compared with all human framework sequences in the Kabat Database (Nuc. Acid Res. 29, 205-206 (2001)) of antibody amino acid sequences. As a result, an HuMc3 antibody was selected as an acceptor based on a sequence homology of 75% in the framework region. The amino acid residues in the framework region of HuMc3 were aligned with

the amino acid residues of #1G5, and the positions where different amino acids were used were identified. The positions of these residues were analyzed using the three-dimensional model of #1G5 constructed above. Then, donor residues to be grafted onto the acceptor were selected according to the criteria provided by Queen et al. (Proc. Natl. Acad. Sci. USA 86, 10029-10033 (1989)). By transferring some selected donor residues to the acceptor antibody, humanized #1G5 sequences were constructed as described in the following Example.

[0161]

5)-2 Humanization of #1G5 Heavy Chain

5)-2-1 h#1G5-H1 Type Heavy Chain:

A humanized #1G5 heavy chain designed by substituting amino acid number 24 (glutamine) with valine, amino acid number 26 (proline) with serine, amino acid number 30 (leucine) with valine, amino acid number 31 (valine) with lysine, amino acid number 39 (leucine) with valine, amino acid number 57 (lysine) with arginine, amino acid number 59 (arginine) with alanine, amino acid number 67 (isoleucine) with methionine, amino acid number 86 (lysine) with arginine, amino acid number 87 (alanine) with valine, amino acid number 89 (leucine) with isoleucine, amino acid number 91 (valine) with alanine, amino acid number 95 (serine) with threonine, amino acid number 101 (glutamine) with glutamic acid, amino acid number 106 (threonine) with arginine, amino acid number 108 (aspartic acid) with glutamic acid, amino

acid number 110 (serine) with threonine, amino acid number 116 (serine) with alanine, amino acid number 139 (serine) with leucine, and amino acid number 141 (isoleucine) with threonine of the #1G5 heavy chain shown in SEQ ID NO: 37 in the Sequence Listing was named "h#1G5-H1 type heavy chain".

[0162]

The amino acid sequence of the h#1G5-H1 type heavy chain is set forth in SEQ ID NO: 57 in the Sequence Listing. A sequence comprising amino acid residues 1 to 19 of the amino acid sequence of SEQ ID NO: 57, a sequence comprising amino acid residues 20 to 144 of the amino acid sequence of SEQ ID NO: 57, and a sequence comprising amino acid residues 145 to 474 of the amino acid sequence of SEQ ID NO: 57 correspond to the signal sequence, the heavy chain variable region, and the heavy chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 57 is set forth in SEQ ID NO: 56 in the Sequence Listing. A sequence comprising nucleotides 1 to 57 of the nucleotide sequence of SEQ ID NO: 56, a sequence comprising nucleotides 58 to 432 of the nucleotide sequence of SEQ ID NO: 56, and a sequence comprising nucleotides 433 to 1422 of the nucleotide sequence of SEQ ID NO: 56 encode the signal sequence, the heavy chain variable region sequence, and the heavy chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 56 and the amino acid sequence of SEQ ID NO: 57 are also described in Fig. 9.

[0163]

5)-2-2 h#1G5-H2 Type Heavy Chain:

A humanized #1G5 heavy chain designed by substituting amino acid number 24 (glutamine) with valine, amino acid number 26 (proline) with serine, amino acid number 30 (leucine) with valine, amino acid number 31 (valine) with lysine, amino acid number 39 (leucine) with valine, amino acid number 57 (lysine) with arginine, amino acid number 59 (arginine) with alanine, amino acid number 67 (isoleucine) with methionine, amino acid number 86 (lysine) with arginine, amino acid number 87 (alanine) with valine, amino acid number 95 (serine) with threonine, amino acid number 101 (glutamine) with glutamic acid, amino acid number 106 (threonine) with arginine, amino acid number 108 (aspartic acid) with glutamic acid, amino acid number 110 (serine) with threonine, amino acid number 139 (serine) with leucine, and amino acid number 141 (isoleucine) with threonine of the #1G5 heavy chain shown in SEQ ID NO: 37 in the Sequence Listing was named "h#1G5-H2 type heavy chain".

[0164]

The amino acid sequence of the h#1G5-H2 type heavy chain is set forth in SEQ ID NO: 59 in the Sequence Listing. A sequence comprising amino acid residues 1 to 19 of the amino acid sequence of SEQ ID NO: 59, a sequence comprising amino acid residues 20 to 144 of the amino acid sequence of SEQ ID NO: 59, and a sequence comprising amino acid residues 145 to 474 of the amino acid

sequence of SEQ ID NO: 59 correspond to the signal sequence, the heavy chain variable region, and the heavy chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 59 is set forth in SEQ ID NO: 58 in the Sequence Listing. A sequence comprising nucleotides 1 to 57 of the nucleotide sequence of SEQ ID NO: 58, a sequence comprising nucleotides 58 to 432 of the nucleotide sequence of SEQ ID NO: 58, and a sequence comprising nucleotides 433 to 1422 of the nucleotide sequence of SEQ ID NO: 58 encode the signal sequence, the heavy chain variable region sequence, and the heavy chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 58 and the amino acid sequence of SEQ ID NO: 59 are also described in Fig. 10.

[0165]

5)-2-3 h#1G5-H3 Type Heavy Chain:

A humanized #1G5 heavy chain designed by substituting amino acid number 30 (leucine) with valine, amino acid number 31 (valine) with lysine, amino acid number 39 (leucine) with valine, amino acid number 57 (lysine) with arginine, amino acid number 59 (arginine) with alanine, amino acid number 67 (isoleucine) with methionine, amino acid number 86 (lysine) with arginine, amino acid number 95 (serine) with threonine, amino acid number 101 (glutamine) with glutamic acid, amino acid number 108 (aspartic acid) with glutamic acid, amino acid number 110 (serine) with threonine, amino acid number 139 (serine) with

leucine, and amino acid number 141 (isoleucine) with threonine of the #1G5 heavy chain shown in SEQ ID NO: 37 in the Sequence Listing was named "h#1G5-H3 type heavy chain".

[0166]

The amino acid sequence of the h#1G5-H3 type heavy chain is set forth in SEQ ID NO: 61 in the Sequence Listing. A sequence comprising amino acid residues 1 to 19 of the amino acid sequence of SEQ ID NO: 61, a sequence comprising amino acid residues 20 to 144 of the amino acid sequence of SEQ ID NO: 61, and a sequence comprising amino acid residues 145 to 474 of the amino acid sequence of SEQ ID NO: 61 correspond to the signal sequence, the heavy chain variable region, and the heavy chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 61 is set forth in SEQ ID NO: 60 in the Sequence Listing. A sequence comprising nucleotides 1 to 57 of the nucleotide sequence of SEQ ID NO: 60, a sequence comprising nucleotides 58 to 432 of the nucleotide sequence of SEQ ID NO: 60, and a sequence comprising nucleotides 433 to 1422 of the nucleotide sequence of SEQ ID NO: 60 encode the signal sequence, the heavy chain variable region sequence, and the heavy chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 60 and the amino acid sequence of SEQ ID NO: 61 are also described in Fig. 11.

[0167]

5)-2-4 h#1G5-H4 Type Heavy Chain

A humanized #1G5 heavy chain designed by substituting amino acid number 31 (valine) with lysine, amino acid number 39 (leucine) with valine, amino acid number 57 (lysine) with arginine, amino acid number 59 (arginine) with alanine, amino acid number 139 (serine) with leucine, and amino acid number 141 (isoleucine) with threonine of the #1G5 heavy chain shown in SEQ ID NO: 37 in the Sequence Listing was named "h#1G5-H4 type heavy chain".

[0168]

The amino acid sequence of the h#1G5-H4 type heavy chain is set forth in SEQ ID NO: 63 in the Sequence Listing. A sequence comprising amino acid residues 1 to 19 of the amino acid sequence of SEQ ID NO: 63, a sequence comprising amino acid residues 20 to 144 of the amino acid sequence of SEQ ID NO: 63, and a sequence comprising amino acid residues 145 to 474 of the amino acid sequence of SEQ ID NO: 63 correspond to the signal sequence, the heavy chain variable region, and the heavy chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 63 is set forth in SEQ ID NO: 62 in the Sequence Listing. A sequence comprising nucleotides 1 to 57 of the nucleotide sequence of SEQ ID NO: 62, a sequence comprising nucleotides 58 to 432 of the nucleotide sequence of SEQ ID NO: 62, and a sequence comprising nucleotides 433 to 1422 of the nucleotide sequence of SEQ ID NO: 62 encode the signal sequence, the heavy chain variable region sequence, and the heavy

chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 62 and the amino acid sequence of SEQ ID NO: 63 are also described in Fig. 12.

[0169]

5)-2-5 h#1G5-H5 Type Heavy Chain

A humanized #1G5 heavy chain designed by substituting amino acid number 31 (valine) with lysine, and amino acid number 39 (leucine) with valine of the #1G5 heavy chain shown in SEQ ID NO: 37 in the Sequence Listing was named "h#1G5-H5 type heavy chain".

[0170]

The amino acid sequence of the h#1G5-H5 type heavy chain is set forth in SEQ ID NO: 65 in the Sequence Listing. A sequence comprising amino acid residues 1 to 19 of the amino acid sequence of SEQ ID NO: 65, a sequence comprising amino acid residues 20 to 144 of the amino acid sequence of SEQ ID NO: 65, and a sequence comprising amino acid residues 145 to 474 of the amino acid sequence of SEQ ID NO: 65 correspond to the signal sequence, the heavy chain variable region, and the heavy chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 65 is set forth in SEQ ID NO: 64 in the Sequence Listing. A sequence comprising nucleotides 1 to 57 of the nucleotide sequence of SEQ ID NO: 64, a sequence comprising nucleotides 58 to 432 of the nucleotide sequence of SEQ ID NO: 64, and a sequence comprising nucleotides 433 to 1422

of the nucleotide sequence of SEQ ID NO: 64 encode the signal sequence, the heavy chain variable region sequence, and the heavy chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 64 and the amino acid sequence of SEQ ID NO: 65 are also described in Fig. 13.

[0171]

5)-3 Humanization of #1G5 Light Chain

5)-3-1 h#1G5-L1 Type Light Chain

A humanized #1G5 light chain designed by substituting amino acid number 21 (asparagine) with aspartic acid, amino acid number 29 (lysine) with aspartic acid, amino acid number 31 (methionine) with leucine, amino acid number 32 (serine) with alanine, amino acid number 33 (methionine) with valine, amino acid number 35 (valine) with leucine, amino acid number 39 (valine) with alanine, amino acid number 41 (leucine) with isoleucine, amino acid number 42 (serine) with asparagine, amino acid number 60 (alanine) with proline, amino acid number 61 (glutamic acid) with glycine, amino acid number 63 (serine) with proline, amino acid number 66 (proline) with leucine, amino acid number 83 (threonine) with serine, amino acid number 88 (alanine) with glycine, amino acid number 98 (valine) with leucine, amino acid number 103 (leucine) with valine, amino acid number 105 (aspartic acid) with valine, amino acid number 107 (histidine) with tyrosine, amino acid number 120 (glycine) with glutamine, amino acid number 124 (leucine) with valine, and amino acid number 129 (alanine) with

threonine of the #1G5 light chain shown in SEQ ID NO: 35 in the Sequence Listing was named “h#1G5-L1 type light chain”.

[0172]

The amino acid sequence of the h#1G5-L1 type light chain is set forth in SEQ ID NO: 67 in the Sequence Listing. A sequence comprising amino acid residues 1 to 20 of the amino acid sequence of SEQ ID NO: 67, a sequence comprising amino acid residues 21 to 129 of the amino acid sequence of SEQ ID NO: 67, and a sequence comprising amino acid residues 130 to 234 of the amino acid sequence of SEQ ID NO: 67 correspond to the signal sequence, the light chain variable region, and the light chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 67 is set forth in SEQ ID NO: 66 in the Sequence Listing. A sequence comprising nucleotides 1 to 60 of the nucleotide sequence of SEQ ID NO: 66, a sequence comprising nucleotides 61 to 387 of the nucleotide sequence of SEQ ID NO: 66, and a sequence comprising nucleotides 388 to 702 of the nucleotide sequence of SEQ ID NO: 66 encode the signal sequence, the light chain variable region sequence, and the light chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 66 and the amino acid sequence of SEQ ID NO: 67 are also described in Fig. 14.

[0173]

5)-3-2 h#1G5-L2 Type Light Chain:

A humanized #1G5 light chain designed by substituting amino

acid number 29 (lysine) with aspartic acid, amino acid number 31 (methionine) with leucine, amino acid number 32 (serine) with alanine, amino acid number 33 (methionine) with valine, amino acid number 35 (valine) with leucine, amino acid number 39 (valine) with alanine, amino acid number 41 (leucine) with isoleucine, amino acid number 60 (alanine) with proline, amino acid number 61 (glutamic acid) with glycine, amino acid number 83 (threonine) with serine, amino acid number 98 (valine) with leucine, amino acid number 103 (leucine) with valine, amino acid number 105 (aspartic acid) with valine, amino acid number 124 (leucine) with valine, and amino acid number 129 (alanine) with threonine of the #1G5 light chain shown in SEQ ID NO: 35 in the Sequence Listing was named "h#1G5-L2 type light chain".

[0174]

The amino acid sequence of the h#1G5-L2 type light chain is set forth in SEQ ID NO: 69 in the Sequence Listing. A sequence comprising amino acid residues 1 to 20 of the amino acid sequence of SEQ ID NO: 69, a sequence comprising amino acid residues 21 to 129 of the amino acid sequence of SEQ ID NO: 69, and a sequence comprising amino acid residues 130 to 234 of the amino acid sequence of SEQ ID NO: 69 correspond to the signal sequence, the light chain variable region, and the light chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 69 is set forth in SEQ ID NO: 68 in the Sequence Listing. A sequence comprising nucleotides

1 to 60 of the nucleotide sequence of SEQ ID NO: 68, a sequence comprising nucleotides 61 to 387 of the nucleotide sequence of SEQ ID NO: 68, and a sequence comprising nucleotides 388 to 702 of the nucleotide sequence of SEQ ID NO: 68 encode the signal sequence, the light chain variable region sequence, and the light chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 68 and the amino acid sequence of SEQ ID NO: 69 are also described in Fig. 15.

[0175]

5)-3-3 h#1G5-L3 Type Light Chain

A humanized #1G5 light chain designed by substituting amino acid number 29 (lysine) with aspartic acid, amino acid number 31 (methionine) with leucine, amino acid number 32 (serine) with alanine, amino acid number 33 (methionine) with valine, amino acid number 35 (valine) with leucine, amino acid number 39 (valine) with alanine, amino acid number 41 (leucine) with isoleucine, amino acid number 60 (alanine) with proline, amino acid number 61 (glutamic acid) with glycine, amino acid number 83 (threonine) with serine, amino acid number 98 (valine) with leucine, amino acid number 103 (leucine) with valine, amino acid number 124 (leucine) with valine, and amino acid number 129 (alanine) with threonine of the #1G5 light chain shown in SEQ ID NO: 35 in the Sequence Listing was named "h#1G5-L3 type light chain".

[0176]

The amino acid sequence of the h#1G5-L3 type light chain is set forth in SEQ ID NO: 71 in the Sequence Listing. A sequence comprising amino acid residues 1 to 20 of the amino acid sequence of SEQ ID NO: 71, a sequence comprising amino acid residues 21 to 129 of the amino acid sequence of SEQ ID NO: 71, and a sequence comprising amino acid residues 130 to 234 of the amino acid sequence of SEQ ID NO: 71 correspond to the signal sequence, the light chain variable region, and the light chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 71 is set forth in SEQ ID NO: 70 in the Sequence Listing. A sequence comprising nucleotides 1 to 60 of the nucleotide sequence of SEQ ID NO: 70, a sequence comprising nucleotides 61 to 387 of the nucleotide sequence of SEQ ID NO: 70, and a sequence comprising nucleotides 388 to 702 of the nucleotide sequence of SEQ ID NO: 70 encode the signal sequence, the light chain variable region sequence, and the light chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 70 and the amino acid sequence of SEQ ID NO: 71 are also described in Fig. 16.

[0177]

5)-3-4 h#1G5-L4 Type Light Chain

A humanized h#1G5 light chain designed by substituting amino acid number 29 (lysine) with aspartic acid, amino acid number 39 (valine) with alanine, amino acid number 98 (valine) with leucine, and amino acid number 129 (alanine) with threonine

of the #1G5 light chain shown in SEQ ID NO: 35 in the Sequence Listing was named "h#1G5-L4 type light chain".

[0178]

The amino acid sequence of the h#1G5-L4 type light chain is set forth in SEQ ID NO: 73 in the Sequence Listing. A sequence comprising amino acid residues 1 to 20 of the amino acid sequence of SEQ ID NO: 73, a sequence comprising amino acid residues 21 to 129 of the amino acid sequence of SEQ ID NO: 73, and a sequence comprising amino acid residues 130 to 234 of the amino acid sequence of SEQ ID NO: 73 correspond to the signal sequence, the light chain variable region, and the light chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 73 is set forth in SEQ ID NO: 72 in the Sequence Listing. A sequence comprising nucleotides 1 to 60 of the nucleotide sequence of SEQ ID NO: 72, a sequence comprising nucleotides 61 to 387 of the nucleotide sequence of SEQ ID NO: 72, and a sequence comprising nucleotides 388 to 702 of the nucleotide sequence of SEQ ID NO: 72 encode the signal sequence, the light chain variable region sequence, and the light chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 72 and the amino acid sequence of SEQ ID NO: 73 are also described in Fig. 17.

[0179]

5)-3-5 h#1G5-L5 Type Light Chain

A humanized #1G5 light chain designed by substituting amino

acid number 29 (lysine) with aspartic acid, amino acid number 98 (valine) with leucine, and amino acid number 129 (alanine) with threonine of the #1G5 light chain shown in SEQ ID NO: 35 in the Sequence Listing was named “h#1G5-L5 type light chain”.

[0180]

The amino acid sequence of the h#1G5-L5 type light chain is set forth in SEQ ID NO: 75 in the Sequence Listing. A sequence comprising amino acid residues 1 to 20 of the amino acid sequence of SEQ ID NO: 75, a sequence comprising amino acid residues 21 to 129 of the amino acid sequence of SEQ ID NO: 75, and a sequence comprising amino acid residues 130 to 234 of the amino acid sequence of SEQ ID NO: 75 correspond to the signal sequence, the light chain variable region, and the light chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 75 is set forth in SEQ ID NO: 74 in the Sequence Listing. A sequence comprising nucleotides 1 to 60 of the nucleotide sequence of SEQ ID NO: 74, a sequence comprising nucleotides 61 to 387 of the nucleotide sequence of SEQ ID NO: 74, and a sequence comprising nucleotides 388 to 702 of the nucleotide sequence of SEQ ID NO: 74 encode the signal sequence, the light chain variable region sequence, and the light chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 74 and the amino acid sequence of SEQ ID NO: 75 are also described in Fig. 18.

[0181]

5)-4 Designing of Humanized Version of #4C13K

5)-4-1 Molecular Modeling of #4C13K Variable Region

The molecular modeling of the #4C13K variable regions was performed according to a method generally known as homology modeling (Methods in Enzymology, 203, 121-153, (1991)). The primary sequences (three-dimensional structures derived from the X-ray crystal structures are available) of the variable regions of human immunoglobulin registered in Protein Data Bank (Nuc. Acid Res. 35, D301-D303 (2007)) were compared with the #4C13K variable regions determined above. As a result, 1UYW was selected as a sequence having the highest sequence homology with the #4C13K light chain variable region among the antibodies which similarly have a deletion in the framework. Further, 1CIC was selected as a sequence having the highest sequence homology with the #4C13K heavy chain variable region. The three-dimensional structure of a framework region was prepared based on a "framework model" by combining the coordinates of 1UYW corresponding to the #4C13K light chain with the coordinates of 1CIC corresponding to the #4C13K heavy chain. Then, representative conformations for each CDR were incorporated into the framework model.

[0182]

Finally, in order to obtain a possible molecular model of the #4C13K variable region in terms of energy, an energy calculation was performed for excluding disadvantageous

interatomic contact. The above procedure was carried out using commercially available protein conformational analysis program Discovery Studio (Accelrys, Inc).

[0183]

5)-4-2 Designing of Amino Acid Sequence of Humanized #4C13K

A humanized #4C13K antibody was constructed according to a method generally known as CDR grafting (Proc. Natl. Acad. Sci. USA 86, 10029-10033 (1989)). An acceptor antibody was selected based on the amino acid homology within the framework region. The sequence of the framework region of #4C13K was compared with all human framework sequences in the Kabat Database (Nuc. Acid Res. 29, 205-206 (2001)) of antibody amino acid sequences. As a result, an HuMc3 antibody was selected as an acceptor based on a sequence homology of 73% in the framework region. The amino acid residues in the framework region of HuMc3 were aligned with the amino acid residues of #4C13K, and the positions where different amino acids were used were identified. The positions of these residues were analyzed using the three-dimensional model of #4C13K constructed above. Then, donor residues to be grafted onto the acceptor were selected according to the criteria provided by Queen et al. (Proc. Natl. Acad. Sci. USA 86, 10029-10033 (1989)). By transferring some selected donor residues to the acceptor antibody, humanized #4C13K sequences were constructed as described in the following Example. The light chain of the humanized #4C13K sequence was completely

common with the light chain of the humanized #1G5 sequence.

[0184]

5)-5 Humanization of #4C13K Heavy Chain

5)-5-1 h#4C13K-H1 Type Heavy Chain

A humanized #4C13K heavy chain designed by substituting amino acid number 24 (glutamine) with valine, amino acid number 26 (proline) with serine, amino acid number 30 (leucine) with valine, amino acid number 31 (valine) with lysine, amino acid number 39 (leucine) with valine, amino acid number 56 (methionine) with valine, amino acid number 57 (glutamine) with arginine, amino acid number 59 (arginine) with alanine, amino acid number 67 (isoleucine) with methionine, amino acid number 86 (lysine) with arginine, amino acid number 87 (alanine) with valine, amino acid number 89 (leucine) with isoleucine, amino acid number 91 (valine) with alanine, amino acid number 95 (serine) with threonine, amino acid number 101 (glutamine) with glutamic acid, amino acid number 103 (asparagine) with serine, amino acid number 106 (threonine) with arginine, amino acid number 108 (aspartic acid) with glutamic acid, amino acid number 110 (serine) with threonine, amino acid number 116 (threonine) with alanine, amino acid number 135 (threonine) with leucine, and amino acid number 136 (leucine) with valine of the #4C13K heavy chain shown in SEQ ID NO: 41 in the Sequence Listing was named “h#4C13K-H1 type heavy chain”.

[0185]

The amino acid sequence of the h#4C13K-H1 type heavy chain is set forth in SEQ ID NO: 77 in the Sequence Listing. A sequence comprising amino acid residues 1 to 19 of the amino acid sequence of SEQ ID NO: 77, a sequence comprising amino acid residues 20 to 140 of the amino acid sequence of SEQ ID NO: 77, and a sequence comprising amino acid residues 141 to 470 of the amino acid sequence of SEQ ID NO: 77 correspond to the signal sequence, the heavy chain variable region, and the heavy chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 77 is set forth in SEQ ID NO: 76 in the Sequence Listing. A sequence comprising nucleotides 1 to 57 of the nucleotide sequence of SEQ ID NO: 76, a sequence comprising nucleotides 58 to 420 of the nucleotide sequence of SEQ ID NO: 76, and a sequence comprising nucleotides 421 to 1410 of the nucleotide sequence of SEQ ID NO: 76 encode the signal sequence, the heavy chain variable region sequence, and the heavy chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 76 and the amino acid sequence of SEQ ID NO: 77 are also described in Fig. 19.

[0186]

5)-5-2 h#4C13K-H2 Type Heavy Chain

A humanized #4C13K heavy chain designed by substituting amino acid number 24 (glutamine) with valine, amino acid number 26 (proline) with serine, amino acid number 30 (leucine) with valine, amino acid number 31 (valine) with lysine, amino acid

number 39 (leucine) with valine, amino acid number 56 (methionine) with valine, amino acid number 57 (glutamine) with arginine, amino acid number 59 (arginine) with alanine, amino acid number 67 (isoleucine) with methionine, amino acid number 86 (lysine) with arginine, amino acid number 87 (alanine) with valine, amino acid number 95 (serine) with threonine, amino acid number 101 (glutamine) with glutamic acid, amino acid number 103 (asparagine) with serine, amino acid number 106 (threonine) with arginine, amino acid number 108 (aspartic acid) with glutamic acid, amino acid number 110 (serine) with threonine, amino acid number 135 (threonine) with leucine, and amino acid number 136 (leucine) with valine of the #4C13K heavy chain shown in SEQ ID NO: 41 in the Sequence Listing was named "h#4C13K-H2 type heavy chain".

[0187]

The amino acid sequence of the h#4C13K-H2 type heavy chain is set forth in SEQ ID NO: 79 in the Sequence Listing. A sequence comprising amino acid residues 1 to 19 of the amino acid sequence of SEQ ID NO: 79, a sequence comprising amino acid residues 20 to 140 of the amino acid sequence of SEQ ID NO: 79, and a sequence comprising amino acid residues 141 to 470 of the amino acid sequence of SEQ ID NO: 79 correspond to the signal sequence, the heavy chain variable region, and the heavy chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 79 is set forth in SEQ ID NO:

78 in the Sequence Listing. A sequence comprising nucleotides 1 to 57 of the nucleotide sequence of SEQ ID NO: 78, a sequence comprising nucleotides 58 to 420 of the nucleotide sequence of SEQ ID NO: 78, and a sequence comprising nucleotides 421 to 1410 of the nucleotide sequence of SEQ ID NO: 78 encode the signal sequence, the heavy chain variable region sequence, and the heavy chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 78 and the amino acid sequence of SEQ ID NO: 79 are also described in Fig. 20.

[0188]

5)-5-3 h#4C13K-H3 Type Heavy Chain

A humanized #4C13K heavy chain designed by substituting amino acid number 30 (leucine) with valine, amino acid number 31 (valine) with lysine, amino acid number 39 (leucine) with valine, amino acid number 67 (isoleucine) with methionine, amino acid number 86 (lysine) with arginine, amino acid number 95 (serine) with threonine, amino acid number 101 (glutamine) with glutamic acid, amino acid number 103 (asparagine) with serine, amino acid number 108 (aspartic acid) with glutamic acid, amino acid number 110 (serine) with threonine, amino acid number 135 (threonine) with leucine, and amino acid number 136 (leucine) with valine of the #4C13K heavy chain shown in SEQ ID NO: 41 in the Sequence Listing was named “h#4C13K-H3 type heavy chain”.

[0189]

The amino acid sequence of the h#4C13K-H3 type heavy chain

is set forth in SEQ ID NO: 81 in the Sequence Listing. A sequence comprising amino acid residues 1 to 19 of the amino acid sequence of SEQ ID NO: 81, a sequence comprising amino acid residues 20 to 140 of the amino acid sequence of SEQ ID NO: 81, and a sequence comprising amino acid residues 141 to 470 of the amino acid sequence of SEQ ID NO: 81 correspond to the signal sequence, the heavy chain variable region, and the heavy chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 81 is set forth in SEQ ID NO: 80 in the Sequence Listing. A sequence comprising nucleotides 1 to 57 of the nucleotide sequence of SEQ ID NO: 80, a sequence comprising nucleotides 58 to 420 of the nucleotide sequence of SEQ ID NO: 80, and a sequence comprising nucleotides 421 to 1410 of the nucleotide sequence of SEQ ID NO: 80 encode the signal sequence, the heavy chain variable region sequence, and the heavy chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 80 and the amino acid sequence of SEQ ID NO: 81 are also described in Fig. 21.

[0190]

5)-5-4 h#4C13K-H4 Type Heavy Chain

A humanized #4C13K heavy chain designed by substituting amino acid number 31 (valine) with lysine, amino acid number 39 (leucine) with valine, amino acid number 101 (glutamine) with glutamic acid, amino acid number 103 (asparagine) with serine, amino acid number 135 (threonine) with leucine, and amino acid

number 136 (leucine) with valine of the #4C13K heavy chain shown in SEQ ID NO: 41 in the Sequence Listing was named "h#4C13K-H4 type heavy chain".

[0191]

The amino acid sequence of the h#4C13K-H4 type heavy chain is set forth in SEQ ID NO: 83 in the Sequence Listing. A sequence comprising amino acid residues 1 to 19 of the amino acid sequence of SEQ ID NO: 83, a sequence comprising amino acid residues 20 to 140 of the amino acid sequence of SEQ ID NO: 83, and a sequence comprising amino acid residues 141 to 470 of the amino acid sequence of SEQ ID NO: 83 correspond to the signal sequence, the heavy chain variable region, and the heavy chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 83 is set forth in SEQ ID NO: 82 in the Sequence Listing. A sequence comprising nucleotides 1 to 57 of the nucleotide sequence of SEQ ID NO: 82, a sequence comprising nucleotides 58 to 420 of the nucleotide sequence of SEQ ID NO: 82, and a sequence comprising nucleotides 421 to 1410 of the nucleotide sequence of SEQ ID NO: 82 encode the signal sequence, the heavy chain variable region sequence, and the heavy chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 82 and the amino acid sequence of SEQ ID NO: 83 are also described in Fig. 22.

[0192]

5)-5-5 h#4C13K-H5 Type Heavy Chain

A humanized #4C13K heavy chain designed by substituting amino acid number 31 (valine) with lysine, amino acid number 39 (leucine) with valine, amino acid number 135 (threonine) with leucine, and amino acid number 136 (leucine) with valine of the #4C13K heavy chain shown in SEQ ID NO: 41 in the Sequence Listing was named "h#4C13K-H5 type heavy chain".

[0193]

The amino acid sequence of the h#4C13K-H5 type heavy chain is set forth in SEQ ID NO: 85 in the Sequence Listing. A sequence comprising amino acid residues 1 to 19 of the amino acid sequence of SEQ ID NO: 85, a sequence comprising amino acid residues 20 to 140 of the amino acid sequence of SEQ ID NO: 85, and a sequence comprising amino acid residues 141 to 470 of the amino acid sequence of SEQ ID NO: 85 correspond to the signal sequence, the heavy chain variable region, and the heavy chain constant region, respectively. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 85 is set forth in SEQ ID NO: 84 in the Sequence Listing. A sequence comprising nucleotides 1 to 57 of the nucleotide sequence of SEQ ID NO: 84, a sequence comprising nucleotides 58 to 420 of the nucleotide sequence of SEQ ID NO: 84, and a sequence comprising nucleotides 421 to 1410 of the nucleotide sequence of SEQ ID NO: 84 encode the signal sequence, the heavy chain variable region sequence, and the heavy chain constant region sequence, respectively. The nucleotide sequence of SEQ ID NO: 84 and the amino acid sequence of SEQ

ID NO: 85 are also described in Fig. 23.

[0194]

Example 6 Preparation of Humanized Antibodies #1G5 and #4C13K

6)-1 Construction of Humanized #1G5 Antibody Light Chain Expression Vector

6)-1-1 Construction of h#1G5-L2 Type Light Chain Expression Vector

A DNA fragment containing a sequence encoding the h#1G5-L2 type light chain variable region shown in nucleotide numbers 38 to 402 of the nucleotide sequence of the h#1G5-L2 type light chain shown in ID SEQ NO: 68 was synthesized (GENEART Inc., Artificial Gene Synthesis Service). By using the synthesized DNA fragment as a template and also using KOD-Plus-(TOYOBO, Co., Ltd.) and the following primer set, a DNA fragment comprising a DNA sequence encoding the h#1G5-L2 type light chain variable region was amplified, and a chimeric and humanized antibody light chain expression vector pCMA-LK was inserted at the site cleaved with the restriction enzyme BsiWI using an In-Fusion HD PCR cloning kit (Clontech, Inc.), and thus, a human chimeric h#1G5-L2 type light chain expression vector was constructed. The obtained expression vector was named “pCMA-LK/h#1G5-L2”.

Primer set for humanized antibody light chain

5’-ctgtggatctccggcgcgtacggc-3’ (CM-LKF)(SEQ ID NO: 112)

5’-ggaggggcggccaccgtacg-3’ (KCL-Inf-R)(SEQ ID NO: 113)

[0195]

6)-1-2 Construction of h#1G5-L3 Type Light Chain Expression Vector

A DNA fragment containing a sequence encoding the h#1G5-L3 type light chain variable region shown in nucleotide numbers 38 to 402 of the nucleotide sequence of the #1G5-L3 type light chain shown in ID SEQ NO: 70 was synthesized (GENEART Inc., Artificial Gene Synthesis Service). A h#1G5-L3 type light chain expression vector was constructed in the same manner as in Example 6)-1-1. The obtained expression vector was named "pCMA-LK/h#1G5-L3".

[0196]

6)-2 Construction of Humanized Antibody #1G5 Heavy Chain Expression Vector

6)-2-1 Construction of h#1G5-H2 Type Heavy Chain Expression Vector

A DNA fragment containing a sequence encoding the h#1G5-H2 type heavy chain variable region shown in nucleotide numbers 36 to 432 of the nucleotide sequence of the h#1G5-H2 type heavy chain shown in ID SEQ NO: 58 was synthesized (GENEART Inc., Artificial Gene Synthesis Service). By using the synthesized DNA fragment as a template and also using KOD-Plus- (TOYOBO, Co., Ltd.) and the following primer set, a DNA fragment comprising a DNA sequence encoding the h#1G5-H2 type heavy chain variable region was amplified, and a chimeric and humanized antibody IgG1 type heavy chain expression vector pCMA-G1 was inserted at the

site cleaved with the restriction enzyme BlpI using an In-Fusion HD PCR cloning kit (Clontech, Inc.), and thus, a h#1G5-H2 type heavy chain expression vector was constructed. The obtained expression vector was named "pCMA-G1/h#1G5-H2".

Primer set for humanized antibody heavy chain

5'-agctcccagatgggtgctgagc-3' (EG-Inf-F) (SEQ ID NO: 110)

5'-cttggtggaggctgagctcacggtcacgagggtgccctggcc-3' (H-R) (SEQ ID NO: 122)

[0197]

6)-2-2 Construction of h#1G5-H3 Type Heavy Chain Expression Vector

A DNA fragment containing a sequence encoding the h#1G5-H3 type heavy chain variable region as shown in the nucleotide numbers 36 to 432 of the nucleotide sequence of the #1G5-H3 type heavy chain shown in ID SEQ NO: 60 was synthesized (GENEART Inc., Artificial Gene Synthesis Service). A h#1G5-H3 type heavy chain expression vector was constructed in the same manner as in Example 6)-2-1. The obtained expression vector was named "pCMA-G1/h#1G5-H3".

[0198]

6)-3 Construction of Humanized Antibody #4C13K Heavy Chain Expression Vector

6)-3-1 Construction of h#4C13K-H2 Type Heavy Chain Expression Vector

A DNA fragment containing a sequence encoding the

h#4C13K-H2 type heavy chain variable region as shown in nucleotide numbers 36 to 420 of the nucleotide sequence of the h#4C13K-H2 type heavy chain shown in ID SEQ NO: 78 was synthesized (GENEART Inc., Artificial Gene Synthesis Service). A h#4C13K-H2 type heavy chain expression vector was constructed in the same manner as in Example 6)-2-1. The obtained expression vector was named "pCMA-G1/h#4C13K-H2".

[0199]

6)-3-2 Construction of h#4C13K-H3 Type Heavy Chain Expression Vector

A DNA fragment containing a sequence encoding the h#4C13K-H3 type heavy chain variable region shown in nucleotide numbers 36 to 420 of the nucleotide sequence of the #4C13K-H3 type heavy chain shown in ID SEQ NO: 80 was synthesized (GENEART Inc., Artificial Gene Synthesis Service). A #4C13K-H3 type heavy chain expression vector was constructed in the same manner as in Example 6)-2-1. The obtained expression vector was named "pCMA-G1/#4C13K-H3".

[0200]

Example 7 Preparation of Humanized Antibodies of #1G5 and #4C13K

[0201]

7)-1 Production of Humanized Antibodies of #1G5 and #4C13K

These humanized antibodies were produced in the same manner as in Example 2)-3-7-1.

[0202]

In the present specification, it is to be noted that, for example, an antibody having a h#1G5-H1 heavy chain and a h#1G5-L1 is referred to as "h#1G5-H1/L1" or "h#1G5-H1/L1 antibody", and an antibody having a h#4C13K-H1 heavy chain and h#1G5-L1 is referred to as "h#4C13K-H1/L1" or "h#4C13K-H1/L1 antibody".

[0203]

The h#1G5-H2/L2 was obtained by the combination of pCMA-G1/h#1G5-H2 and pCMA-LK/h#1G5-L2; the h#1G5-H3/L3 was obtained by the combination of pCMA-G1/h#1G5-H3 and pCMA-LK/h#1G5-L3; the h#4C13K-H2/L2 was obtained by the combination of pCMA-G1/h#4C13K-H2 and pCMA-LK/h#1G5-L2; the h#4C13K-H3/L3 was obtained by the combination of pCMA-G1/h#4C13K-H3 and pCMA-LK/h#1G5-L3.

7)-2 Construction of Humanized Antibodies of #1G5 and #4C13K

The culture supernatant obtained in the above 7)-1 was purified in the same manner as in Example 2)-3-7-2.

[0204]

Example 8

Measurement of In Vitro Activity of Humanized Antibodies of #1G5 and #4C13K

8)-1 Opsonophagocytic Killing Activity Against Pseudomonas aeruginosa ATCC 29260

In accordance with the method of 1)-3-2, the opsonophagocytic killing activities of h#1G5-H2/L2, h#1G5-H3/L3, h#4C13K-H2/L2, and h#4C13k-H3/L3 against ATCC

29260 were measured, and then compared to those of c#1G5 and c#4C13. The opsonophagocytic killing activity was expressed in terms of 50% minimum growth inhibitory concentration. The results are shown in Table 7.

[0205]

[Table 7]

Strain No.	Opsonophagocytic killing activity (ng/mL)					
	c#1G5	c#4C13	h#1G5-H2/L2	h#1G5-H3/L3	h#4C13K-H2/L2	h#4C13K-H3/L3
ATCC 29260	4.1	4.1	4.1	4.1	4.1	4.1

[0206]

The four humanized antibodies showed activities equivalent to those of c#1G5 and c#4C13.

[0207]

8)-2 Binding Specificity to Pseudomonas aeruginosa O11 Strains

The binding specificities of h#1G5-H2/L2, h#1G5-H3/L3, h#4C13K-H2/L2, and h#4C13K-H3/L3 to Pseudomonas aeruginosa carrying various O antigens were confirmed according to the method of 3)-2. The four humanized antibodies showed high specific activities only to the O11 strains as with c#1G5 and c#4C13 (Fig. 25).

[0208]

8)-3 Binding Activity against Pseudomonas aeruginosa O11 Clinical Isolates

The binding activities of h#1G5-H2/L2, h#1G5-H3/L3, h#4C13K-H2/L2, and h#4C13K-H3/L3 to 31 Pseudomonas aeruginosa

O11 clinical isolates held by Daiichi Sankyo Co., Ltd. were evaluated according to the method of 3)-3. The four humanized antibodies showed binding activities to all of the O11 clinical isolates as with c#1G5 and c#4C13 (Fig. 26).

[0209]

Example 9 Evaluation of In Vivo Activity of Humanized Antibodies of #1G5 and #4C13K

The in vivo activities of c#1G5, c#4C13, h#1G5-H2/L2, h#1G5-H3/L3, h#4C13K-H2/L2, and h#4C13K-H3/L3 against ATCC 29260 were evaluated according to the method of 4)-1. In comparison with the control group to which the antibody was not administered, all of c#1G5, c#4C13, and the four humanized antibodies showed therapeutic efficacies, and such efficacies were greater than the efficacy of Meiji 1656 (Fig. 27).

[0210]

Example 10 Competition assay

A competition assay between No.76 mIgG2a and cNO.76, c#1G5, c#4C13, Meiji 1640, Meiji 1656, or 1BO11 hIgG1 was performed with Pseudomonas aeruginosa ATCC 29260 using a binding activity in the whole cell ELISA as an index. According to the method of 3)-2, No.76 mIgG2a (final concentration of 0.01 μg/ml) and any one of cNO.76, c#1G5, c#4C13, Meiji 1640, Meiji 1656, and 1BO11 hIgG1 (final concentration of 0.01. 0.1, 1, 10, 100, 1,000 μg/ml) were respectively added as a primary antibody, and Goat anti-Mouse IgG2a HRP conjugated (Bethyl Laboratories, Inc.) was

used as a secondary antibody. The No.76 mIgG2a competed with cNO.76, c#1G4, and c#4C13, but did not compete with the Meiji 1640, Meiji 1656, and 1BO11 hIgG1. From this, it was revealed that the epitope of the cNO.76, c#1G5, and c#4C13 was different from the epitope of the Meiji 1640, Meiji 1656, and 1BO11 hIgG1 (Fig. 28).

Industrial Applicability

[0211]

Since the chimeric or humanized anti-LPS O11 antibody of the present invention has an opsonophagocytic killing action and/or complement dependent killing action, a pharmaceutical composition containing the anti-LPS O11 antibody can be a therapeutic agent or a prophylactic agent for pseudomonal infection.

Sequence Listing Free Text

[0212]

SEQ ID NO: 1: Nucleotide sequence of pJON mIgG2a

SEQ ID NO: 2: Nucleotide sequence of pJON mIgG2a-hIgG1

SEQ ID NO: 3: Nucleotide sequence of pJON mIgκ

SEQ ID NO: 4: Amino acid sequence of No.76 light chain variable region

SEQ ID NO: 5: Amino acid sequence of No.76 light chain CDR1

SEQ ID NO: 6: Amino acid sequence of No.76 light chain CDR2

SEQ ID NO: 7: Amino acid sequence of No.76 light chain CDR3

SEQ ID NO: 8: Amino acid sequence of No.76 heavy chain variable region

SEQ ID NO: 9: Amino acid sequence of No.76 heavy chain CDR1

SEQ ID NO: 10: Amino acid sequence of No.76 heavy chain CDR2

SEQ ID NO: 11: Amino acid sequence of No.76 heavy chain CDR3

SEQ ID NO: 12: Amino acid sequence of #1G5 light chain variable region

SEQ ID NO: 13: Amino acid sequence of #1G5 light chain CDR1

SEQ ID NO: 14: Amino acid sequence of #1G5 light chain CDR2

SEQ ID NO: 15: Amino acid sequence of #1G5 light chain CDR3

SEQ ID NO: 16: Amino acid sequence of #1G5 heavy chain variable region

SEQ ID NO: 17: Amino acid sequence of #1G5 heavy chain CDR1

SEQ ID NO: 18: Amino acid sequence of #1G5 heavy chain CDR2

SEQ ID NO: 19: Amino acid sequence of #1G5 heavy chain CDR3

SEQ ID NO: 20: Amino acid sequence of #4C12 light chain variable region

SEQ ID NO: 21: Amino acid sequence of #4C12 light chain CDR1

SEQ ID NO: 22: Amino acid sequence of #4C12 light chain CDR2

SEQ ID NO: 23: Amino acid sequence of #4C12 light chain CDR3

SEQ ID NO: 24: Amino acid sequence of #4C12 heavy chain variable region

SEQ ID NO: 25: Amino acid sequence of #4C12 heavy chain CDR1

SEQ ID NO: 26: Amino acid sequence of #4C12 heavy chain CDR2

SEQ ID NO: 27: Amino acid sequence of #4C12 heavy chain CDR3

SEQ ID NO: 28: DNA fragment comprising a DNA sequence encoding the amino acids of the human light chain secretion signal and the human light chain constant region

SEQ ID NO: 29: DNA fragment comprising a DNA sequence encoding the amino acids of the human heavy chain signal sequence and the human IgG1 constant region

SEQ ID NO: 30: Nucleotide sequence of human chimeric No.76 light chain

SEQ ID NO: 31: Amino acid sequence of human chimeric No.76 light chain

SEQ ID NO: 32: Nucleotide sequence of human chimeric No.76 heavy chain

SEQ ID NO: 33: Amino acid sequence of human chimeric No.76 heavy chain

SEQ ID NO: 34: Nucleotide sequence of DNA fragment comprising DNA sequence encoding human chimeric #1G5 light chain.

SEQ ID NO: 35: Amino acid sequence of human chimeric #1G5 light chain

SEQ ID NO: 36: Nucleotide sequence of human chimeric #1G5 heavy chain

SEQ ID NO: 37: Amino acid sequence of human chimeric #1G5 heavy chain

SEQ ID NO: 38: Nucleotide sequence of DNA fragment comprising DNA sequence encoding human chimeric #4C13 light chain.

SEQ ID NO: 39: Amino acid sequence of human chimeric #4C13 light

chain

SEQ ID NO: 40: Nucleotide sequence of human chimeric #4C13 heavy chain

SEQ ID NO: 41: Amino acid sequence of human chimeric #4C13 heavy chain

SEQ ID NO: 42: Nucleotide sequence of Meiji 1640 light chain

SEQ ID NO: 43: Amino acid sequence of Meiji 1640 light chain

SEQ ID NO: 44: Nucleotide sequence of Meiji 1640 heavy chain

SEQ ID NO: 45: Amino acid sequence of Meiji 1640 heavy chain

SEQ ID NO: 46: Nucleotide sequence of Meiji 1656 light chain

SEQ ID NO: 47: Amino acid sequence of Meiji 1656 light chain

SEQ ID NO: 48: Nucleotide sequence of Meiji 1656 heavy chain

SEQ ID NO: 49: Amino acid sequence of Meiji 1656 heavy chain

SEQ ID NO: 50: Nucleotide sequence of DNA fragment encoding mouse IgG2b type chimeric 1BO11 light chain

SEQ ID NO: 51: Nucleotide sequence of human IgG1 type 1BO11 light chain

SEQ ID NO: 52: Amino acid sequence of human IgG1 type 1BO11 light chain

SEQ ID NO: 53: Nucleotide sequence of DNA fragment encoding mouse IgG2b type chimeric 1BO11 heavy chain

SEQ ID NO: 54: Nucleotide sequence of human IgG1 type 1BO11 heavy chain

SEQ ID NO: 55: Amino acid sequence of human IgG1 type 1BO11 heavy chain

SEQ ID NO: 56: Nucleotide sequence of h#1G5-H1

SEQ ID NO: 57: Amino acid sequence of h#1G5-H1

SEQ ID NO: 58: Nucleotide sequence of h#1G5-H2

SEQ ID NO: 59: Amino acid sequence of h#1G5-H2

SEQ ID NO: 60: Nucleotide sequence of h#1G5-H3

SEQ ID NO: 61: Amino acid sequence of h#1G5-H3

SEQ ID NO: 62: Nucleotide sequence of h#1G5-H4

SEQ ID NO: 63: Amino acid sequence of h#1G5-H4

SEQ ID NO: 64: Nucleotide sequence of h#1G5-H5

SEQ ID NO: 65: Amino acid sequence of h#1G5-H5

SEQ ID NO: 66: Nucleotide sequence of h#1G5-L1

SEQ ID NO: 67: Amino acid sequence of h#1G5-L1

SEQ ID NO: 68: Nucleotide sequence of h#1G5-L2

SEQ ID NO: 69: Amino acid sequence of h#1G5-L2

SEQ ID NO: 70: Nucleotide sequence of h#1G5-L3

SEQ ID NO: 71: Amino acid sequence of h#1G5-L3

SEQ ID NO: 72: Nucleotide sequence of h#1G5-L4

SEQ ID NO: 73: Amino acid sequence of h#1G5-L4

SEQ ID NO: 74: Nucleotide sequence of h#1G5-L5

SEQ ID NO: 75: Amino acid sequence of h#1G5-L5

SEQ ID NO: 76: Nucleotide sequence of h#4C13K-H1

SEQ ID NO: 77: Amino acid sequence of h#4C13K-H1

SEQ ID NO: 78: Nucleotide sequence of h#4C13K-H2

SEQ ID NO: 79: Amino acid sequence of h#4C13K-H2

SEQ ID NO: 80: Nucleotide sequence of h#4C13K-H3

SEQ ID NO: 81: Amino acid sequence of h#4C13K-H3

SEQ ID NO: 82: Nucleotide sequence of h#4C13K-H4

SEQ ID NO: 83: Amino acid sequence of h#4C13K-H4

SEQ ID NO: 84: Nucleotide sequence of h#4C13K-H5

SEQ ID NO: 85: Amino acid sequence of h#4C13K-H5

SEQ ID NO: 86: Amino acid sequence of #4C13 heavy chain CDR1

SEQ ID NO: 87: Amino acid sequence of #4C13 heavy chain CDR2

SEQ ID NO: 88: Amino acid sequence of #4C13 heavy chain CDR3

SEQ ID NO: 89: AP3DC-S

SEQ ID NO: 90: mIgγRT1 1111-AS

SEQ ID NO: 91: mIgκ 1st 589-AS

SEQ ID NO: 92: MCS-AP3-S

SEQ ID NO: 93: mIgγ 3rd 656T-AS

SEQ ID NO: 94: mIgκ 3rd 525-AS

SEQ ID NO: 95: mIgG joint PCR-S

SEQ ID NO: 96: polyG-AS

SEQ ID NO: 97: mIgκ joint PCR-S

SEQ ID NO: 98: miniCMV f1-S

SEQ ID NO: 99: miniCMV f1-AS

SEQ ID NO: 100: mIgG sequence

SEQ ID NO: 101: mIgκ sequence

SEQ ID NO: 102: 3.3-F1

SEQ ID NO: 103: 3.3-R1

SEQ ID NO: 104: 76L-F

SEQ ID NO: 105: 76L-R

SEQ ID NO: 106: 76H-F

SEQ ID NO: 107: 76H-R

SEQ ID NO: 108: CM-inf-F

SEQ ID NO: 109: CM-inf-R

SEQ ID NO: 110: EG-inf-F

SEQ ID NO: 111: EG1-inf-R

SEQ ID NO: 112: CM-LKF

SEQ ID NO: 113: KCL-inf-R

SEQ ID NO: 114: h1BO-LF

SEQ ID NO: 115: h1BO-LR

SEQ ID NO: 116: h1BO-HF

SEQ ID NO: 117: h1BO-HR

SEQ ID NO: 118: Nucleotide sequence comprising a DNA fragment comprising a sequence encoding No.76 mIgG2a light chain

SEQ ID NO: 119: Amino acid sequence of No.76 mIgG2a light chain

SEQ ID NO: 120: Nucleotide sequence comprising a DNA fragment comprising a sequence encoding No.76 mIgG2a heavy chain

SEQ ID NO: 121: Amino acid sequence of No.76 mIgG2a heavy chain

SEQ ID NO: 122: H-R

SEQ ID NO: 123: Amino acid sequence of #4C13 light chain CDR1

SEQ ID NO: 124: Amino acid sequence of #4C13 light chain CDR2

SEQ ID NO: 125: Amino acid sequence of #4C13 light chain CDR3

CLAIMS

1. An antibody , or an antigen-binding fragment thereof, that recognizes LPS of Pseudomonas aeruginosa, binds to O11 antigen and does not bind to O1, O2, O3, O4, O5, O6, O7, O8, O9, O10, O13, O14, O15, O16, O17, O18, O19, O20 antigens, and O antigen-deficient strain, and has a coverage ratio of 85% or more for the O11 clinical isolates.

2. The antibody or an antigen-binding fragment thereof according to claim 1, wherein the coverage ratio for the O11 clinical isolates is 95% or more.

3. An antibody , or an antigen-binding fragment thereof, that recognizes LPS of Pseudomonas aeruginosa, binds to the O11 antigen, and does not bind to O1, O2, O3, O4, O5, O6, O7, O8, O9, O10, O13, O14, O15, O16, O17, O18, O19, O20 antigens and O antigen-deficient strain, and exhibits 50% minimum growth inhibitory concentration of opsonophagocytic killing activity against Pseudomonas aeruginosa which is identified by ATCC 29260 is 12 ng/mL or less.

4. The antibody or an antigen-binding fragment thereof according to claim 3, wherein the 50% minimum growth inhibitory concentration of opsonophagocytic killing activity against Pseudomonas aeruginosa identified by ATCC 29260 is 4.1 ng/mL

or less.

5. An antibody, or an antigen-binding fragment thereof, characterized in that it cross-competes with at least one of the antibodies selected from the group consisting of an antibody containing a heavy chain variable region sequence comprising amino acid residues 20 to 140 of the amino acid sequence shown in SEQ ID NO: 8 and a light chain variable region sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 4; an antibody containing a heavy chain variable region sequence comprising amino acid residues 20 to 144 of the amino acid sequence shown in SEQ ID NO: 16 and a light chain variable region sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 12; and an antibody containing a heavy chain variable region sequence comprising amino acid residues 20 to 140 of the amino acid sequence shown in SEQ ID NO: 24 and a light chain variable region sequence comprising 21 to 129 amino acid residues of the amino acid sequence shown in SEQ ID NO: 12.

6. The antibody or an antigen-binding fragment thereof according to claim 5, characterized in that it binds to an epitope to which at least one antibody binds, wherein the antibody is selected from the group consisting of an antibody containing a heavy chain variable region sequence comprising amino acid

residues 20 to 140 of the amino acid sequence shown in SEQ ID NO: 8 and a light chain variable region sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 4; an antibody containing a heavy chain variable region sequence comprising amino acid residues 20 to 144 of the amino acid sequence shown in SEQ ID NO: 16 and a light chain variable region sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 12; and an antibody containing a heavy chain variable region sequence comprising amino acid residues 20 to 140 of the amino acid sequence shown in SEQ ID NO: 24 and a light chain variable region sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 12.

7. The antibody or an antigen-binding fragment thereof according to any one of claims 1 to 6, characterized in that:

the heavy chain sequence contains a variable region having CDRH1, CDRH2, and CDRH3, and the CDRH1 comprises the amino acid sequence shown in SEQ ID NO: 9, the CDRH2 comprises the amino acid sequence shown in SEQ ID NO: 10, and the CDRH3 comprises the amino acid sequence shown in SEQ ID NO: 11, and

the light chain sequence contains a variable region having CDRL1, CDRL2, and CDRL3, and the CDRL1 comprises the amino acid sequence shown in SEQ ID NO: 5, the CDRL2 comprises the amino acid sequence shown in SEQ ID NO: 6, and the CDRL3 comprises

the amino acid sequence shown in SEQ ID NO: 7.

8. The antibody or an antigen-binding fragment thereof according to claim 7, characterized by containing a heavy chain variable region sequence comprising amino acid residues 20 to 140 of the amino acid sequence shown in SEQ ID NO: 8 and a light chain variable region sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 4.

9. The antibody or an antigen-binding fragment thereof according to any one of claims 1 to 6, characterized in that:

the heavy chain sequence contains a variable region having CDRH1, CDRH2, and CDRH3, and the CDRH1 comprises the amino acid sequence shown in SEQ ID NO: 17, the CDRH2 comprises the amino acid sequence shown in SEQ ID NO: 18, and the CDRH3 comprises the amino acid sequence shown in SEQ ID NO: 19, and

the light chain sequence contains a variable region having CDRL1, CDRL2, and CDRL3, and the CDRL1 comprises the amino acid sequence shown in SEQ ID NO: 13, the CDRL2 comprises the amino acid sequence shown in SEQ ID NO: 14, and the CDRL3 comprises the amino acid sequence shown in SEQ ID NO: 15.

10. The antibody or an antigen-binding fragment thereof according to claim 9, characterized by containing a heavy chain variable region sequence comprising amino acid residues 20 to

144 of the amino acid sequence shown in SEQ ID NO: 16 and a light chain variable region sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 12.

11. The antibody or an antigen-binding fragment thereof according to any one of claims 1 to 6, characterized in that:

the heavy chain sequence contains a variable region having CDRH1, CDRH2, and CDRH3, and the CDRH1 comprises the amino acid sequence shown in SEQ ID NO: 25, the CDRH2 comprises the amino acid sequence shown in SEQ ID NO: 26, and the CDRH3 comprises the amino acid sequence shown in SEQ ID NO: 27, and

the light chain sequence contains a variable region having CDRL1, CDRL2, and CDRL3, and the CDRL1 comprises the amino acid sequence shown in SEQ ID NO: 13, the CDRL2 comprises the amino acid sequence shown in SEQ ID NO: 14, and the CDRL3 comprises the amino acid sequence shown in SEQ ID NO: 15.

12. The antibody or an antigen-binding fragment thereof according to claim 11, characterized by containing a heavy chain variable region sequence comprising amino acid residues 20 to 140 of the amino acid sequence shown in SEQ ID NO: 24 and a light chain variable region sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 12.

13. An antigen-binding fragment of the antibody according to

any one of claims 1 to 12, characterized by being selected from the group consisting of Fab, F(ab')2, Fab' and Fv.

14. The antibody according to any one of claims 1 to 12, characterized by being scFv.

15. The antibody or an antigen-binding fragment thereof according to claims 1 to 12, characterized by being a chimeric antibody.

16. The antibody or an antigen-binding fragment thereof according to claims 1 to 12, characterized by being a humanized antibody.

17. The antibody according to any one of claims 1 to 16, wherein the heavy chain contains a constant region of a human immunoglobulin G1 heavy chain, and the light chain contains a constant region of a human immunoglobulin κ light chain.

18. An antibody, or an antigen-binding fragment thereof, which has a binding activity to the LPS O11 antigen of Pseudomonas aeruginosa characterized in that the antibody contains:

(a) a heavy chain variable region sequence selected from the group consisting of the following amino acid sequences:

a1) an amino acid sequence comprising amino acid residues

20 to 144 of the amino acid sequence shown in SEQ ID NO: 57;

a2) an amino acid sequence comprising amino acid residues 20 to 144 of the amino acid sequence shown in SEQ ID NO: 59;

a3) an amino acid sequence comprising amino acid residues 20 to 144 of the amino acid sequence shown in SEQ ID NO: 61;

a4) an amino acid sequence comprising amino acid residues 20 to 144 of the amino acid sequence shown in SEQ ID NO: 63;

a5) an amino acid sequence comprising amino acid residues 20 to 144 of the amino acid sequence shown in SEQ ID NO: 65;

a6) an amino acid sequence having a homology of at least 95% with any one of the amino acid sequences selected from a1) to a5);

a7) an amino acid sequence having a homology of at least 99% with any one of the amino acid sequences selected from a1) to a5); and

a8) an amino acid sequence including substitution, deletion, or addition of one to several amino acid residues in any one of the amino acid sequences selected from a1) to a5); and

(b) a light chain variable region sequence selected from the group consisting of the following amino acid sequences:

b1) an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 67;

b2) an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 69;

b3) an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 71;

b4) an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 73;

b5) an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 75;

b6) an amino acid sequence having a homology of at least 95% with any one of the amino acid sequences selected from b1) to b5);

b7) an amino acid sequence having a homology of at least 99% with any one of the amino acid sequences selected from b1) to b5); and

b8) an amino acid sequence including substitution, deletion, or addition of one to several amino acid residues in any one of the amino acid sequences selected from b1) to b5).

19. The antibody or an antigen-binding fragment thereof according to claim 18, characterized by containing a heavy chain variable region sequence comprising amino acid residues 20 to 144 of the amino acid sequence shown in SEQ ID NO: 59 and a light chain variable region sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 69.

20. The antibody or an antigen-binding fragment thereof according to claim 18, characterized by containing a heavy chain

variable region sequence comprising amino acid residues 20 to 144 of the amino acid sequence shown in SEQ ID NO: 61 and a light chain variable region sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 71.

21. An antibody or an antigen-binding fragment thereof having a binding activity to the LPS O11 antigen of Pseudomonas aeruginosa, characterized by containing:

(a) a heavy chain variable region sequence selected from the group consisting of the following amino acid sequences:

a1) an amino acid sequence comprising amino acid residues 20 to 140 of the amino acid sequence shown in SEQ ID NO: 77;

a2) an amino acid sequence comprising amino acid residues 20 to 140 of the amino acid sequence shown in SEQ ID NO: 79;

a3) an amino acid sequence comprising amino acid residues 20 to 140 of the amino acid sequence shown in SEQ ID NO: 81;

a4) an amino acid sequence comprising amino acid residues 20 to 140 of the amino acid sequence shown in SEQ ID NO: 83;

a5) an amino acid sequence comprising amino acid residues 20 to 140 of the amino acid sequence shown in SEQ ID NO: 85;

a6) an amino acid sequence having a homology of at least 95% with any one of the amino acid sequences selected from a1) to a5);

a7) an amino acid sequence having a homology of at least 99% with any one of the amino acid sequences selected from a1)

to a5); and

a8) an amino acid sequence including substitution, deletion, or addition of one to several amino acid residues in any one of the amino acid sequences selected from a1) to a5); and

(b) a light chain variable region sequence selected from the group consisting of the following amino acid sequences:

b1) an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 67;

b2) an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 69;

b3) an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 71;

b4) an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 73;

b5) an amino acid sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 75;

b6) an amino acid sequence having a homology of at least 95% with any one of the amino acid sequences selected from b1) to b5);

b7) an amino acid sequence having a homology of at least 99% with any one of the amino acid sequences selected from b1) to b5); and

b8) an amino acid sequence including substitution, deletion, or addition of one to several amino acid residues in

any one of the amino acid sequences selected from b1) to b5).

22. The antibody or an antigen-binding fragment thereof according to claim 21, characterized by containing a heavy chain variable region sequence comprising amino acid residues 20 to 140 of the amino acid sequence shown in SEQ ID NO: 79 and a light chain variable region sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 69.

23. The antibody or an antigen-binding fragment thereof according to claim 21, characterized by containing a heavy chain variable region sequence comprising amino acid residues 20 to 140 of the amino acid sequence shown in SEQ ID NO: 81 and a light chain variable region sequence comprising amino acid residues 21 to 129 of the amino acid sequence shown in SEQ ID NO: 71.

24. The antibody according to any one of claims 18 to 23, comprising a heavy chain including deletion of one to several amino acids from the carboxyl terminus.

25. A pharmaceutical composition comprising at least one of the antibody or the antigen-binding fragment thereof according to claims 1 to 24.

26. The pharmaceutical composition according to claim 25,

characterized by being a therapeutic and/or prophylactic agent for pseudomonas infection.

27. A pharmaceutical composition for the treatment and/or prophylaxis of pseudomonal infection, characterized by comprising at least one of the antibody or the antigen-binding fragment thereof according to any one of claims 1 to 24 and at least one selected from the group consisting of penicillin, cephem, carbapenem, monobactam, quinolone, aminoglycoside, polymyxin, rifampicin, and macrolide antimicrobial agents.

28. The pharmaceutical composition according to claim 26 or 27, characterized in that the pseudomonal infection is a systemic infectious disease caused by a multidrug-resistant Pseudomonas aeruginosa infection.

29. The pharmaceutical composition according to claim 26 or 27, characterized in that the pseudomonal infection is:

bloodstream infection, sepsis, meningitis, endocarditis, otitis media, sinusitis, pneumonia, lung abscess, empyema, chronic respiratory tract infection, peritonitis, post-operative infection, cholecystitis, cholangitis, eyelid tumor, dacryocystitis, conjunctivitis, corneal ulcer, corneal abscess, panophthalmitis, orbital infection, urinary tract infection, catheter infection, perianal abscess, secondary

infection of burn wounds, decubitus infection, cystic fibrosis disease, lymphangitis, lymphadenitis, osteomyelitis, arthritis, tonsillitis, liver abscess, skin and soft tissue infections, intrauterine infection, uterine adnexitis, parametritis, perimandibular phlegmon, or jaw inflammation.

30. The pharmaceutical composition according to claim 26 or 27, characterized in that the pseudomonal infection is pneumonia.

31. A method for the treatment and/or prophylaxis of pseudomonal infection, characterized by administering at least one of the antibody, or the antigen-binding fragment thereof, according to claims 1 to 24, or the pharmaceutical composition according to claim 26 or 27.

32. A method for the treatment and/or prophylaxis of pseudomonal infection, characterized by simultaneously or sequentially administering at least one of the antibodies, or antigen-binding fragment thereof, according to any one of claims 1 to 24, or the pharmaceutical composition according to claim 26, and at least one selected from the group consisting of penicillin, cephem, carbapenem, monobactam, quinolone, aminoglycoside, polymyxin, rifampicin, and macrolide antimicrobial agents.

33. The method for the treatment and/or prophylaxis of pseudomonal infection according to claim 31 or 32, characterized in that the pseudomonal infection is a systemic infectious disease caused by a multidrug-resistant Pseudomonas aeruginosa infection.

34. The method for the treatment and/or prophylaxis of pseudomonal infection according to claim 33, characterized in that the pseudomonal infection is bloodstream infection, sepsis, meningitis, endocarditis, otitis media, sinusitis, pneumonia, lung abscess, empyema, chronic respiratory tract infection, peritonitis, post-operative infection, cholecystitis, cholangitis, eyelid tumor, dacryocystitis, conjunctivitis, corneal ulcer, corneal abscess, panophthalmitis, orbital infection, urinary tract infection, catheter infection, perianal abscess, secondary infection of burn wounds, decubitus infection, cystic fibrosis disease, lymphangitis, lymphadenitis, osteomyelitis, arthritis, tonsillitis, liver abscess, skin and soft tissue infections, intrauterine infection, uterine adnexitis, parametritis, perimandibular phlegmon, or jaw inflammation.

35. The method for the treatment and/or prophylaxis of pseudomonal infection according to claim 33, characterized in that the pseudomonal infection is pneumonia.

36. A diagnostic agent for pseudomonal infection, characterized by containing at least one of the antibodies or the antigen-binding fragments thereof according to claims 1 to 24.

37. A detection kit for Pseudomonas aeruginosa, characterized by containing at least one of the antibodies or the antigen-binding fragments thereof according to claims 1 to 24.

38. A polynucleotide encoding any one of the antibodies according to claims 1 to 24.

39. The polynucleotide according to claim 38, characterized by containing:

(a) a polynucleotide selected from the group consisting of the following nucleotide sequences:

a1) a nucleotide sequence comprising nucleotides 58 to 432 of the nucleotide sequence shown in SEQ ID NO: 56;

a2) a nucleotide sequence comprising nucleotides 58 to 432 of the nucleotide sequence shown in SEQ ID NO: 58;

a3) a nucleotide sequence comprising nucleotides 58 to 432 of the nucleotide sequence shown in SEQ ID NO: 60;

a4) a nucleotide sequence comprising nucleotides 58 to 432 of the nucleotide sequence shown in SEQ ID NO: 62;

a5) a nucleotide sequence comprising nucleotides 58 to 432 of the nucleotide sequence shown in SEQ ID NO: 64;

a6) a nucleotide sequence having a homology of at least 95% with any one of the nucleotide sequences selected from a1) to a5);

a7) a nucleotide sequence having a homology of at least 99% with any one of the nucleotide sequences selected from a1) to a5);

a8) a nucleotide sequence of a polynucleotide which hybridizes to a polynucleotide comprising a nucleotide sequence complementary to any one of the nucleotide sequences selected from a1) to a5) under stringent conditions; and

a9) a nucleotide sequence including substitution, deletion, or addition of one to several amino acid residues in any one of the nucleotide sequences selected from a1) to a5); and

(b) a polynucleotide selected from the group consisting of the following nucleotide sequences:

b1) a nucleotide sequence comprising nucleotides 61 to 387 of the nucleotide sequence shown in SEQ ID NO: 66;

b2) a nucleotide sequence comprising nucleotides 61 to 387 of the nucleotide sequence shown in SEQ ID NO: 68;

b3) a nucleotide sequence comprising nucleotides 61 to 387 of the nucleotide sequence shown in SEQ ID NO: 70;

b4) a nucleotide sequence comprising nucleotides 61 to

387 of the nucleotide sequence shown in SEQ ID NO: 72;

b5) a nucleotide sequence comprising nucleotides 61 to 387 of the nucleotide sequence shown in SEQ ID NO: 74;

b6) a nucleotide sequence having a homology of at least 95% with any one of the nucleotide sequences selected from b1) to b5);

b7) a nucleotide sequence having a homology of at least 99% with any one of the nucleotide sequences selected from b1) to b5);

b8) a nucleotide sequence of a polynucleotide which hybridizes to a polynucleotide comprising a nucleotide sequence complementary to any one of the nucleotide sequences selected from b1) to b5) under stringent conditions; and

b9) a nucleotide sequence including substitution, deletion, or addition of one to several nucleotides in any one of the nucleotide sequences selected from b1) to b5).

40. The polynucleotide according to claim 39, characterized by containing a polynucleotide comprising nucleotides 58 to 432 of the nucleotide sequence shown in SEQ ID NO: 58 and a polynucleotide comprising nucleotides 61 to 387 of the nucleotide sequence shown in SEQ ID NO: 68.

41. The polynucleotide according to claim 39, characterized by containing a polynucleotide comprising nucleotides 58 to 432

of the nucleotide sequence shown in SEQ ID NO: 60 and a polynucleotide comprising nucleotides 61 to 387 of the nucleotide sequence shown in SEQ ID NO: 70.

42. The polynucleotide according to claim 38, characterized by containing:

(a) a polynucleotide selected from the group consisting of the following nucleotide sequences:

a1) a nucleotide sequence comprising nucleotides 58 to 420 of the nucleotide sequence shown in SEQ ID NO: 76;

a2) a nucleotide sequence comprising nucleotides 58 to 420 of the nucleotide sequence shown in SEQ ID NO: 78;

a3) a nucleotide sequence comprising nucleotides 58 to 420 of the nucleotide sequence shown in SEQ ID NO: 80;

a4) a nucleotide sequence comprising nucleotides 58 to 420 of the nucleotide sequence shown in SEQ ID NO: 82;

a5) a nucleotide sequence comprising nucleotides 58 to 420 of the nucleotide sequence shown in SEQ ID NO: 84;

a6) a nucleotide sequence having a homology of at least 95% with any one of the nucleotide sequences selected from a1) to a5);

a7) a nucleotide sequence having a homology of at least 99% with any one of the nucleotide sequences selected from a1) to a5);

a8) a nucleotide sequence of a polynucleotide which

hybridizes to a polynucleotide comprising a nucleotide sequence complementary to any one of the nucleotide sequences selected from a1) to a5) under stringent conditions; and

a9) a nucleotide sequence including substitution, deletion, or addition of one to several amino acid residues in any one of the nucleotide sequences selected from a1) to a5); and

(b) a polynucleotide selected from the group consisting of the following nucleotide sequences:

b1) a nucleotide sequence comprising nucleotides 61 to 387 of the nucleotide sequence shown in SEQ ID NO: 66;

b2) a nucleotide sequence comprising nucleotides 61 to 387 of the nucleotide sequence shown in SEQ ID NO: 68;

b3) a nucleotide sequence comprising nucleotides 61 to 387 of the nucleotide sequence shown in SEQ ID NO: 70;

b4) a nucleotide sequence comprising nucleotides 61 to 387 of the nucleotide sequence shown in SEQ ID NO: 72;

b5) a nucleotide sequence comprising nucleotides 61 to 387 of the nucleotide sequence shown in SEQ ID NO: 74;

b6) a nucleotide sequence having a homology of at least 95% with any one of the nucleotide sequences selected from b1) to b5);

b7) a nucleotide sequence having a homology of at least 99% with any one of the nucleotide sequences selected from b1) to b5);

b8) a nucleotide sequence of a polynucleotide which hybridizes to a polynucleotide comprising a nucleotide sequence complementary to any one of the nucleotide sequences selected from b1) to b5) under stringent conditions; and

b9) a nucleotide sequence including substitution, deletion, or addition of one to several nucleotides in any one of the nucleotide sequences selected from b1) to b5).

43. The polynucleotide according to claim 42, characterized by containing a polynucleotide comprising nucleotides 58 to 420 of the nucleotide sequence shown in SEQ ID NO: 78 and a polynucleotide comprising nucleotides 61 to 387 of the nucleotide sequence shown in SEQ ID NO: 68.

44. The polynucleotide according to claim 42, characterized by containing a polynucleotide comprising nucleotides 58 to 420 of the nucleotide sequence shown in SEQ ID NO: 80 and a polynucleotide comprising nucleotides 61 to 387 of the nucleotide sequence shown in SEQ ID NO: 70.

45. A vector containing any one of the polynucleotides according to claims 38 to 44.

46. A transformed host cell containing any one of the polynucleotides according to claims 38 to 44.

47. A transformed host cell containing the vector according to claim 45.

48. A method for producing the antibody according to any one of claims 1 to 24, containing the steps of culturing the host cell according to claim 46 or 47 and purifying the antibody from the resulting cultured product.