

Description

Title of Invention: ANTI-HUMAN TLR7 ANTIBODY

Technical Field

[0001]

The present invention relates to anti-human Toll-like receptor (TLR) 7 antibodies for treating disorders and diseases.

Background Art

[0002]

In humans, 10 types of TLRs, which are pattern recognition molecules, are known to be present and form one family. Among them, TLR7 (variant 1) is a single transmembrane protein consisting of 1049 amino acids, and the possibility of it playing an important role in the pathogenesis of various inflammatory diseases and autoimmune diseases, by detecting single-stranded RNAs and playing a role in the biological defense reaction through production of type I interferons and cytokines and the like, has been suggested (Non Patent Literature 1). Further, TLR7 (variant 2), which is a single transmembrane protein consisting of 1049 amino acids, is also known to have the same function as TLR7 (variant 1) above (Non Patent Literature 2). In particular, there are many reports suggesting the involvement of TLR7 in

diseases such as psoriasis and systemic lupus erythematosus, and it is expected that it is possible to treat such diseases by inhibiting the functions of TLR7 (Non Patent Literature 3).

[0003]

While TLR7 deficiency acts to reduce symptoms in disease models, drugs capable of specifically inhibiting TLR7 are considered promising in treating inflammatory diseases or the like, based on reports suggesting that TLR9 deficiency is involved in disease progression (Non Patent Literature 4).

[0004]

Until now, research and development of nucleic acids, low-molecular weight compounds, and the like for inhibiting human TLR7 have been attempted, but it is difficult to inhibit human TLR7 specifically using nucleic acids or low-molecular weight compounds. Further, a mouse anti-mouse TLR7 antibody is known as a TLR7 antibody (Patent Literature 1), but research and development of new human TLR7-specific inhibitors have been required.

Citation List

Patent Literature

[0005]

Patent Literature 1: International publication No. WO 2014/174704

Non Patent Literature

[0006]

Non Patent Literature 1: Takeuchi O, Akira S. Cell. 2010 Mar 19; 140 (6): 805-2

Non Patent Literature 2: Chuang TH, Ulevitch RJ, Eur Cytokine Netw. 2000 Sep; 11 (3): 372-8

Non Patent Literature 3: Lyn-Cook BD, Xie C, Oates J, Treadwell E, Word B, Hammons G, Wiley K. Mol Immunol. 2014 Sep; 61 (1): 38-43.

Non Patent Literature 4: Christensen SR, Shupe J, Nickerson K, Kashgarian M, Flavell RA, Shlomchik MJ Immunity. 2006 Sep; 25 (3): 417-28.

Summary of Invention

Technical Problem

[0007]

It is an object of the present invention to provide an agent for treating and/or preventing immune inflammation-related diseases, allergic diseases, infections, and cancers.

Solution to Problem

[0008]

In order to solve the aforementioned problems, the inventors have searched for a substance having the effect of treating and/or preventing immune inflammation-related diseases, allergic diseases, infections, and cancers, and

acquired a mouse anti-human TLR7 antibody. They have accomplished the present invention by humanizing the mouse anti-human TLR7 antibody obtained and further modifying CDRs, frameworks, and variable regions of the humanized antibody.

[0009]

That is, the present invention includes the following aspects.

(1) An antibody or an antigen-binding fragment of the antibody having the properties of:

(a) specifically binding to human TLR7 or monkey TLR7 and not binding to mouse TLR7 or rat TLR7; and

(b) inhibiting a function of human TLR7 or monkey TLR7.

(2) The antibody or the antigen-binding fragment of the antibody according to (1) above, wherein the human TLR7 is a molecule consisting of the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4, the monkey TLR7 is a molecule consisting of the amino acid sequence set forth in SEQ ID NO: 85, the mouse TLR7 is a molecule consisting of the amino acid sequence set forth in SEQ ID NO: 83, or the rat TLR7 is a molecule consisting of the amino acid sequence set forth in SEQ ID NO: 84.

(3) The antibody or the antigen-binding fragment of the antibody according to (1) or (2) above, having a competitive inhibitory activity for binding to human TLR7 with:

an antibody comprising a heavy chain with a heavy-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 5 and a light chain with a light-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 11;

an antibody comprising a heavy chain with a heavy-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 7 and a light chain with a light-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 13; or

an antibody comprising a heavy chain with a heavy-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 9 and a light chain with a light-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 15.

(4) The antibody or the antigen-binding fragment of the antibody according to any one of (1) to (3) above, comprising:

(a) CDRH1 consisting of the amino acid sequence set forth in SEQ ID NO: 17, CDRH2 consisting of the amino acid sequence set forth in SEQ ID NO: 18 and CDRH3 consisting of the amino acid sequence set forth in SEQ ID NO: 19, as complementarity determining regions in the heavy chain, and CDRL1 consisting of the amino acid sequence set forth in SEQ ID NO: 20, CDRL2 consisting of the amino acid sequence set forth in SEQ ID NO: 21 and CDRL3 consisting of the amino acid sequence set forth in

SEQ ID NO: 22, as complementarity determining regions in the light chain;

(b) CDRH1 consisting of the amino acid sequence set forth in SEQ ID NO: 23, CDRH2 consisting of the amino acid sequence set forth in SEQ ID NO: 24 and CDRH3 consisting of the amino acid sequence set forth in SEQ ID NO: 25, as complementarity determining regions in the heavy chain, and CDRL1 consisting of the amino acid sequence set forth in SEQ ID NO: 26, CDRL2 consisting of the amino acid sequence set forth in SEQ ID NO: 27 and CDRL3 consisting of the amino acid sequence set forth in SEQ ID NO: 28, as complementarity determining regions in the light chain; or

(c) CDRH1 consisting of the amino acid sequence set forth in SEQ ID NO: 29, CDRH2 consisting of the amino acid sequence set forth in SEQ ID NO: 30 and CDRH3 consisting of the amino acid sequence set forth in SEQ ID NO: 31, as complementarity determining regions in the heavy chain, and CDRL1 consisting of the amino acid sequence set forth in SEQ ID NO: 32, CDRL2 consisting of the amino acid sequence set forth in SEQ ID NO: 33 and CDRL3 consisting of the amino acid sequence set forth in SEQ ID NO: 34, as complementarity determining regions in the light chain.

(5) The antibody or the antigen-binding fragment of the antibody according to (4) above, comprising:

CDRH1 consisting of the amino acid sequence set forth in SEQ ID NO: 17, CDRH2 consisting of the amino acid sequence set forth in SEQ ID NO: 18 and CDRH3 consisting of the amino acid sequence set forth in SEQ ID NO: 19, as complementarity determining regions in the heavy chain, and

CDRL1 consisting of the amino acid sequence set forth in SEQ ID NO: 20, CDRL2 consisting of the amino acid sequence set forth in SEQ ID NO: 21 and CDRL3 consisting of the amino acid sequence set forth in SEQ ID NO: 22, as complementarity determining regions in the light chain.

(6) The antibody or the antigen-binding fragment of the antibody according to any one of (1) to (5) above, comprising:

(a) a heavy-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 5 and a light-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 11;

(b) a heavy-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 7 and a light-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 13; or

(c) a heavy-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 9 and a light-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 15.

(7) The antibody or the antigen-binding fragment of the antibody according to any one of (1) to (6) above, wherein a constant region is a human-derived constant region.

(8) The antibody or the antigen-binding fragment of the antibody according to (7) above, comprising:

(a) a heavy chain consisting of an amino acid sequence at positions 20 to 465 in the amino acid sequence set forth in SEQ ID NO: 35 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 36;

(b) a heavy chain consisting of an amino acid sequence at positions 20 to 471 in the amino acid sequence set forth in SEQ ID NO: 37 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 38, or

(c) a heavy chain consisting of an amino acid sequence at positions 20 to 470 in the amino acid sequence set forth in SEQ ID NO: 39 and a light chain consisting of an amino acid sequence at positions 21 to 234 in the amino acid sequence set forth in SEQ ID NO: 40.

(9) The antibody or the antigen-binding fragment of the antibody according to any one of (1) to (8) above, being humanized.

(10) The antibody or the antigen-binding fragment of the antibody according to (9) above, comprising:

(I) a heavy-chain variable region consisting of an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence set forth in SEQ ID NO: 41;

(b) the amino acid sequence set forth in SEQ ID NO: 42;

(c) an amino acid sequence having a homology of at least 95% or more to the sequence (a) or (b); and

(d) an amino acid sequence derived from the sequence (a) or (b) by deletion, substitution or addition of 1 to 10 amino acids; and

a light-chain variable region consisting of an amino acid sequence selected from the group consisting of:

(e) the amino acid sequence set forth in SEQ ID NO: 43;

(f) the amino acid sequence set forth in SEQ ID NO: 44;

(g) an amino acid sequence having a homology of at least 95% or more to the sequence (e) or (f); and

(h) an amino acid sequence derived from the sequence (e) or (f) by deletion, substitution or addition of 1 to 10 amino acids; or

(II) a heavy-chain variable region consisting of an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence set forth in SEQ ID NO: 50;

(b) an amino acid sequence having a homology of at least 95% or more to the sequence (a); and

(c) an amino acid sequence derived from the sequence (a) by deletion, substitution or addition of 1 to 10 amino acids; and

a light-chain variable region consisting of an amino acid sequence selected from the group consisting of:

(d) the amino acid sequence set forth in SEQ ID NO: 51;

(e) an amino acid sequence having a homology of at least 95% or more to the sequence (d); and

(f) an amino acid sequence derived from the sequence (d) by deletion, substitution or addition of 1 to 10 amino acids.

(11) The antibody or the antigen-binding fragment of the antibody according to (10) above, comprising:

(a) a heavy-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 41 and a light-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 43;

(b) a heavy-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 41 and a

light-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 44;

(c) a heavy-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 42 and a light-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 43;

(d) a heavy-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 42 and a light-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 44; or

(e) a heavy-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 50 and a light-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 51.

(12) The antibody or the antigen-binding fragment of the antibody according to (11) above, comprising a heavy-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 42 and a light-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 44.

(13) The antibody or the antigen-binding fragment of the antibody according to (11) above, comprising:

(a) a heavy chain consisting of an amino acid sequence at positions 20 to 465 in the amino acid sequence set forth in SEQ ID NO: 45 and a light chain consisting of an amino acid sequence at positions 21 to

233 in the amino acid sequence set forth in SEQ ID NO: 48;

(b) a heavy chain consisting of an amino acid sequence at positions 20 to 465 in the amino acid sequence set forth in SEQ ID NO: 45 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 49;

(c) a heavy chain consisting of an amino acid sequence at positions 20 to 465 in the amino acid sequence set forth in SEQ ID NO: 46 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 48;

(d) a heavy chain consisting of an amino acid sequence at positions 20 to 465 in the amino acid sequence set forth in SEQ ID NO: 46 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 49;

(e) a heavy chain consisting of an amino acid sequence at positions 20 to 462 in the amino acid sequence set forth in SEQ ID NO: 47 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 48;

(f) a heavy chain consisting of an amino acid sequence at positions 20 to 462 in the amino acid sequence set forth in SEQ ID NO: 47 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 49;

(g) a heavy chain consisting of an amino acid sequence at positions 20 to 471 in the amino acid sequence set forth in SEQ ID NO: 52 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 53; or

(h) a heavy chain consisting of an amino acid sequence at positions 20 to 468 in the amino acid sequence set forth in SEQ ID NO: 54 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 53.

(14) The antibody or the antigen-binding fragment of the antibody according to (13) above, comprising a heavy chain consisting of an amino acid sequence at positions 20 to 465 in the amino acid sequence set forth in SEQ ID NO: 46 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 49.

(15) An antibody or an antigen-binding fragment of the antibody having the properties of:

(a) specifically binding to human TLR7,

(b) inhibiting a function of human TLR7, and

(c) comprising:

(1) CDRH1 consisting of the amino acid sequence set forth in SEQ ID NO: 17, CDRH2 consisting of the amino acid sequence set forth in SEQ ID NO: 18 and CDRH3 consisting of the amino acid sequence set forth in SEQ ID NO: 19, as complementarity determining regions in the heavy chain, and CDRL1 consisting of the amino acid sequence set forth in SEQ ID NO: 20, CDRL2 consisting of the amino acid sequence set forth in SEQ ID NO: 21 and CDRL3 consisting of the amino acid sequence set forth in SEQ ID NO: 22, as complementarity determining regions in the light chain;

(2) CDRH1 consisting of the amino acid sequence set forth in SEQ ID NO: 23, CDRH2 consisting of the amino acid sequence set forth in SEQ ID NO: 24 and CDRH3 consisting of the amino acid sequence set forth in SEQ ID NO: 25, as complementarity determining regions in the heavy chain, and CDRL1 consisting of the amino acid sequence set forth in SEQ ID NO: 26, CDRL2 consisting of the amino acid sequence set forth in SEQ ID NO: 27 and CDRL3 consisting of the amino acid sequence set forth in SEQ ID NO: 28, as complementarity determining regions in the light chain; or

(3) CDRH1 consisting of the amino acid sequence set forth in SEQ ID NO: 29, CDRH2 consisting of the amino acid sequence set forth in SEQ ID NO: 30 and CDRH3

consisting of the amino acid sequence set forth in SEQ ID NO: 31, as complementarity determining regions in the heavy chain, and CDRL1 consisting of the amino acid sequence set forth in SEQ ID NO: 32, CDRL2 consisting of the amino acid sequence set forth in SEQ ID NO: 33 and CDRL3 consisting of the amino acid sequence set forth in SEQ ID NO: 34, as complementarity determining regions in the light chain.

(16) The antibody or the antigen-binding fragment of the antibody according to (15) above, comprising:

CDRH1 consisting of the amino acid sequence set forth in SEQ ID NO: 17, CDRH2 consisting of the amino acid sequence set forth in SEQ ID NO: 18 and CDRH3 consisting of the amino acid sequence set forth in SEQ ID NO: 19, as complementarity determining regions in the heavy chain, and

CDRL1 consisting of the amino acid sequence set forth in SEQ ID NO: 20, CDRL2 consisting of the amino acid sequence set forth in SEQ ID NO: 21 and CDRL3 consisting of the amino acid sequence set forth in SEQ ID NO: 22, as complementarity determining regions in the light chain.

(17) The antibody or the antigen-binding fragment of the antibody according to (15) or (16) above, comprising:

(a) a heavy-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 5 and a

light-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 11;

(b) a heavy-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 7 and a light-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 13; or

(c) a heavy-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 9 and a light-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 15.

(18) The antibody or the antigen-binding fragment of the antibody according to any one of (15) to (17) above, wherein a constant region is a human-derived constant region.

(19) The antibody or the antigen-binding fragment of the antibody according to (18) above, comprising:

(a) a heavy chain consisting of an amino acid sequence at positions 20 to 465 in the amino acid sequence set forth in SEQ ID NO: 35 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 36;

(b) a heavy chain consisting of an amino acid sequence at positions 20 to 471 in the amino acid sequence set forth in SEQ ID NO: 37 and a light chain consisting of an amino acid sequence at positions 21 to

233 in the amino acid sequence set forth in SEQ ID NO: 38,
or

(c) a heavy chain consisting of an amino acid sequence at positions 20 to 470 in the amino acid sequence set forth in SEQ ID NO: 39 and a light chain consisting of an amino acid sequence at positions 21 to 234 in the amino acid sequence set forth in SEQ ID NO: 40.

(20) The antibody or the antigen-binding fragment of the antibody according to any one of (15) to (19) above, being humanized.

(21) The antibody or the antigen-binding fragment of the antibody according to (20) above, comprising:

(I) a heavy-chain variable region consisting of an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence set forth in SEQ ID NO: 41;

(b) the amino acid sequence set forth in SEQ ID NO: 42;

(c) an amino acid sequence having a homology of at least 95% or more to the sequence (a) or (b); and

(d) an amino acid sequence derived from the sequence (a) or (b) by deletion, substitution or addition of 1 to 10 amino acids; and

a light-chain variable region consisting of an amino acid sequence selected from the group consisting of:

(e) the amino acid sequence set forth in SEQ ID NO: 43;

(f) the amino acid sequence set forth in SEQ ID NO: 44;

(g) an amino acid sequence having a homology of at least 95% or more to the sequence (e) or (f); and

(h) an amino acid sequence derived from the sequence (e) or (f) by deletion, substitution or addition of 1 to 10 amino acids; or

(II) a heavy-chain variable region consisting of an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence set forth in SEQ ID NO: 50;

(b) an amino acid sequence having a homology of at least 95% or more to the sequence (a); and

(c) an amino acid sequence derived from the sequence (a) by deletion, substitution or addition of 1 to 10 amino acids; and

a light-chain variable region consisting of an amino acid sequence selected from the group consisting of:

(d) the amino acid sequence set forth in SEQ ID NO: 51;

(e) an amino acid sequence having a homology of at least 95% or more to the sequence (d); and

(f) an amino acid sequence derived from the sequence (d) by deletion, substitution or addition of 1 to 10 amino acids.

(22) The antibody or the antigen-binding fragment of the antibody according to (21) above, comprising:

(a) a heavy-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 41 and a light-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 43;

(b) a heavy-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 41 and a light-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 44;

(c) a heavy-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 42 and a light-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 43;

(d) a heavy-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 42 and a light-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 44; or

(e) a heavy-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 50 and a light-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 51.

(23) The antibody or the antigen-binding fragment of the antibody according to (22) above, comprising a heavy-

chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 42 and a light-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 44.

(24) The antibody or the antigen-binding fragment of the antibody according to (22) above, comprising:

(a) a heavy chain consisting of an amino acid sequence at positions 20 to 465 in the amino acid sequence set forth in SEQ ID NO: 45 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 48;

(b) a heavy chain consisting of an amino acid sequence at positions 20 to 465 in the amino acid sequence set forth in SEQ ID NO: 45 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 49;

(c) a heavy chain consisting of an amino acid sequence at positions 20 to 465 in the amino acid sequence set forth in SEQ ID NO: 46 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 48;

(d) a heavy chain consisting of an amino acid sequence at positions 20 to 465 in the amino acid sequence set forth in SEQ ID NO: 46 and a light chain

consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 49;

(e) a heavy chain consisting of an amino acid sequence at positions 20 to 462 in the amino acid sequence set forth in SEQ ID NO: 47 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 48;

(f) a heavy chain consisting of an amino acid sequence at positions 20 to 462 in the amino acid sequence set forth in SEQ ID NO: 47 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 49;

(g) a heavy chain consisting of an amino acid sequence at positions 20 to 471 in the amino acid sequence set forth in SEQ ID NO: 52 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 53; or

(h) a heavy chain consisting of an amino acid sequence at positions 20 to 468 in the amino acid sequence set forth in SEQ ID NO: 54 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 53.

(25) The antibody or the antigen-binding fragment of the antibody according to (24) above, comprising a heavy chain consisting of an amino acid sequence at positions 20 to 465 in the amino acid sequence set forth in SEQ ID NO: 46 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 49.

(26) An antibody or an antigen-binding fragment of the antibody comprising a heavy chain consisting of an amino acid sequence at positions 20 to 465 in the amino acid sequence set forth in SEQ ID NO: 45 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 48.

(27) An antibody or an antigen-binding fragment of the antibody comprising a heavy chain consisting of an amino acid sequence at positions 20 to 465 in the amino acid sequence set forth in SEQ ID NO: 46 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 48.

(28) An antibody or an antigen-binding fragment of the antibody comprising a heavy chain consisting of an amino acid sequence at positions 20 to 465 in the amino acid sequence set forth in SEQ ID NO: 46 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 49.

(29) An antibody or an antigen-binding fragment of the antibody comprising a heavy chain consisting of an amino

acid sequence at positions 20 to 462 in the amino acid sequence set forth in SEQ ID NO: 47 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 49.

(30) An antibody or an antigen-binding fragment of the antibody comprising a heavy chain consisting of an amino acid sequence at positions 20 to 471 in the amino acid sequence set forth in SEQ ID NO: 52 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 53.

(31) An antibody or an antigen-binding fragment of the antibody comprising a heavy chain consisting of an amino acid sequence at positions 20 to 468 in the amino acid sequence set forth in SEQ ID NO: 54 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 53.

(32) The antibody or the antigen-binding fragment of the antibody according to any one of (26) to (31) above, having the properties of:

(a) specifically binding to human TLR7; and

(b) inhibiting a function of human TLR7.

(33) The antigen-binding fragment of the antibody according to any one of (1) to (32) above, wherein the antigen-binding fragment is selected from the group consisting of Fab, F(ab)₂, Fab' and Fv.

(34) A polynucleotide comprising a polynucleotide sequence encoding the antibody or the antigen-binding

fragment of the antibody according to any one of (1) to (33) above.

(35) The polynucleotide according to (34) above, comprising:

(1) a polynucleotide sequence encoding CDRH1 consisting of the amino acid sequence set forth in SEQ ID NO: 17, a polynucleotide sequence encoding CDRH2 consisting of the amino acid sequence set forth in SEQ ID NO: 18 and a polynucleotide sequence encoding CDRH3 consisting of the amino acid sequence set forth in SEQ ID NO: 19, and a polynucleotide sequence encoding CDRL1 consisting of the amino acid sequence set forth in SEQ ID NO: 20, a polynucleotide sequence encoding CDRL2 consisting of the amino acid sequence set forth in SEQ ID NO: 21 and a polynucleotide sequence encoding CDRL3 consisting of the amino acid sequence set forth in SEQ ID NO: 22;

(2) a polynucleotide sequence encoding CDRH1 consisting of the amino acid sequence set forth in SEQ ID NO: 23, a polynucleotide sequence encoding CDRH2 consisting of the amino acid sequence set forth in SEQ ID NO: 24 and a polynucleotide sequence encoding CDRH3 consisting of the amino acid sequence set forth in SEQ ID NO: 25, and a polynucleotide sequence encoding CDRL1 consisting of the amino acid sequence set forth in SEQ ID NO: 26, a polynucleotide sequence encoding CDRL2 consisting of the amino acid sequence set forth in SEQ ID

NO: 27 and a polynucleotide sequence encoding CDRL3 consisting of the amino acid sequence set forth in SEQ ID NO: 28; or

(3) a polynucleotide sequence encoding CDRH1 consisting of the amino acid sequence set forth in SEQ ID NO: 29, a polynucleotide sequence encoding CDRH2 consisting of the amino acid sequence set forth in SEQ ID NO: 30 and a polynucleotide sequence encoding CDRH3 consisting of the amino acid sequence set forth in SEQ ID NO: 31, and a polynucleotide sequence encoding CDRL1 consisting of the amino acid sequence set forth in SEQ ID NO: 32, a polynucleotide sequence encoding CDRL2 consisting of the amino acid sequence set forth in SEQ ID NO: 33 and a polynucleotide sequence encoding CDRL3 consisting of the amino acid sequence set forth in SEQ ID NO: 34.

(36) The polynucleotide according to (35) above, comprising:

(I) a polynucleotide sequence selected from the group consisting of:

(a) a polynucleotide sequence encoding the heavy-chain variable region set forth in SEQ ID NO: 77;

(b) a polynucleotide sequence encoding the heavy-chain variable region set forth in SEQ ID NO: 78; and

(c) a polynucleotide sequence of a polynucleotide which hybridizes with a polynucleotide consisting of a polynucleotide sequence complementary to the

polynucleotide sequence (a) or (b) under stringent conditions; and

a polynucleotide sequence selected from the group consisting of:

(d) a polynucleotide sequence encoding the light-chain variable region set forth in SEQ ID NO: 79;

(e) a polynucleotide sequence encoding the light-chain variable region set forth in SEQ ID NO: 80; and

(f) a polynucleotide sequence of a polynucleotide which hybridizes with a polynucleotide consisting of a polynucleotide sequence complementary to the polynucleotide sequence (d) or (e) under stringent conditions; or

(II) a polynucleotide sequence selected from the group consisting of:

(a) a polynucleotide sequence encoding the heavy-chain variable region set forth in SEQ ID NO: 81; and

(b) a polynucleotide sequence of a polynucleotide which hybridizes with a polynucleotide consisting of a polynucleotide sequence complementary to the polynucleotide sequence (a) under stringent conditions; and

a polynucleotide sequence selected from the group consisting of:

(c) a polynucleotide sequence encoding the light-chain variable region set forth in SEQ ID NO: 82; and

(d) a polynucleotide sequence of a polynucleotide which hybridizes with a polynucleotide consisting of a polynucleotide sequence complementary to the polynucleotide sequence (c) under stringent conditions.

(37) The polynucleotide according to (36) above, comprising:

(a) a polynucleotide sequence encoding the heavy-chain variable region set forth in SEQ ID NO: 77 and a polynucleotide sequence encoding the light-chain variable region set forth in SEQ ID NO: 79;

(b) a polynucleotide sequence encoding the heavy-chain variable region set forth in SEQ ID NO: 77 and a polynucleotide sequence encoding the light-chain variable region set forth in SEQ ID NO: 80;

(c) a polynucleotide sequence encoding the heavy-chain variable region set forth in SEQ ID NO: 78 and a polynucleotide sequence encoding the light-chain variable region set forth in SEQ ID NO: 79;

(d) a polynucleotide sequence encoding the heavy-chain variable region set forth in SEQ ID NO: 78 and a polynucleotide sequence encoding the light-chain variable region set forth in SEQ ID NO: 80; or

(e) a polynucleotide sequence encoding the heavy-chain variable region set forth in SEQ ID NO: 81 and a polynucleotide sequence encoding the light-chain variable region set forth in SEQ ID NO: 82.

(38) An expression vector comprising the polynucleotide according to any one of (34) to (37) above.

(39) A host cell transformed with the expression vector according to (38) above.

(40) The host cell according to (39) above, wherein the host cell is a eukaryotic cell.

(41) A method for producing an antibody or an antigen-binding fragment of the antibody, comprising the steps of:

culturing the host cell according to (39) or (40) above; and

collecting the antibody or the fragment of interest from the culture obtained in the above step.

(42) An antibody or an antigen-binding fragment of the antibody obtained by the production method according to (41).

(43) The antibody according to any one of (1) to (32) above, comprising one or more modifications selected from the group consisting of glycosylation to N-linkage, glycosylation to O-linkage, N-terminal processing, C-terminal processing, deamidation, isomerization of aspartic acid, oxidation of methionine, addition of a methionine residue to N-terminal, amidation of a proline residue, and deletion of one or two amino acids at the carboxyl terminus of the heavy chain.

(44) The antibody according to (43) above, comprising: two heavy chains consisting of one kind of or a

combination of any two kinds of heavy chains selected from the group consisting of the full length and deletion variants with one or two amino acids deleted at the carboxyl terminus.

(45) The antibody according to (44) above, wherein one amino acid is deleted at the carboxyl terminus in each of the two heavy chains.

(46) The antibody according to any one of (43) to (45) above, wherein a proline residue at the carboxyl terminus of the heavy chain is further amidated.

(47) A pharmaceutical composition for treating and/or preventing a disease, comprising: at least one of the antibodies or the antigen-binding fragments of the antibodies according to (1) to (33) and (42) to (46) above, or the expression vector according to (38) as an active ingredient.

(48) The pharmaceutical composition according to (47) above, for treating a disease caused by a function of human TLR7.

(49) The pharmaceutical composition according to (48) above, wherein the disease caused by a function of human TLR7 is an immune inflammation-related disease, an allergic disease, an infection or a cancer.

(50) The pharmaceutical composition according to (49) above, wherein the immune inflammation-related disease is systemic lupus erythematosus, rheumatoid arthritis, juvenile idiopathic arthritis, adult-onset Still's

disease, ankylosing spondylitis, systemic scleroderma, polymyositis, dermatomyositis, psoriatic arthritis, osteoarthritis, mixed connective tissue disease or muscular dystrophy.

(51) The pharmaceutical composition according to (49) or (50) above, wherein the immune inflammation-related disease is systemic lupus erythematosus.

(52) The pharmaceutical composition according to any one of (47) to (51) above, to be used in combination with an immunosuppressant, an anti-inflammatory drug, an antiallergic agent, an anti-infective agent, or an anticancer agent.

(53) A method for treating a disease caused by a function of human TLR7, comprising: administering at least one of the antibodies or the antigen-binding fragments of the antibodies according to (1) to (33) and (42) to (46) above to an individual.

(54) A method for treating a disease caused by a function of human TLR7, comprising administering at least one of the antibodies or the antigen-binding fragments of the antibodies according to (1) to (33) and (42) to (46) above together with an immunosuppressant, an anti-inflammatory drug, an antiallergic agent, an anti-infective agent or an anticancer agent, simultaneously, separately or sequentially to an individual.

(55) The treatment method according to (53) or (54) above, wherein the disease caused by a function of human TLR7 is

an immune inflammation-related disease, an allergic disease, an infection or a cancer.

(56) The treatment method according to (55) above, wherein the immune inflammation-related disease is systemic lupus erythematosus, rheumatoid arthritis, juvenile idiopathic arthritis, adult-onset Still's disease, ankylosing spondylitis, systemic scleroderma, polymyositis, dermatomyositis, psoriatic arthritis, osteoarthritis, mixed connective tissue disease or muscular dystrophy.

(57) The treatment method according to (55) or (56) above, wherein the immune inflammation-related disease is systemic lupus erythematosus.

(58) The antibody or the antigen-binding fragment of the antibody according to any one of (1) to (33) and (42) to (46) above, or the expression vector according to (38) above, to be used for treating or preventing a disease caused by a function of human TLR7.

(59) The antibody, the antigen-binding fragment of the antibody, or the expression vector according to (58) above, wherein the disease caused by a function of human TLR7 is an immune inflammation-related disease, an allergic disease, an infection or a cancer.

(60) The antibody, the antigen-binding fragment of the antibody, or the expression vector according to (59) above, wherein the immune inflammation-related disease is systemic lupus erythematosus, rheumatoid arthritis,

juvenile idiopathic arthritis, adult-onset Still's disease, ankylosing spondylitis, systemic scleroderma, polymyositis, dermatomyositis, psoriatic arthritis, osteoarthritis, mixed connective tissue disease or muscular dystrophy.

(61) The antibody, the antigen-binding fragment of the antibody, or the expression vector according to (59) or (60) above, wherein the immune inflammation-related disease is systemic lupus erythematosus.

(62) The antibody, the antigen-binding fragment of the antibody, or the expression vector according to any one of (58) to (61) above, to be used in combination with an immunosuppressant, an anti-inflammatory drug, an antiallergic agent, an anti-infective agent or an anticancer agent.

(63) Use of the antibody or the antigen-binding fragment of the antibody according to any one of (1) to (33) and (42) to (46) above, or the expression vector according to (38) above, for producing a therapeutic agent or a preventive agent for a disease caused by a function of human TLR7.

(64) The use according to (63) above, wherein the disease caused by a function of human TLR7 is an immune inflammation-related disease, an allergic disease, an infection or a cancer.

(65) The use according to (64) above, wherein the immune inflammation-related disease is systemic lupus

erythematosus, rheumatoid arthritis, juvenile idiopathic arthritis, adult-onset Still's disease, ankylosing spondylitis, systemic scleroderma, polymyositis, dermatomyositis, psoriatic arthritis, osteoarthritis, mixed connective tissue disease or muscular dystrophy.

(66) The use according to (64) or (65) above, wherein the immune inflammation-related disease is systemic lupus erythematosus.

(67) The use according to any one of (63) to (66) above, wherein the therapeutic agent or the preventive agent is used in combination with an immunosuppressant, an anti-inflammatory drug, an antiallergic agent, an anti-infective agent or an anticancer agent.

Advantageous Effects of Invention

[0010]

According to the present invention, an agent having a mechanism of action of inhibiting a function of TLR7 for treating and/or preventing immune inflammation-related diseases, allergic diseases, infections, and cancers can be obtained.

Brief Description of Drawings

[0011]

[Figure 1] Figure 1 shows the amino acid sequence (SEQ ID NO: 2) of human TLR7 (variant 1).

[Figure 2] Figure 2 shows the amino acid sequence (SEQ ID NO: 4) of human TLR7 (variant 2).

[Figure 3] Figure 3 shows the amino acid sequence (SEQ ID NO: 83) of mouse TLR7.

[Figure 4] Figure 4 shows the amino acid sequence (SEQ ID NO: 84) of rat TLR7.

[Figure 5] Figure 5 shows the amino acid sequence (SEQ ID NO: 85) of monkey TLR7.

[Figure 6] Figure 6 is a graph showing that the AT01 antibody, the NB7 antibody, and the FAN2 antibody suppress the production of IL-6 from human peripheral blood mononuclear cells (which will be hereinafter referred to as PBMCs) treated with CL-264 in a concentration dependent manner.

[Figure 7] Figure 7 shows the amino acid sequence (SEQ ID NO: 35) of the heavy chain of the cAT01 antibody, which includes the signal sequence thereof, and the amino acid sequence (SEQ ID NO: 36) of the light chain of the cAT01 antibody, which includes the signal sequence thereof. In the heavy-chain amino acid sequence, the amino acid sequence at positions 1 to 19 is the signal sequence, the amino acid sequence at positions 20 to 135 is the heavy-chain variable region, and the amino acid sequence at positions 136 to 465 is the heavy-chain constant region. In the light-chain amino acid sequence, the amino acid sequence at positions 1 to 20 is the signal sequence, the amino acid sequence at positions 21 to 126 is the light-

chain variable region, and the amino acid sequence at positions 127 to 233 is the light-chain constant region.

[Figure 8] Figure 8 shows the amino acid sequence (SEQ ID NO: 37) of the heavy chain of the cNB7 antibody, which includes the signal sequence thereof, and the amino acid sequence (SEQ ID NO: 38) of the light chain of the cNB7 antibody, which includes the signal sequence thereof. In the heavy-chain amino acid sequence, the amino acid sequence at positions 1 to 19 is the signal sequence, the amino acid sequence at positions 20 to 141 is the heavy-chain variable region, and the amino acid sequence at positions 142 to 471 is the heavy-chain constant region. In the light-chain amino acid sequence, the amino acid sequence at positions 1 to 20 is the signal sequence, the amino acid sequence at positions 21 to 126 is the light-chain variable region, and the amino acid sequence at positions 127 to 233 is the light-chain constant region.

[Figure 9] Figure 9 shows the amino acid sequence (SEQ ID NO: 39) of the heavy chain of the cFAN2 antibody, which includes the signal sequence thereof, and the amino acid sequence (SEQ ID NO: 40) of the light chain of the cFAN2 antibody, which includes the signal sequence thereof. In the heavy-chain amino acid sequence, the amino acid sequence at positions 1 to 19 is the signal sequence, the amino acid sequence at positions 20 to 140 is the heavy-chain variable region, and the amino acid sequence at positions 141 to 470 is the heavy-chain constant region.

In the light-chain amino acid sequence, the amino acid sequence at positions 1 to 20 is the signal sequence, the amino acid sequence at positions 21 to 127 is the light-chain variable region, and the amino acid sequence at positions 128 to 234 is the light-chain constant region.

[Figure 10] Figure 10 is a graph showing that cAT01, cNB7, and cFAN2, which are chimeric anti-human TLR7 antibodies, suppress the production of IL-6 from human PBMCs treated with CL-264 in a concentration dependent manner.

[Figure 11] Figure 11 shows the amino acid sequence (SEQ ID NO: 45) of the heavy chain of huAT01_H1_IgG1LALA, which includes the signal sequence thereof, and which is a humanized heavy chain. In the sequence, the amino acid sequence at positions 1 to 19 is the signal sequence, the amino acid sequence at positions 20 to 135 is the variable region, and the amino acid sequence at positions 136 to 465 is the constant region. Further, the amino acid sequence at positions 45 to 54 is CDRH1, the amino acid sequence at positions 69 to 78 is CDRH2, and the amino acid sequence at positions 118 to 124 is CDRH3.

[Figure 12] Figure 12 shows the amino acid sequence (SEQ ID NO: 46) of the heavy chain of huAT01_H3_IgG1LALA, which includes the signal sequence thereof, and which is a humanized heavy chain. In the sequence, the amino acid sequence at positions 1 to 19 is the signal sequence, the amino acid sequence at positions 20 to 135 is the variable region, and the amino acid sequence at positions

136 to 465 is the constant region. Further, the amino acid sequence at positions 45 to 54 is CDRH1, the amino acid sequence at positions 69 to 78 is CDRH2, and the amino acid sequence at positions 118 to 124 is CDRH3.

[Figure 13] Figure 13 shows the amino acid sequence (SEQ ID NO: 47) of the heavy chain of huAT01_H3_IgG4Pro, which includes the signal sequence thereof, and which is a humanized heavy chain. In the sequence, the amino acid sequence at positions 1 to 19 is the signal sequence, the amino acid sequence at positions 20 to 135 is the variable region, and the amino acid sequence at positions 136 to 462 is the constant region. Further, the amino acid sequence at positions 45 to 54 is CDRH1, the amino acid sequence at positions 69 to 78 is CDRH2, and the amino acid sequence at positions 118 to 124 is CDRH3.

[Figure 14] Figure 14 shows the comparison of the amino acid sequence (SEQ ID NO: 5) of the variable region of cAT01_H, which is the heavy chain of chimeric antibody cAT01 (which will be hereinafter referred to as cAT01_H), the amino acid sequence (SEQ ID NO: 41) of the variable region of humanized antibody heavy chain

huAT01_H1_IgG1LALA (which will be hereinafter referred to as huAT01_H1), and the amino acid sequence (SEQ ID NO: 42) of the variable region of humanized antibody heavy chain huAT01_H3_IgG1LALA (which will be hereinafter referred to as huAT01_H3). In the sequences of huAT01_H1 and huAT01_H3, the symbol "." represents the same amino

acid residue as that of cAT01_H, and a substituted amino acid residue is shown in the site where the amino acid residue is described.

[Figure 15] Figure 15 shows the amino acid sequence (SEQ ID NO: 48) of the light chain of huAT01_L1, which includes the signal sequence thereof and which is a humanized light chain. In the sequence, the amino acid sequence at positions 1 to 20 is the signal sequence, the amino acid sequence at positions 21 to 126 is the variable region, and the amino acid sequence at positions 127 to 233 is the constant region. Further, the amino acid sequence at positions 44 to 54 is CDRL1, the amino acid sequence at positions 70 to 76 is CDRL2, and the amino acid sequence at positions 109 to 116 is CDRL3.

[Figure 16] Figure 16 shows the amino acid sequence (SEQ ID NO: 49) of the light chain of huAT01_L2, which includes the signal sequence thereof and which is a humanized light chain. In the sequence, the amino acid sequence at positions 1 to 20 is the signal sequence, the amino acid sequence at positions 21 to 126 is the variable region, and the amino acid sequence at positions 127 to 233 is the constant region. Further, the amino acid sequence at positions 44 to 54 is CDRL1, the amino acid sequence at positions 70 to 76 is CDRL2, and the amino acid sequence at positions 109 to 116 is CDRL3.

[Figure 17] Figure 17 shows the comparison of the amino acid sequence (SEQ ID NO: 11) of the variable region of

cAT01_L, which is the light chain of chimeric antibody cAT01 (which will be hereinafter referred to as cAT01_L), the amino acid sequence (SEQ ID NO: 43) of the variable region of humanized antibody light chain huAT01_L1 (which will be hereinafter referred to as huAT01_L1), and the amino acid sequence (SEQ ID NO: 44) of the variable region of humanized antibody light chain huAT01_L2 (which will be hereinafter referred to as huAT01_L2). In the sequences of huAT01_L1 and huAT01_L2, the symbol "." represents the same amino acid residue as that of cAT01_L, and a substituted amino acid residue is shown in the site where the amino acid residue is described.

[Figure 18] Figure 18 shows the amino acid sequence (SEQ ID NO: 52) of the heavy chain of huNB7_H3_IgG1LALA, which includes the signal sequence thereof and which is a humanized heavy chain. In the sequence, the amino acid sequence at positions 1 to 19 is the signal sequence, the amino acid sequence at positions 20 to 141 is the variable region, and the amino acid sequence at positions 142 to 471 is the constant region. Further, the amino acid sequence at positions 45 to 55 is CDRH1, the amino acid sequence at positions 70 to 78 is CDRH2, and the amino acid sequence at positions 118 to 130 is CDRH3.

[Figure 19] Figure 19 shows the amino acid sequence (SEQ ID NO: 54) of the heavy chain of huNB7_H3_IgG4Pro, which includes the signal sequence thereof and which is a humanized heavy chain. In the sequence, the amino acid

sequence at positions 1 to 19 is the signal sequence, the amino acid sequence at positions 20 to 141 is the variable region, and the amino acid sequence at positions 142 to 468 is the constant region. Further, the amino acid sequence at positions 45 to 55 is CDRH1, the amino acid sequence at positions 70 to 78 is CDRH2, and the amino acid sequence at positions 118 to 130 is CDRH3.

[Figure 20] Figure 20 shows the comparison of the amino acid sequence (SEQ ID NO: 7) of the variable region of cNB7_H, which is the heavy chain of chimeric antibody cNB7 (which will be hereinafter referred to as cNB7_H) and the amino acid sequence (SEQ ID NO: 50) of the variable region of humanized antibody heavy chain huNB7_H3_IgG1LALA (which will be hereinafter referred to as huNB7_H3). In huNB7_H3, the symbol "." represents the same amino acid residue as that of cNB7_H, and a substituted amino acid residue is shown in the site where the amino acid residue is described.

[Figure 21] Figure 21 shows the amino acid sequence (SEQ ID NO: 53) of the light chain of huNB7_L3, which includes the signal sequence thereof and which is a humanized light chain. In the sequence, the amino acid sequence at positions 1 to 20 is the signal sequence, the amino acid sequence at positions 21 to 126 is the variable region, and the amino acid sequence at positions 127 to 233 is the constant region. Further, the amino acid sequence at positions 44 to 54 is CDRL1, the amino acid sequence at

positions 70 to 76 is CDRL2, and the amino acid sequence at positions 109 to 116 is CDRL3.

[Figure 22] Figure 22 shows the comparison of the amino acid sequence (SEQ ID NO: 13) of the variable region of cNB7_L, which is the light chain of chimeric antibody cNB7 (which will be hereinafter referred to as cNB7_L) and the amino acid sequence (SEQ ID NO: 51) of the variable region of humanized antibody light chain huNB7_L3 (which will be hereinafter referred to as huNB7_L3). In huNB7_L3, the symbol "." represents the same amino acid residue as that of cNB7_L, and a substituted amino acid residue is shown in the site where the amino acid residue is described.

[Figure 23] Figure 23 is a graph showing that humanized anti-human TLR7 antibodies (huAT01_H1L1_IgG1LALA, huAT01_H3L1_IgG1LALA, huAT01_H3L2_IgG1LALA, huAT01_H3L2_IgG4Pro, huNB7_H3L3_IgG1LALA, and huNB7_H3L3_IgG4Pro) suppress the production of IL-6 from human PBMCs treated with CL-264 in a concentration dependent manner.

[Figure 24] Figure 24-1 shows the amino acid sequence (SEQ ID NO: 17) of CDRH1 of AT01 antibody, the amino acid sequence (SEQ ID NO: 18) of CDRH2 thereof, the amino acid sequence (SEQ ID NO: 19) of CDRH3 thereof, the amino acid sequence (SEQ ID NO: 20) of CDRL1 thereof, the amino acid sequence (SEQ ID NO: 21) of CDRL2 thereof, and the amino acid sequence (SEQ ID NO: 22) of CDRL3 thereof. Figure

24-2 shows the amino acid sequence (SEQ ID NO: 23) of CDRH1 of NB7 antibody, the amino acid sequence (SEQ ID NO: 24) of CDRH2 thereof, the amino acid sequence (SEQ ID NO: 25) of CDRH3 thereof, the amino acid sequence (SEQ ID NO: 26) of CDRL1 thereof, the amino acid sequence (SEQ ID NO: 27) of CDRL2 thereof, and the amino acid sequence (SEQ ID NO: 28) of CDRL3 thereof. Figure 24-3 shows the amino acid sequence (SEQ ID NO: 29) of CDRH1 of FAN2 antibody, the amino acid sequence (SEQ ID NO: 30) of CDRH2 thereof, the amino acid sequence (SEQ ID NO: 31) of CDRH3 thereof, the amino acid sequence (SEQ ID NO: 32) of CDRL1 thereof, the amino acid sequence (SEQ ID NO: 33) of CDRL2 thereof, and the amino acid sequence (SEQ ID NO: 34) of CDRL3 thereof.

[Figure 25] Figure 25 shows the results of a flow cytometry analysis on binding to an antigen (human TLR7: variant 1) of humanized anti-human TLR7 antibody (huAT01_H3L2_IgG1LALA) and is a graph showing specific binding to the antigen.

[Figure 26] Figure 26 shows the results of a flow cytometry analysis on binding to an antigen (human TLR7: variant 2) of humanized anti-human TLR7 antibody (huAT01_H3L2_IgG1LALA) and is a graph showing specific binding to the antigen.

[Figure 27] Figure 27 shows the results of a flow cytometry analysis on binding to an antigen (monkey TLR7) of humanized anti-human TLR7 antibody

(huAT01_H3L2_IgG1LALA) and is a graph showing specific binding to the antigen.

[Figure 28] Figure 28 shows the results of a flow cytometry analysis on binding to an antigen (mouse TLR7) of humanized anti-human TLR7 antibody (huAT01_H3L2_IgG1LALA) and is a graph showing non-binding to the antigen.

[Figure 29] Figure 29 shows the results of a flow cytometry analysis on binding to an antigen (rat TLR7) of humanized anti-human TLR7 antibody (huAT01_H3L2_IgG1LALA) and is a graph showing non-binding to the antigen.

Description of Embodiments

[0012]

In this description, the term "gene" includes not only DNA but also mRNA, cDNA, and cRNA.

[0013]

In this description, the term "polynucleotide" is used in the same meaning as "nucleic acid" and also includes DNA, RNA, probes, oligonucleotides, and primers.

[0014]

In this description, the terms "polypeptide" and "protein" are used without distinction.

[0015]

In this description, the term "RNA fraction" means a fraction containing RNA.

[0016]

In this description, the term "cells" includes cells in animal individuals and cultured cells.

[0017]

In this description, the term "TLR7" is used to have the same meaning as TLR7 protein.

[0018]

In this description, the term "antigen-binding fragment of an antibody" means a partial fragment of an antibody having binding activity to an antigen and includes Fab, F(ab')₂, Fv, scFv, diabodies, linear antibodies, multispecific antibodies formed from antibody fragments, and the like. Further, an antigen-binding fragment of an antibody also includes Fab' which is a monovalent fragment in a variable region of an antibody obtained by treating F(ab')₂ under reducing conditions. However, there is no limitation to these molecules as long as the fragment has an ability to bind to an antigen. Further, the antigen-binding fragment includes not only those obtained by treating a full-length molecule of an antibody protein with a suitable enzyme but also a protein produced in a suitable host cell using a genetically engineered antibody gene.

[0019]

Complementarity determining regions (CDR: Complementarity Determining Regions) are known to be present at three points in each of the heavy and light chains of an antibody molecule. The complementarity

determining regions, which are also called hypervariable regions, are present in the variable regions of the heavy and light chains of an antibody at sites where the variability of the primary structure is particularly high. In the primary structure of the polypeptide chain in each of the heavy and light chains, the complementarity determining regions are separately present at three points. Concerning the complementarity determining regions of an antibody, the complementarity determining regions of the heavy chain are expressed as CDRH1, CDRH2, and CDRH3 from the amino-terminal side of the heavy-chain amino acid sequence, and the complementarity determining regions of the light chain are expressed as CDRL1, CDRL2, and CDRL3 from the amino-terminal side of the light-chain amino acid sequence, in this description. These sites are adjacent to each other on the three-dimensional structure and determine the specificity for the antigen to be bound. Further, the amino chain sequence of each CDR is described according to the AbM definition (Martin, A. C. R., Cheetham, J. C. and Rees, A. R. (1989) Proc. Natl Acad. Sci. USA, 86, 9268-9272).

[0020]

In the present invention, the phrase "hybridizing under stringent conditions" means hybridizing at 68°C in a commercially available hybridization solution, ExpressHyb Hybridization Solution (Clontech Laboratories, Inc.) or hybridizing under conditions which allow

hybridization at 68°C in the presence of 0.7 to 1.0-M NaCl using a filter to which DNA is immobilized and subsequent identification by washing at 68°C using an SSC solution of 0.1 to 2-fold concentration (SSC of 1-fold concentration composed of 150-mM NaCl and 15-mM sodium citrate) or conditions equivalent thereto.

[0021]

1. TLR7

Human TLR7 used in the present invention can be directly purified from human B cells or dendritic cells or prepared by adjusting the cell membrane fractions of the aforementioned cells for use. Further, human TLR7 can be obtained by in-vitro synthesis or production in host cells by genetic engineering. In genetic engineering, human TLR7 is expressed specifically by being integrated into a vector capable of expressing human TLR7 cDNA, followed by synthesis in a solution containing enzymes, substrates, and energy substances that are necessary for transcription and translation, or human TLR7 is expressed by transformation of host cells of other prokaryotes or eukaryotes, so that such a protein can be obtained.

[0022]

Likewise, monkey TLR7, mouse TLR7, and rat TLR7 can be directly purified from TLR7-expressing cells of monkeys, mice, and rats, respectively, and used, or prepared by adjusting the cell membrane fractions of the

aforementioned cells for use. Further, monkey TLR7, mouse TLR7, and rat TLR7 can be obtained by in-vitro synthesis or production in host cells by genetic engineering.

[0023]

The polynucleotide sequences encoding the amino acid sequences of human TLR7 (variant 1) and human TLR7 (variant 2) are set forth in SEQ ID NO: 1 and SEQ ID NO: 3, respectively in the Sequence Listing, and the amino acid sequences of human TLR7 (variant 1) and human TLR7 (variant 2) are set forth in SEQ ID NO: 2 and SEQ ID NO: 4, respectively in the Sequence Listing.

[0024]

The cDNA of human TLR7 can be obtained, for example, by the so-called PCR method, with polymerase chain reaction (which will be hereinafter referred to as "PCR") (Saiki, R. K., et al., Science, (1988) 239, 487-49) using a cDNA library from an organ expressing the mRNA of human TLR7 as a template and a primer which specifically amplifies the cDNA of human TLR7.

[0025]

The cDNA of human TLR7 also includes a polynucleotide which hybridizes with a polynucleotide consisting of a nucleotide sequence complementary to the nucleotide sequence encoding human TLR7 under stringent conditions and encodes a protein having a biological activity equivalent to human TLR7. Further, the cDNA of

human TLR7 also includes a splicing variant transcribed from the human TLR7 gene locus or a polynucleotide which hybridizes with the splicing variant under stringent conditions and encodes a protein having a biological activity equivalent to human TLR7.

[0026]

Further, human TLR7 also includes a protein which consists of the amino acid sequence of human TLR7 or the amino acid sequence excluding the signal sequence with one, two, three, four, or five amino acids substituted, deleted, or added and has a biological activity equivalent to human TLR7. Further, human TLR7 also includes a protein which consists of the amino acid sequence encoded by the splicing variant transcribed from the human TLR7 gene locus or the amino acid sequence with one, two, three, four, or five amino acids substituted, deleted, or added and has a biological activity equivalent to human TLR7.

[0027]

The polynucleotide sequence encoding the amino acid sequence of monkey TLR7 is set forth in SEQ ID NO: 58 in the Sequence Listing, and the amino acid sequence of monkey TLR7 is set forth in SEQ ID NO: 85 in the Sequence Listing.

[0028]

The cDNA of monkey TLR7 can be obtained, for example, by the PCR method using a cDNA library from an organ

expressing the mRNA of monkey TLR7 as a template and a primer which specifically amplifies the cDNA of monkey TLR7.

[0029]

The cDNA of monkey TLR7 also includes a polynucleotide which hybridizes with a polynucleotide consisting of a nucleotide sequence complementary to the nucleotide sequence encoding monkey TLR7 under stringent conditions and encodes a protein having a biological activity equivalent to monkey TLR7. Further, the cDNA of monkey TLR7 also includes a splicing variant transcribed from the monkey TLR7 gene locus or a polynucleotide which hybridizes with the splicing variant under stringent conditions and encodes a protein having a biological activity equivalent to monkey TLR7.

[0030]

Further, monkey TLR7 also includes a protein which consists of the amino acid sequence of monkey TLR7 or the amino acid sequence excluding the signal sequence with one, two, three, four, or five amino acids substituted, deleted, or added and has a biological activity equivalent to monkey TLR7. Further, monkey TLR7 also includes a protein which consists of the amino acid sequence encoded by the splicing variant transcribed from the monkey TLR7 gene locus or the amino acid sequence with one, two, three, four, or five amino acids

substituted, deleted, or added and has a biological activity equivalent to monkey TLR7.

[0031]

The polynucleotide sequence encoding the amino acid sequence of monkey TLR7 is set forth in SEQ ID NO: 58 in the Sequence Listing, and the amino acid sequence of monkey TLR7 is set forth in SEQ ID NO: 85 in the Sequence Listing.

[0032]

The cDNA of monkey TLR7 can be obtained, for example, by the PCR method using a cDNA library from an organ expressing the mRNA of monkey TLR7 as a template and a primer which specifically amplifies the cDNA of monkey TLR7.

[0033]

The polynucleotide sequence encoding the amino acid sequence of mouse TLR7 is set forth in SEQ ID NO: 56 in the Sequence Listing, and the amino acid sequence of mouse TLR7 is set forth in SEQ ID NO: 83 in the Sequence Listing.

[0034]

The cDNA of mouse TLR7 can be obtained, for example, by the PCR method using a cDNA library from an organ expressing the mRNA of mouse TLR7 as a template and a primer which specifically amplifies the cDNA of mouse TLR7.

[0035]

The polynucleotide sequence encoding the amino acid sequence of rat TLR7 is set forth in SEQ ID NO: 57 in the Sequence Listing, and the amino acid sequence of rat TLR7 is set forth in SEQ ID NO: 84 in the Sequence Listing.

[0036]

The cDNA of rat TLR7 can be obtained, for example, by the PCR method using a cDNA library from an organ expressing the mRNA of rat TLR7 as a template and a primer which specifically amplifies the cDNA of rat TLR7.

[0037]

2. Anti-TLR7 antibody and production thereof

The present invention provides an antibody or an antigen-binding fragment of the antibody having the properties of:

- (a) specifically binding to human TLR7 or monkey TLR7 and not binding to mouse TLR7 or rat TLR7; and
- (b) inhibiting a function of the human TLR7 or the monkey TLR7.

[0038]

The antibody or the antigen-binding fragment of the antibody of the present invention binds specifically to human TLR7 or monkey TLR7 and inhibits the functions thereof.

[0039]

The binding activity of the antibody or the antigen-binding fragment of the antibody to TLR7 is evaluated by

flow cytometry analysis according to a conventional method.

[0040]

In this description, inhibiting a function of TLR7 means suppressing the production of IL-6 and/or type I interferon by TLR7-expressing cells. The activity of inhibiting a function of TLR7 is evaluated by incubating TLR7-expressing cells (for example, PBMCs) in vitro in the presence of the antibody or the antigen-binding fragment of the antibody and measuring the concentration of IL-6 or type I interferon in the culture supernatant.

[0041]

In one embodiment of the present invention, the human TLR7 is a molecule consisting of the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4, the monkey TLR7 is a molecule consisting of the amino acid sequence set forth in SEQ ID NO: 85, the mouse TLR7 is a molecule consisting of the amino acid sequence set forth in SEQ ID NO: 83, or the rat TLR7 is a molecule consisting of the amino acid sequence set forth in SEQ ID NO: 84. For example, the human TLR7 can be a molecule consisting of the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4, the monkey TLR7 can be a molecule consisting of the amino acid sequence set forth in SEQ ID NO: 85, the mouse TLR7 can be a molecule consisting of the amino acid sequence set forth in SEQ ID NO: 83, and the rat TLR7 can be a molecule consisting of the amino

acid sequence set forth in SEQ ID NO: 84, in the present invention.

[0042]

For example, the antibody or the antigen-binding fragment of the antibody of the present invention can be an antibody against human TLR7 or an antigen-binding fragment of the antibody having the properties of:

(a) specifically binding to human TLR7 and not binding to mouse TLR7 and/or rat TLR7; and

(b) inhibiting a function of human TLR7, wherein the human TLR7 is a molecule consisting of the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4, the mouse TLR7 is a molecule consisting of the amino acid sequence set forth in SEQ ID NO: 83, and the rat TLR7 is a molecule consisting of the amino acid sequence set forth in SEQ ID NO: 84.

[0043]

For example, the antibody or the antigen-binding fragment of the antibody of the present invention can be an antibody to monkey TLR7 or an antigen-binding fragment of the antibody having the properties of:

(a) specifically binding to monkey TLR7 and not binding to mouse TLR7 and/or rat TLR7; and

(b) inhibiting a function of monkey TLR7, wherein the monkey TLR7 is a molecule consisting of the amino acid sequence set forth in SEQ ID NO: 85, the mouse TLR7 is a molecule consisting of the amino acid sequence set forth

in SEQ ID NO: 83, and the rat TLR7 is a molecule consisting of the amino acid sequence set forth in SEQ ID NO: 84.

[0044]

Hereinafter, the anti-TLR7 antibody of the present invention and a production method thereof are described with reference to the case of anti-human TLR7 antibody as an example. Anti-monkey TLR7 antibody also can be produced in the same manner as the production method of anti-human TLR7 antibody.

[0045]

The antibody to human TLR7 of the present invention can be obtained by immunizing an animal with human TLR7 or any polypeptide selected from the amino acid sequence of human TLR7, and collecting and purifying the antibody produced in vivo, using conventional methods.

[0046]

Human TLR7 serving as an antigen can be obtained by expressing a human TLR7 gene in a host cell by genetic engineering. Specifically, a vector capable of expressing the TLR7 gene may be prepared and introduced into a host cell to express the gene, and the TLR7 expressed may be purified.

[0047]

The antibody to human TLR7 of the present invention can be obtained also by DNA immunization. The DNA immunization is a technique to induce immunity to an

antigen by transfection of an antigen-expressing plasmid into an animal individual such as a mouse or a rat, thereby allowing the individual to express the antigen.

[0048]

Examples of the transfection technique include a method of directly injecting the plasmid into a muscle, a method of injecting a conjugate of the plasmid with a liposome, polyethyleneimine, or the like into a vein, a technique using viral vectors, a technique of injecting gold particles with the plasmid attached using a Gene Gun, and a hydrodynamic method of rapidly injecting a large amount of a solution of the plasmid into a vein.

[0049]

For improving the expression level in the transfection method by intramuscular injection of the expression plasmid, a technique of intramuscularly injecting the plasmid and thereafter applying electroporation to the same site (Aihara H, Miyazaki J. Nat Biotechnol. 1998 Sep; 16(9): 867-70 or Mir LM, Bureau MF, Gehl J, Rangara R, Rouy D, Caillaud JM, Delaere P, Branellec D, Schwartz B, Scherman D. Proc Natl Acad Sci U S A. 1999 Apr 13; 96(8): 4262-7) is known as in-vivo electroporation. In this technique, the expression level is further improved by treating a muscle with hyaluronidase before intramuscular injection of the plasmid (McMahon JM, Signori E, Wells KE, Fazio VM, Wells DJ. Gene Ther. 2001 Aug; 8(16): 1264-70).

[0050]

Further, a monoclonal antibody can also be obtained, according to a known method (for example, Kohler and Milstein, Nature (1975) 256, p. 495-497, Kennet, R. ed., Monoclonal Antibodies, p. 365-367, Plenum Press, N.Y. (1980)), by fusing an antibody-producing cell which produces an antibody to TLR7 with a myeloma cell to establish a hybridoma. Specific examples of such a method include those described in International publication No. WO 09/48072 (published on April 16, 2009) and International publication No. WO 10/117011 (published on October 14, 2010).

[0051]

Examples of the mouse anti-human TLR7 antibody established as above can include the AT01 antibody, the NB7 antibody, and the FAN2 antibody.

[0052]

The amino acid sequence of the heavy-chain variable region of the AT01 antibody is set forth in SEQ ID NO: 5 in the Sequence Listing, and the polynucleotide sequence encoding the amino acid sequence is set forth in SEQ ID NO: 6 in the Sequence Listing. The amino acid sequence of the heavy-chain variable region of the NB7 antibody is set forth in SEQ ID NO: 7 in the Sequence Listing, and the polynucleotide sequence encoding the amino acid sequence is set forth in SEQ ID NO: 8 in the Sequence Listing. The amino acid sequence of the heavy-chain

variable region of the FAN2 antibody is set forth in SEQ ID NO: 9 in the Sequence Listing, and the polynucleotide sequence encoding the amino acid sequence is set forth in SEQ ID NO: 10 in the Sequence Listing.

[0053]

Further, the amino acid sequence of the light-chain variable region of the AT01 antibody is set forth in SEQ ID NO: 11 in the Sequence Listing, and the polynucleotide sequence encoding the amino acid sequence is set forth in SEQ ID NO: 12 in the Sequence Listing. The amino acid sequence of the light-chain variable region of the NB7 antibody is set forth in SEQ ID NO: 13 in the Sequence Listing, and the polynucleotide sequence encoding the amino acid sequence is set forth in SEQ ID NO: 14 in the Sequence Listing. The amino acid sequence of the light-chain variable region of the FAN2 antibody is set forth in SEQ ID NO: 15 in the Sequence Listing, and the polynucleotide sequence encoding the amino acid sequence is set forth in SEQ ID NO: 16 in the Sequence Listing.

[0054]

The heavy-chain variable region of the AT01 antibody comprises CDRH1 consisting of the amino acid sequence set forth in SEQ ID NO: 17 (GYTFTNNWLH), CDRH2 consisting of the amino acid sequence set forth in SEQ ID NO: 18 (DIYPSNGRTN), and CDRH3 consisting of the amino acid sequence set forth in SEQ ID NO: 19 (ERGYFDY). Further, the light-chain variable region of the antibody comprises

CDRL1 consisting of the amino acid sequence set forth in SEQ ID NO: 20 (KASQDINKYIA), CDRL2 consisting of the amino acid sequence set forth in SEQ ID NO: 21 (YTSTLQP), and CDRL3 consisting of the amino acid sequence set forth in SEQ ID NO: 22 (LQYDYLLT). The amino acid sequences of the aforementioned CDRs are shown also in Figure 24-1.

[0055]

The heavy-chain variable region of the NB7 antibody comprises CDRH1 consisting of the amino acid sequence set forth in SEQ ID NO: 23 (GYSITSDYAWN), CDRH2 consisting of the amino acid sequence set forth in SEQ ID NO: 24 (HISYRGNTN), and CDRH3 consisting of the amino acid sequence set forth in SEQ ID NO: 25 (WNYYGYVDYAMDY). Further, the light-chain variable region of the antibody comprises CDRL1 consisting of the amino acid sequence set forth in SEQ ID NO: 26 (RASQDISNYLN), CDRL2 consisting of the amino acid sequence set forth in SEQ ID NO: 27 (YTSRLHS), and CDRL3 consisting of the amino acid sequence set forth in SEQ ID NO: 28 (QQGDTFPT). The amino acid sequences of the aforementioned CDRs are shown also in Figure 24-2.

[0056]

The heavy-chain variable region of the FAN2 antibody comprises CDRH1 consisting of the amino acid sequence set forth in SEQ ID NO: 29 (GFSLTGYGVN), CDRH2 consisting of the amino acid sequence set forth in SEQ ID NO: 30 (MIWGDGSTD), and CDRH3 consisting of the amino acid

sequence set forth in SEQ ID NO: 31 (DKGYDGYYYAMDY). Further, the light-chain variable region of the antibody comprises CDRL1 consisting of the amino acid sequence set forth in SEQ ID NO: 32 (RASENIYSYLA), CDRL2 consisting of the amino acid sequence set forth in SEQ ID NO: 33 (DAKTLAE), and CDRL3 consisting of the amino acid sequence set forth in SEQ ID NO: 34 (QHGYGIPYT). The amino acid sequences of the aforementioned CDRs are shown also in Figure 24-3.

[0057]

In one embodiment, the antibody or the antigen-binding fragment of the antibody of the present invention can have a competitive inhibitory activity for binding to the human TLR7 with an antibody comprising the amino acid sequences of the heavy-chain variable region and the light-chain variable region of the AT01 antibody, the NB7 antibody or the FAN2 antibody.

[0058]

In another embodiment, the antibody or the antigen-binding fragment of the antibody of the present invention can be an antibody or an antigen-binding fragment of the antibody comprising CDRH1 to CDRH3 of the AT01 antibody as complementarity determining regions in the heavy chain and CDRL1 to CDRL3 of the AT01 antibody as complementarity determining regions in the light chain, an antibody or an antigen-binding fragment of the antibody comprising CDRH1 to CDRH3 of the NB7 antibody as

complementarity determining regions in the heavy chain and CDRL1 to CDRL3 of the NB7 antibody as complementarity determining regions in the light chain, or an antibody or an antigen-binding fragment of the antibody comprising CDRH1 to CDRH3 of the FAN2 antibody as complementarity determining regions in the heavy chain and CDRL1 to CDRL3 of the FAN2 antibody as complementarity determining regions in the light chain. In a preferred aspect, the antibody or the antigen-binding fragment of the antibody of the present invention can be an antibody or an antigen-binding fragment of the antibody comprising CDRH1 to CDRH3 of the AT01 antibody as complementarity determining regions in the heavy chain and CDRL1 to CDRL3 of the AT01 antibody as complementarity determining regions in the light chain.

[0059]

In still another embodiment, the antibody or the antigen-binding fragment of the antibody of the present invention can be an antibody comprising the sequences of the heavy-chain variable region and the light-chain variable region of the AT01 antibody, the NB7 antibody or the FAN2 antibody.

[0060]

The antibody of the present invention includes recombinant antibodies artificially modified for the purpose of reducing the heteroantigenicity to humans and the like, such as chimeric antibodies, humanized

antibodies, and human antibodies, in addition to the aforementioned monoclonal antibodies to human TLR7.

These antibodies can be produced using known methods.

[0061]

Examples of the chimeric antibodies can include an antibody in which the variable region and the constant region of the antibody are heterogeneous to each other, such as a chimeric antibody with a variable region of a mouse- or rat-derived antibody joined to a human-derived constant region (see Proc. Natl. Acad. Sci. U. S. A., 81, 6851-6855, (1984)). Examples of the mouse anti-human TLR7 antibody (AT01 antibody)-derived chimeric antibody can include an antibody consisting of a heavy chain comprising a heavy-chain variable region consisting of the amino acid sequence of SEQ ID NO: 5 and a light chain comprising a light-chain variable region consisting of the amino acid sequence of SEQ ID NO: 11. Examples of the AT01 antibody-derived chimeric antibody can include an antibody consisting of a heavy chain consisting of the amino acid sequence at positions 20 to 465 in SEQ ID NO: 35 and a light chain consisting of an amino acid sequence at positions 21 to 233 in SEQ ID NO: 36. In this description, such an antibody is referred to as "cAT01" or "cAT01 antibody".

[0062]

Further, examples of the mouse anti-human TLR7 antibody (NB7 antibody)-derived chimeric antibody can

include an antibody consisting of a heavy chain comprising a heavy-chain variable region consisting of the amino acid sequence of SEQ ID NO: 7 and a light chain comprising a light-chain variable region consisting of the amino acid sequence of SEQ ID NO: 13. Examples of the NB7 antibody-derived chimeric antibody can include an antibody consisting of a heavy chain consisting of an amino acid sequence at positions 20 to 471 in SEQ ID NO: 37 and a light chain consisting of an amino acid sequence at positions 21 to 233 in SEQ ID NO: 38. In this description, such an antibody is referred to as "cNB7" or "cNB7 antibody".

[0063]

Further, examples of the mouse anti-human TLR7 antibody (FAN2 antibody)-derived chimeric antibody can include an antibody consisting of a heavy chain comprising a heavy-chain variable region consisting of the amino acid sequence of SEQ ID NO: 9 and a light chain comprising a light-chain variable region consisting of the amino acid sequence of SEQ ID NO: 15. Examples of the FAN2 antibody-derived chimeric antibody can include an antibody consisting of a heavy chain consisting of an amino acid sequence at positions 20 to 470 in SEQ ID NO: 39 and a light chain consisting of an amino acid sequence at positions 21 to 234 in SEQ ID NO: 40. In this description, such an antibody is referred to as "cFAN2" or "cFAN2 antibody".

[0064]

Humanized antibodies that are recombinant antibodies can be produced by artificially modifying the sequences of the aforementioned chimeric antibodies to human TLR7 for the purpose of reducing the heteroantigenicity to humans and the like. Further, the antibody of the present invention includes antibodies with modified CDRs of the humanized antibodies. These antibodies can be produced using known methods.

[0065]

Examples of the humanized antibodies can include an antibody with only the CDRs integrated into a human-derived antibody (see Nature (1986) 321, p. 522-525), and an antibody with not only the sequences of CDRs but also the amino acid residues of some parts of the framework grafted into a human antibody (International publication No. WO 90/07861).

[0066]

For example, humanized antibodies derived from the cAT01 antibody and the cNB7 antibody retain all of the 6 CDR sequences derived from cAT01 or cNB7 and have the activity of inhibiting a function of human TLR7.

[0067]

Suitable cases of the aforementioned humanized antibodies can include cAT01 antibody-derived humanized antibodies (huAT01 antibodies) such as: an antibody consisting of a heavy chain comprising a heavy-chain

variable region consisting of the amino acid sequence set forth in SEQ ID NO: 41 and a light chain comprising a light-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 43; an antibody consisting of a heavy chain comprising a heavy-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 41 and a light chain comprising a light-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 44; an antibody consisting of a heavy chain comprising a heavy-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 42 and a light chain comprising a light-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 43; and an antibody consisting of a heavy chain comprising a heavy-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 42 and a light chain comprising a light-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 44.

[0068]

Further, an antibody having an activity equivalent to the aforementioned huAT01 antibodies can be selected by combining sequences exhibiting high homology with the amino acid sequence of the heavy-chain variable region and the amino acid sequence of the light-chain variable region of the aforementioned huAT01 antibodies respectively. The 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 (however, the CDRs are the same as in the aforementioned antibodies). Further, an antibody having an activity equivalent to the aforementioned antibodies can also be selected by combining an amino acid sequence with one to several (for example, 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid residues substituted, deleted, or added in the amino acid sequence of the heavy-chain variable region or the light-chain variable region (however, excluding the CDR sites).
[0069]

Further suitable cases can include: an antibody consisting of a heavy chain consisting of the amino acid sequence at positions 20 to 465 in the amino acid sequence set forth in SEQ ID NO: 45 and a light chain consisting of the amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 48; an antibody consisting of a heavy chain consisting of the amino acid sequence at positions 20 to 465 in the amino acid sequence set forth in SEQ ID NO: 45 and a light chain consisting of the amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 49; an antibody consisting of a heavy chain consisting of the amino acid sequence at positions 20 to 465 in the amino acid sequence set forth in SEQ ID NO: 46 and a light chain consisting of the amino acid sequence

at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 48; an antibody consisting of a heavy chain consisting of the amino acid sequence at positions 20 to 465 in the amino acid sequence set forth in SEQ ID NO: 46 and a light chain consisting of the amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 49; an antibody consisting of a heavy chain consisting of the amino acid sequence at positions 20 to 462 in the amino acid sequence set forth in SEQ ID NO: 47 and a light chain consisting of the amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 48; or an antibody consisting of a heavy chain consisting of the amino acid sequence at positions 20 to 462 in the amino acid sequence set forth in SEQ ID NO: 47 and a light chain consisting of the amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 49.

[0070]

Further, examples of the cNB7 antibody-derived humanized antibody (huNB7 antibody) can include an antibody consisting of a heavy chain comprising a heavy-chain variable region consisting of the amino acid sequence of SEQ ID NO: 50 and a light chain comprising a light-chain variable region consisting of the amino acid sequence of SEQ ID NO: 51.

[0071]

Further, an antibody having an activity equivalent to the aforementioned huNB7 antibody can be selected by combining sequences exhibiting high homology with the amino acid sequence of the heavy-chain variable region and the amino acid sequence of the light-chain variable region of the aforementioned huNB7 antibody. The 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 (however, the CDRs are the same as in the aforementioned antibodies). Further, an antibody having an activity equivalent to the aforementioned antibody can also be selected by combining an amino acid sequence with one to several (for example, 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid residues substituted, deleted, or added in the amino acid sequence of the heavy-chain variable region or the light-chain variable region (however, excluding the CDR sites).

[0072]

Further suitable cases can include: an antibody consisting of a heavy chain consisting of the amino acid sequence at positions 20 to 471 in the amino acid sequence set forth in SEQ ID NO: 52 and a light chain consisting of the amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 53; or an antibody consisting of a heavy chain consisting of the amino acid sequence at positions 20 to 468 in the

amino acid sequence set forth in SEQ ID NO: 54 and a light chain consisting of the amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 53.

[0073]

The antibody of the present invention may be obtained by introducing further mutation into the aforementioned humanized antibodies to change the binding ability with respect to human TLR7. Such a technique is called affinity maturation, and specific examples thereof can include the ribosome display method. The ribosome display method is a method of isolating a gene sequence of a protein which binds to a target molecule, using a tripartite complex in which the protein and mRNA carrying the genetic information of the protein are bound together via ribosomes (Stafford RL. et. al. Protein Eng. Des. Sel. 2014 (4): 97-109).

[0074]

In order to avoid injuring normal human TLR7-expressing cells, the antibody desirably has low effector activity. The effector activity is known to differ depending on the subclass of the antibody. For example, a feature of IgG4 is that it has low ADCC and CDC activities, and a feature of IgG2 is that it has CDC activity while having low ADCC activity. Based on such features, an antibody with reduced ADCC and CDC activities can be produced by substituting the constant

region of IgG1 with the constant region of IgG2 or IgG4. Further, an IgG1 antibody with reduced ADCC and CDC activities can be produced by partially substituting the sequence of the constant region of IgG1 with reference to IgG2 or IgG4. As an example, Marjan Hezareh et. al. Journal of Virology, 75 (24): 12161-12168 (2001) shows that the ADCC and CDC activities are reduced by substituting leucine residues 234 and 235 (the numerals represent EU indices by Kabat et. al.) of IgG1 respectively with alanine residues.

[0075]

The present invention includes modified forms of the antibody or the antigen-binding fragment of the antibody. The modified forms mean those formed by chemically or biologically modifying the antibody or the antigen-binding fragment of the antibody of the present invention. The chemically modified forms include chemically modified forms of carbohydrate chains by attachment of a chemical moiety to the amino acid backbone, N-linkage, or O-linkage. The biologically modified forms include those modified after translation (for example, by glycosylation to N-linkage or O-linkage, N-terminal or C-terminal processing, deamidation, isomerization of aspartic acid, and oxidation of methionine), and those with a methionine residue added at the N-terminal by expression using prokaryote host cells. Further, the meaning of the modified products also includes those labeled for

enabling detection or isolation of the antibody of the present invention or antigen, such as enzyme-labeled bodies, fluorescence-labeled bodies, and affinity-labeled bodies. Such modified products of the antibody or the antigen-binding fragment of the antibody of the present invention are useful for improving the stability and the blood retention of the original antibody of the present invention, reducing the antigenicity, and detecting or isolating the antibody or antigen.

[0076]

It is known that a lysine residue at the carboxyl terminus of the heavy chain of antibodies produced in cultured mammalian cells is deleted (Journal of Chromatography A, 705: 129-134 (1995)). Further, it is also known that two amino acid residues, glycine and lysine, at the carboxyl terminus of the same heavy chain are deleted, and a proline residue newly located at the carboxyl terminus is amidated (Analytical Biochemistry, 360: 75-83 (2007)).

[0077]

However, such deletions and modifications of the heavy-chain sequence have no influence on the antigen-binding ability and the effector functions (such as complement activation and antibody-dependent cellular cytotoxicity) of the antibody.

[0078]

Accordingly, examples of modified forms of the antibody and the antigen-binding fragment of the antibody of the present invention can include: deletion variants with one or two amino acids deleted at the carboxyl terminus of the heavy chain; and amidated deletion variants (such as a heavy chain with an amidated proline residue at the carboxyl terminal part). However, the deletion variants at the carboxyl terminus of the heavy chain of the antibody according to the present invention are not limited to the aforementioned types, as long as the antigen-binding ability and the effector functions are maintained.

[0079]

Two heavy chains constituting the antibody according to the present invention may be a combination of any one or two types of heavy chains selected from the group consisting of the full length and the aforementioned deletion variants. The quantitative ratio of the deletion variants can be affected by the type of cultured mammalian cells and culture conditions for producing the antibody according to the present invention, but examples of the main component of the antibody according to the present invention can include a component with one amino acid residue deleted at the carboxyl terminus in each of the two heavy chains. That is, heavy chains consisting of amino acid sequences with one or two amino acids deleted at the carboxyl terminus of each heavy-chain

sequence represented by an amino acid sequence at positions 20 to 465 in SEQ ID NO: 35, an amino acid sequence at positions 20 to 471 in SEQ ID NO: 37, an amino acid sequence at positions 20 to 470 in SEQ ID NO: 39, an amino acid sequence at positions 20 to 465 in SEQ ID NO: 45, an amino acid sequence at positions 20 to 465 in SEQ ID NO: 46, an amino acid sequence at positions 20 to 462 in SEQ ID NO: 47, an amino acid sequence at positions 20 to 471 in SEQ ID NO: 52, and an amino acid sequence at positions 20 to 468 in SEQ ID NO: 54, in the Sequence Listing can also be used for the antibody of the present invention.

[0080]

The antibodies obtained by the aforementioned methods are evaluated for their binding property with respect to an antigen, so that a suitable antibody can be selected therefrom. Another indicator for comparing the properties of the antibodies can be the stability of the antibodies, for example. Differential scanning calorimetry (DSC) is a method that enables quick and accurate measurement of the thermal denaturation midpoint (T_m), which is a good indicator of the relative structural stability of proteins. The differences in thermostability can be compared by measuring the T_m values using DSC and comparing the values. It is known that the storage stability of antibodies shows some correlation with the thermostability of antibodies (Lori

Burton, et. al., *Pharmaceutical Development and Technology* (2007) 12, p. 265-273), and a suitable antibody can be selected using the thermostability as an indicator. Examples of other indicators for selecting an antibody can include high yield in a suitable host cell and low aggregability in an aqueous solution. Since the antibody, for example, with the highest yield does not necessarily show the highest thermostability, comprehensive determination needs to be made based on the aforementioned indicators to select an antibody that is most suitable for administration to humans.

[0081]

Further, a method of obtaining a single-chain immunoglobulin by linking the full-length sequences of the heavy and light chains of an antibody using a suitable linker is also known (Lee, H-S, et. al., *Molecular Immunology* (1999) 36, p. 61-71; Schirrmann, T. et. al., *mAbs* (2010), 2, (1) p. 73-76). Such a single-chain immunoglobulin is dimerized and thus can maintain a structure and an activity that are similar to those of an antibody that was originally a tetramer. Further, the antibody of the present invention may be an antibody comprising a single heavy-chain variable region and no light-chain sequences. Such an antibody is called a single domain antibody (sdAb) or nanobody and is reported to be actually observed in camels or llamas and maintain antigen-binding ability (Muyldermans S. et. al., *Protein*

Eng. (1994) 7 (9), 1129-35, Hamers-Casterman C. et. al., Nature (1993) 363 (6428) 446-8). The aforementioned antibody can also be understood to be a kind of antigen-binding fragment of the antibody of the present invention. [0082]

Further, the antibody-dependent cellular cytotoxicity can be enhanced by adjusting the glycosylation bound to the antibody of the present invention. International publication No. WO 99/54342, International publication No. WO 00/61739, International publication No. WO 02/31140, International publication No. WO 2007/133855, International publication No. WO 2013/120066, and the like are known as techniques for adjusting glycosylation of antibodies, but there is no limitation to these. [0083]

In the case of producing an antibody by isolating an antibody gene once and thereafter introducing it into a suitable host cell, a combination of such a suitable host cell and an expression vector can be used. [0084]

Specific examples of the antibody gene can include a combination of a gene encoding the heavy-chain sequence and a gene encoding the light-chain sequence of the antibodies in this specification. More specifically, examples of the antibody gene can include a combination of a polynucleotide comprising a polynucleotide sequence

encoding the antibody or the antigen-binding fragment of the antibody of the present invention, a polynucleotide comprising a polynucleotide sequence encoding the amino acid sequence of the heavy-chain variable region of the antibody or the antigen-binding fragment of the antibody of the present invention, and a polynucleotide comprising a polynucleotide sequence encoding the amino acid sequence of the light-chain variable region of the antibody or the antigen-binding fragment of the antibody of the present invention. The heavy-chain sequence gene constituting the antibody gene also includes a polynucleotide comprising a polynucleotide sequence of a polynucleotide which hybridizes with a polynucleotide consisting of a polynucleotide sequence complementary to the aforementioned polynucleotide sequence encoding the heavy-chain variable region under stringent conditions. Further, the light-chain sequence gene constituting the antibody gene also includes a polynucleotide comprising a polynucleotide sequence of a polynucleotide which hybridizes with a polynucleotide consisting of a polynucleotide sequence complementary to the aforementioned polynucleotide sequence encoding the light-chain variable region under stringent conditions.

[0085]

When transforming the host cell, the heavy-chain sequence gene and the light-chain sequence gene can be inserted in the same expression vector or in separate

expression vectors. In the case of using a eukaryotic cell as a host cell, animal cells, plant cells, and eukaryotic microorganisms can be used. Examples of the animal cells can include (1) mammalian cells such as COS cells (Gluzman, Y. Cell (1981) 23, p. 175-182, ATCC CRL-1650) which are monkey cells, and dihydrofolate reductase enzyme-deficient strains (Urlaub, G. and Chasin, L. A. Proc. Natl. Acad. Sci. U. S. A. (1980) 77, p. 4126-4220) of mouse fibroblasts NIH3T3 (ATCC No. CRL-1658) and Chinese hamster ovary cells (CHO cells, ATCC CCL-61). Further, in the case of using prokaryotic cells, examples can include Escherichia coli and Bacillus subtilis. An antibody is obtained by introducing a target antibody gene into such a cell by transformation and culturing the transformed cell in vitro. Since the yield may differ depending on the sequence of the antibody in the aforementioned culture method, those easily produced as pharmaceuticals can be selected from amongst antibodies having the same binding activity using the yield as an indicator.

[0086]

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

[0087]

Further, the antibody of the present invention may be an antigen-binding fragment of an antibody comprising an antigen-binding site of the antibody or a modified product thereof. A fragment of the antibody can be obtained by treating the antibody with a protease such as papain and pepsin or modifying the antibody gene with a genetically engineered technique to allow expression in a suitable cultured cell. Among such antibody fragments, fragments maintaining all or part of the functions of the full-length molecules of the antibody can be called antigen-binding fragments of the antibody.

[0088]

Examples of the functions of antibodies can generally include antigen-binding activity, the activity of neutralizing antigen activity, the activity of enhancing antigen activity, antibody-dependent cytotoxicity, complement-dependent cytotoxicity, and complement-dependent cellular cytotoxicity. The function of the antigen-binding fragment of the antibody in the present invention is binding activity to human TLR7, preferably the activity of inhibiting a function of human TLR7, more preferably the activity of suppressing production of IL-6 and/or type I interferon by TLR7-expressing cells.

[0089]

Examples of the fragment of the antibody can include Fab, F(ab')₂, Fv, or single-chain Fv (scFv) obtained by

linking heavy- and light-chain Fvs with a suitable linker, diabodies, linear antibodies, and multispecific antibodies formed from antibody fragments. Further, the fragment of the antibody also includes a monovalent Fab' fragment in a variable region of the antibody obtained by treating F(ab')₂ under reduced conditions.

[0090]

Further, the antibody of the present invention may be a multispecific antibody having specificity to at least two types of different antigens. In general, such a molecule binds to two types of antigens (that is, a bispecific antibody), but the "multispecific antibody" in the present invention includes antibodies having specificity to more (for example, three types of) antigens.

[0091]

The multispecific antibody of the present invention may be an antibody consisting of the full-length or a fragment of such an antibody (for example, F(ab')₂ bispecific antibody). The bispecific antibody can be produced also by binding the heavy and light chains (HL pairs) of two types of antibodies to each other or producing a bispecific antibody-producing fusion cell by fusing hybridomas which produce different monoclonal antibodies (Millstein et al., Nature (1983) 305, p. 537-539).

[0092]

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

[0093]

Methods for producing single-chain antibodies are known in this technical field (for example, see U.S. Patent No. 4,946,778, U.S. Patent No. 5,260,203, U.S. Patent No. 5,091,513, and U.S. Patent No. 5,455,030). In such a scFv, the heavy-chain variable region and the light-chain variable region are linked via a linker which does not produce conjugates, preferably a polypeptide linker (Huston, J. S. et al., *Proc. Natl. Acad. Sci. U. S. A.* (1988), 85, p. 5879-5883). The heavy-chain variable region and the light-chain variable region in the scFv may be derived from the same antibody or separate antibodies. Any single-chain peptide consisting of 12 to 19 residues is, for example, used as the polypeptide linker which links the variable regions.

[0094]

DNA encoding the scFv is obtained by PCR amplification using a DNA element encoding the whole or a desired part of the amino acid sequence of DNA encoding the heavy chain or the heavy-chain variable region of the antibody and DNA encoding the light chain or the light-chain variable region thereof as a template and a primer pair defining both ends thereof, and further amplification by combining DNA encoding a polypeptide linker portion and a primer pair defining both ends thereof so as to be linked respectively with the heavy chain and the light chain.

[0095]

Further, once DNA encoding the scFv is produced, an expression vector containing the DNA and a host cell transformed by the expression vector can be obtained according to conventional methods. Further, the scFv can be obtained using the host cell according to a conventional method. Such an antibody fragment can be produced by obtaining and expressing a gene thereof in a host cell as described above.

[0096]

The antibody of the present invention may be an antibody multimerized to increase the affinity for the antigen. The antibody to be multimerized may be one antibody or a plurality of antibodies which recognize a plurality of epitopes of the same antigen. Examples of the method for multimerizing the antibody can include

binding of an IgG CH3 domain to two scFvs, binding to streptavidin, and introduction of a helix-turn-helix motif.

[0097]

The antibody of the present invention may be a polyclonal antibody which is a mixture of a plurality of types of anti-human TLR7 antibodies comprising different amino acid sequences. As an example of the polyclonal antibody, there is a mixture of a plurality of types of antibodies comprising different CDRs. As such a polyclonal antibody, an antibody purified from a culture obtained by culturing a mixture of cells which produce different antibodies can be used (see International publication No. WO 2004/061104).

[0098]

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

[0099]

The antibody of the present invention may further be an immunoconjugate formed by such an antibody and another drug. Examples of the antibody can include the antibody bound to a radioactive substance or a compound having a pharmacological action (Nature Biotechnology (2005) 23, p. 1137-1146).

[0100]

The antibody obtained can be purified to homogeneity. For separating and purifying the antibody, common separation and purification methods used for proteins may be used. For example, the antibody can be separated and purified by appropriately selecting and combining column chromatography, filter filtration, ultrafiltration, salting-out, dialysis, preparative polyacrylamide gel electrophoresis, isoelectric focusing, 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 there is no limitation to these.

[0101]

Examples of the chromatography can include affinity chromatography, ion exchange chromatography, hydrophobic chromatography, gel filtration chromatography, reverse-phase chromatography, and adsorption chromatography. Such chromatography can be performed using liquid chromatography such as HPLC and FPLC. Examples of the column used for the affinity chromatography can include a protein A column and a protein G column. Examples of a column which is a protein A column can include Hyper D, POROS, Sepharose F. F. (pharmacia). Further, the antibody can also be purified, using a carrier with an

immobilized antigen, by utilizing the binding property to the antigen.

[0102]

3. Pharmaceutical containing anti-human TLR7 antibody

Antibodies that inhibit a function of human TLR7 can be obtained from the anti-human TLR7 antibodies obtained by the methods disclosed in "2. Anti-TLR7 antibody and production thereof" above. These antibodies that inhibit a function of human TLR7 can inhibit the biological activity of human TLR7 in vivo, that is, activation of human TLR7-expressing cells typified by blood cells by human TLR7 ligands, and therefore can be used as pharmaceuticals such as agents for treating and/or preventing diseases caused by a function of human TLR7.

[0103]

The diseases and conditions caused by a function of human TLR7 can include immune inflammation-related diseases, allergic diseases, infections, or cancers.

[0104]

Examples of the immune inflammation-related diseases can include diseases of the connective tissue and the musculoskeletal system (such as systemic lupus erythematosus, rheumatoid arthritis, juvenile idiopathic arthritis, adult-onset Still's disease, ankylosing spondylitis, systemic scleroderma, polymyositis, dermatomyositis, psoriatic arthritis, osteoarthritis, mixed connective tissue disease, and muscular dystrophy),

the blood system (such as autoimmune hemolytic anemia, aplastic anemia, and idiopathic thrombocytopenic purpura), the digestive tract system (such as Crohn's disease, ulcerative colitis, and ileitis), the hepatobiliary pancreatic system and the endocrine system (such as autoimmune hepatitis, viral hepatitis, alcoholic hepatitis, nonalcoholic steatohepatitis, primary sclerosing cholangitis, primary biliary cirrhosis, Sjogren's syndrome, type 1 diabetes, autoimmune thyroiditis, Graves' disease, and Hashimoto's thyroiditis), the respiratory system (such as chronic obstructive pulmonary disease, cystic fibrosis, and interstitial pneumonia), the cranial nervous system (such as multiple sclerosis, myasthenia gravis, meningitis, encephalomyelitis, and autoimmune encephalitis), the visual system (such as uveitis, trachoma, and endophthalmitis), the cardiovascular system (such as vasculitis syndrome, polyangiitis granulomatosis Wegener's granulomatosis, myocarditis, ischemic heart disease, and atherosclerosis), the skin epidermis system (such as psoriasis, pemphigus, vitiligo, contact dermatitis, and eczema), the renal system (such as glomerulonephritis, diabetic nephropathy, IgA nephropathy, purpura nephritis, nephrosis, and interstitial cystitis), and the endocrine system (such as type 1 diabetes, autoimmune thyroiditis, Graves' disease, and Hashimoto's thyroiditis), and systemic inflammation (such as Behcet's

disease, antiphospholipid antibody syndrome, IgG4-related diseases, sepsis, hemorrhage, hypersensitivity, transplantation rejection, and shock symptoms caused by, for example, cancer chemotherapy).

[0105]

Examples of the allergic diseases can include atopic dermatitis, asthma, anaphylaxis, anaphylactoid reactions, food allergy, rhinitis, otitis media, drug reactions, insect sting reactions, plant reactions, latex allergy, conjunctivitis, and urticaria.

[0106]

Examples of the infections can include diseases caused by infections by viruses (such as a single-stranded RNA virus, a double-stranded RNA virus, a single-stranded DNA virus, and a double-stranded DNA virus), bacteria (such as gram-negative bacteria, gram-positive bacteria, acid-fast bacteria, actinomycetes, spirochetes, spiral bacteria, Rickettsia, Chlamydia, and Mycoplasma), fungi (such as Trichophyton, Candida, Cryptococcus, Aspergillus, Pneumocystis, and Malassezia), and parasites (such as filariae, trematodes, cestodes, Distoma, Echinococcus, Entamoeba histolytica, fleas, lice, mites, Ascaridida, and Oxyuridae).

[0107]

Examples of the cancer treatment can include treatments for lymphoma, leukemia, breast cancer, lung cancer, and skin cancer.

[0108]

Examples of the anti-human TLR7 antibodies as pharmaceuticals can include chimeric antibodies and humanized antibodies, which are produced from the AT01 antibody and/or the NB7 antibody, and CDR modified forms thereof.

[0109]

The inhibitory activity against a function of human TLR7 by the anti-human TLR7 antibodies in vitro can be measured, for example, based on the activity of suppressing the activation of blood cells expressing human TLR7. For example, the activity of suppressing IL-6 release from human PBMCs by CL264 stimulation can be measured by adding each anti-human TLR7 antibody to human PBMCs at various concentrations.

[0110]

It has been experimentally proved and widely recognized that, in various immune inflammation-related diseases including systemic lupus erythematosus, the ligand concentration of human TLR7 is increased, human TLR7 expression is enhanced, or human TLR7 stimulation is caused. Inflammatory responses due to inflammatory cytokines such as interleukin 6 (IL-6) produced by the action of human TLR7 ligands on human TLR7-expressing cells and release of type I interferons due to enhancement and activation of antibody production cause systemic immune inflammatory responses and cause

development of, or worsen, autoimmunity such as systemic lupus erythematosus. Accordingly, suppressing the production of cytokines which depend on human TLR7 stimulation leads to prevention and treatment of systemic lupus erythematosus and the like, and the usefulness of a human TLR7 antibody as a remedy can be determined using suppression activity as an indicator.

[0111]

Examples of a suitable antibody in the present invention can include an antibody or an antigen-binding fragment of the antibody which binds specifically to human TLR7 comprising the amino acid sequence set forth in SEQ ID NO: 2 or 4 and reduces the amount of IL-6 released by CL264-treated human PBMCs in a concentration dependent manner.

[0112]

Further, the treatment or prevention effects by the anti-human TLR7 antibody on various diseases can be checked based on an in-vivo evaluation system or ex-vivo evaluation system by administration of the anti-human TLR7 antibody to monkeys cross-reactive with the antibody, or also based on the following in-vivo evaluation system by administration of the anti-human TLR7 antibody to human TLR7 transgenic and mouse TLR7 knockout mice.

[0113]

The effects on inflammatory cytokine production are evaluated by comparing the ability to produce cytokine in

the peripheral blood between the anti-TLR7 antibody group and the non-administration group, in inflammation induced by intraperitoneally or intravenously administering TLR7 ligands to mice.

[0114]

The effects on atopic dermatitis are evaluated by quantifying the behavior caused by atopic dermatitis spontaneously developed or induced by a method such as repeatedly applying a mite antigen cream to the auricle or the back 3 to 6 times every 3 days to 2 weeks, applying haptens to the auricle, the abdomen, or the back daily to once a week, administering a pruritus inducer intradermally in the auricle, subcutaneously in the back, or intrathecally, or using NC/Nga mice which are spontaneous atopic mice, through measurement of the frequency of pruritus using magnets mounted on both insteps of mice and a pruritus behavior measuring device, or inspection of the pathological features of the skin, the peripheral blood, the spinal cord tissue, and the like, and comparing the results between the anti-human TLR7 antibody group and the non-administration group.

[0115]

The effects on psoriasis are evaluated by quantifying psoriasiform dermatitis induced by a method such as applying Imiquimod or R848 to the auricle and the back after shaving, administering cytokines such as IL-23 to mice intradermally in the auricle, through such as

measurement of the weight and the thickness of the inflammation site or the myeloperoxidase activity of neutrophils infiltrating the site, flow cytometry analysis of the infiltrated cells, gene analysis, or measurement of the cytokine concentration, and comparing the results between the anti-TLR7 antibody group and the non-administration group.

[0116]

The effects on arthritis are evaluated by inducing arthritis by a method such as administering an emulsion obtained by mixing a bovine type II collagen solution and a complete Freund's adjuvant intradermally to the tail base of mice and thereafter administering an emulsion obtained by mixing a bovine type II collagen solution and an incomplete Freund's adjuvant 2 to 3 weeks later, or administering an anti-bovine type II collagen antibody and TLR ligands, and comparing the scoring of subsequent joint swelling, measurement of the thickness of the footpad, the concentrations of antibodies, cytokines or blood biomarkers in the blood or tissues, or the proliferative activity, the ability to produce cytokine or the surface antigens of the cells obtained from the peripheral blood, the spleen, the lymph nodes, the bone marrow, the joint sites, or the like, between the anti-human TLR7 antibody group and the non-administration group.

[0117]

The effects on colitis are evaluated by inducing colitis by a method such as administering trinitrobenzene sulfonic acid into the intestine of mice fasted for 24 hours, allowing ad libitum access to a 1 to 10% dextran sodium sulfate aqueous solution from a supply bottle for 4 days to 2 weeks, or transferring CD4+CD25-CD45RBhi T cells collected from the lymph nodes and the spleen of human TLR7 transgenic mice after purification intraperitoneally to Rag2-deficient mice, and comparing the body weight during the observation period, the thickening degree, the number and size of polyps, and the histopathological features of the intestine by autopsy after the completion of the test, the concentrations of antibodies, cytokines or blood biomarkers in the blood or tissues, the proliferative activity, the ability to produce cytokine or the surface antigens of the cells obtained from the intestine, the peripheral blood, the thymus, the spleen, the lymph nodes, the bone marrow, or Peyer's patches, or the like, between the anti-human TLR7 antibody group and the non-administration group.

[0118]

The effects on systemic lupus erythematosus are evaluated by comparing between the anti-human TLR7 antibody group and the non-administration group: the concentrations of antibodies, cytokines or blood biomarkers in the blood or tissues collected from NZB/WF1 mice, MRL/lpr mice and BXSB mice which are spontaneous

models over time, the antibody titer or the biomarker concentration in their urine, or a symptom induced by transferring cells prepared from an organ such as the spleen and the lymph nodes collected after the completion of the above test to untreated mice, or the like.

[0119]

The effects on hepatitis are evaluated by inducing hepatitis by a method such as administering D-galactosamine alone or in combination with lipopolysaccharide intraperitoneally to mice and administering concanavalin A alone into the tail vein, and comparing the AST and ALT concentrations in the blood collected 1 hour to 1 week after administering an inflammatory substance, the cytokine concentration, and the histopathological condition of liver lesions, between the anti-human TLR7 antibody group and the non-administration group.

[0120]

The thus obtained antibody that inhibits the biological activity of human TLR7 is useful as a pharmaceutical, particularly, as an antibody for preventing or treating immune inflammation-related diseases including systemic lupus erythematosus, allergic diseases, infections, or cancers.

[0121]

As one example, the anti-human TLR7 antibody can be administered alone or in combination with at least one

other therapeutic agent, for treating or preventing immune inflammation-related diseases, allergic diseases, infections, or cancers. Examples of the other therapeutic agent which can be administered in combination with the anti-human TLR7 antibody can include corticosteroids, non-steroidal anti-inflammatory drugs, nucleic acid antimetabolites, nucleic acid synthesis inhibitors, antifolates, calcineurin inhibitors, anti-malarial drugs, antithymocyte globulins, biologics targeting cell surface antigens, or biologics targeting cytokine interferons or cytokine interferon receptors, but there is no limitation to these examples.

[0122]

Specific examples of the therapeutic agent can include Methylprednisolone as a corticosteroid, Loxoprofen Sodium Hydrate, Diclofenac sodium, Indomethacin, and Acetyl salicylic acid as non-steroidal anti-inflammatory drugs, Mycophenolate mofetil as a nucleic acid antimetabolite, Cyclophosphamide as a nucleic acid synthesis inhibitor, Cyclosporin and Tacrolimus as calcineurin inhibitors, Hydroxychloroquine as an anti-malarial drug, Methotrexate as an antifolate, Zetbulin, Lymphoglobuline, and Thymoglobulin as antithymocyte globulins, Alemtuzumab, Rituximab, and Abatacept as biologics targeting cell surface antigens, and Infliximab, Etanercept, Adalimumab, Tocilizumab,

Belimumab and Anifrolumab as biologics targeting cytokine interferons or cytokine interferon receptors.

[0123]

Depending on the condition of immune inflammation-related diseases, allergic diseases, infections or cancers and the degree of treatment and/or prevention targeted, two, three or more types of other therapeutic agents can also be administered, and the other therapeutic agents can be encapsulated in the same preparation so as to be administered at the same time. The other therapeutic agents and the anti-human TLR7 antibody can also be encapsulated in the same preparation so as to be administered at the same time. Further, the anti-human TLR7 antibody and the other therapeutic agents can also be encapsulated in separate preparations and administered at the same time. Further, the other therapeutic agents and the anti-human TLR7 antibody can also be administered separately one after another. That is, a therapeutic agent containing the anti-human TLR7 antibody or the antigen-binding fragment of the antibody as an active ingredient may be administered after the other therapeutic agents are administered, or the other therapeutic agents may be administered after a therapeutic agent containing the anti-human TLR7 antibody or the antigen-binding fragment of the antibody as an active ingredient is administered. In the case of administration in gene therapy, the gene of a protein

therapeutic agent and the gene of the anti-human TLR7 antibody can be inserted downstream of the same or separate promoter regions and can be introduced into the same or separate vectors.

[0124]

The targeted drug conjugates disclosed in M. C. Garnet "Targeted drug conjugates: principles and progress", Advanced Drug Delivery Reviews, (2001) 53, 171-216 can be produced by binding the therapeutic agents to the anti-human TLR7 antibody or its fragment. For this purpose, any antibody fragment can be applied, other than antibody molecules, as long as it does not completely lose T-cell recognition. Examples thereof can include fragments such as Fab, F(ab')₂, and Fv, and the antibody and such fragments can be used in the same manner as in the present invention. The binding mode of the anti-human TLR7 antibody, or the fragment of the antibody, to the therapeutic agents can be in various forms disclosed 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, etc. That is, examples thereof can include a mode in which the anti-human TLR7 antibody is chemically bound to the therapeutic agents directly or

via a spacer such as an oligopeptide, or bound via a suitable drug carrier. Examples of the drug carrier can include liposomes and water-soluble polymers. More specifically, examples of the mode of binding via such a drug carrier can include a mode in which the antibody and the therapeutic agents are included in a liposome, and the liposome is bound to the antibody, and a mode in which the therapeutic agents are chemically bound to a water-soluble polymer (compound with a molecular weight of about 1000 to 100000) directly or via a spacer such as an oligopeptide, and the antibody is bound to the water-soluble polymer. The antibody (or the fragment) can be bound to the therapeutic agents or the drug carriers such as liposomes and water-soluble polymers by a method known to those skilled in the art such as the method according to 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. The therapeutic agents can be included in the liposome by a method known to those skilled in the art such as the method according to D. D. Lasic "Liposomes: From Physics to Applications", Elsevier Science Publishers B. V., Amsterdam (1993). The therapeutic agents can be bound to the water-soluble polymer by a method known to those skilled in the art such as the method according to D. Putnam and J Kopecek "Polymer Conjugates with Anticancer Activity" Advances in

Polymer Science (1995) 122, 55-123. The conjugates of the antibody (or the fragment) and a protein therapeutic agent (or the fragment) can be produced by a genetic engineering method known to those skilled in the art other than the aforementioned method.

[0125]

The present invention also provides a pharmaceutical composition comprising an expression vector which comprises a polynucleotide comprising a polynucleotide sequence encoding at least one or those of the anti-human TLR7 antibody or the antigen-binding fragment of the antibody, as an active ingredient in an amount effective for treating and/or preventing disease, and a diluent, a carrier, a solubilizer, an emulsifier, a preservative and/or an additive, which are pharmaceutically acceptable.

[0126]

The present invention also provides a pharmaceutical composition comprising an expression vector which comprises a polynucleotide comprising a polynucleotide sequence encoding at least one or those of the anti-human TLR7 antibody or the antigen-binding fragment of the antibody, in an amount effective for treating and/or preventing disease, at least one therapeutic agent in an amount effective for treating and/or preventing disease, and a diluent, a carrier, a solubilizer, an emulsifier, a preservative, and/or an additive, which are pharmaceutically acceptable.

[0127]

Examples of the therapeutic agent can include antifolates, calcineurin inhibitors, corticosteroids, antithymocyte globulins, nucleic acid antimetabolites, nucleic acid synthesis inhibitors, biologics targeting cell surface antigens, or biologics targeting cytokines or cytokine receptors described above, but there is no limitation to these examples.

[0128]

The substances used for preparations which are acceptable in the pharmaceutical composition of the present invention are preferably non-toxic, preferably at the administration dosage and the administration concentration, to a person to whom the pharmaceutical composition is administered.

[0129]

The pharmaceutical composition of the present invention can contain substances used for preparations for changing or maintaining the pH, the osmotic pressure, the viscosity, the transparency, the color, the isotonicity, the sterility, the stability, the dissolution rate, the sustained release rate, the absorption rate and the penetration rate. Examples of the substances used for preparations can include, but are not limited to: amino acids such as glycine, alanine, glutamine, asparagine, arginine or lysine, antibacterial agents, antioxidants such as ascorbic acid, sodium

sulfate or sodium bisulfite, buffers such as phosphoric acid, citric acid, boric acid buffer, sodium bicarbonate or a tris-hydrochloric acid (Tris-Hcl) solution, fillers such as mannitol or glycine, chelating agents such as ethylenediaminetetraacetic acid (EDTA), complexing agents such as caffeine, polyvinyl pyrrolidone, β -cyclodextrin, or hydroxypropyl- β -cyclodextrin, extenders such as glucose, mannose or dextrin, other carbohydrates such as monosaccharides or disaccharides, coloring agents, flavoring agents, diluents, emulsifiers, hydrophilic polymers such as polyvinyl pyrrolidone, preservatives such as low-molecular weight polypeptides, salt-forming counterions, benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid or hydrogen peroxide, solvents such as glycerin, propylene glycol or polyethylene glycol, sugar alcohols such as mannitol or sorbitol, suspending agents, polysorbates such as sorbitan ester, polysorbate 20 or polysorbate 80, surfactants such as triton, tromethamine, lecithin or cholesterol, stability enhancers such as sucrose or sorbitol, elasticity enhancers such as sodium chloride, potassium chloride, mannitol or sorbitol, transport agents, excipients, and/or pharmaceutical auxiliary agents. The amount of the substances used for preparations is to be added at preferably 0.01 to 100 times, particularly 0.1 to 10 times, with respect to the

weight of the anti-human TLR7 antibody. The suitable composition of the pharmaceutical composition in preparations can be appropriately determined by those skilled in the art, corresponding to the disease to be applied, the administration route to be applied, or the like.

[0130]

Excipients or carriers in the pharmaceutical composition may be liquid or solid. Suitable excipients or carriers may be water for injection, normal saline, artificial cerebrospinal fluid, or other substances generally used for parenteral administration. Neutral normal saline or normal saline containing serum albumin can also be used for carriers. The pharmaceutical composition can contain a Tris buffer with a pH of 7.0 to 8.5, an acetate buffer with a pH of 4.0 to 5.5 or a citric acid buffer with a pH of 3.0 to 6.2. Further, these buffers can also contain sorbitol or other compounds. Examples of the pharmaceutical composition of the present invention can include a pharmaceutical composition comprising anti-human TLR7 antibody and a pharmaceutical composition comprising anti-human TLR7 antibody and at least one therapeutic agent, and the pharmaceutical composition of the present invention is prepared as a drug having a selected composition and a necessary purity, in the form of a freeze-dried product or liquid. A pharmaceutical composition comprising anti-

human TLR7 antibody and a pharmaceutical composition comprising anti-human TLR7 antibody and at least one bone metabolism disorder therapeutic drug can be formed as a freeze-dried product using a suitable excipient such as sucrose.

[0131]

The pharmaceutical composition of the present invention can be prepared for parenteral administration or can be prepared for oral gastrointestinal absorption. The composition and the concentration of the preparation can be determined depending on the administration method. Alternatively, an affinity that is a higher affinity (lower K_d value), in terms of the dissociation constant (K_d value) to human TLR7 of the anti-human TLR7 antibodies contained in the pharmaceutical composition of the present invention, enables the drug efficacy to be exerted with reduced dosage in humans, and therefore the dosage of the pharmaceutical composition of the present invention in humans can also be determined based on this affinity. The dosage in the present invention may be about 0.1 to 100 mg/kg once every 1 to 180 days when the anti-human TLR7 antibody is administered to a human.

[0132]

Examples of embodiments of the pharmaceutical composition of the present invention can include injections including intravenous drip, suppositories,

nasal preparations, sublingual preparations and transdermal drugs.

[0133]

Although most approved antibody preparations are intravenously administered, subcutaneous administration is preferred in many medical settings. In such a case, the volume is limited to 1.0 to 1.5 mL, and thus a high-concentration antibody solution is required depending on the dosage. However, when the concentration is increased, the viscosity of the drug solution increases, and therefore the injection may be impossible due to the high viscosity with the thickness of commonly used injection needles. That is, in the case of selecting an injection, the property of low viscosity has an important meaning to be prioritized as an embodiment of the pharmaceutical composition, and a suitable antibody can be selected using the viscosity as an indicator.

Examples

[0134]

Hereinafter, the present invention will be specifically described by way of examples, but the present invention is not limited to these examples. In the following examples, each operation related to genetic engineering is performed according to the method disclosed in "Molecular Cloning" (Sambrook, J., Fritsch, E. F. and Maniatis, T., Cold Spring Harbor Laboratory

Press, 1989) or performed using a commercially available reagent or kit according to the instruction of the commercially available product, unless otherwise specified.

[0135]

[Example 1]

Production of mouse anti-human TLR7 antibody

1)-1 Immunization

1)-1-1 Establishment of Ba/F3 cell line expressing human TLR7-Flag-His×6/human Unc93B1-HA×2

The human TLR7 (variant 2) gene (SEQ ID NO: 3) was integrated into retroviral vectors, pMXs (Cell Biolabs, Inc.) tagged with Flag-His×6 added to the C-terminus of the gene using an enzyme, In Fusion (registered trademark) (Takara Bio Inc). The retroviral vectors were transfected into a packaging cell line based on the HEK293 cell line, Plat-E (Cell Biolabs, Inc.) using a transfection reagent, Fugene (registered trademark) 6 (Roche). The culture supernatant was collected 24 hours later and used as a virus suspension. The virus suspension was mixed with a transfection reagent, DOTAP (Roche), the mixture was added to a Ba/F3 cell line (RIKEN, BRC), and high-speed centrifugation was performed at 2000 rpm for 1 hour, to establish a Ba/F3 cell line expressing human TLR7-Flag-His×6.

[0136]

The human TLR7 encoded by the human TLR7 (variant 2) gene (SEQ ID NO: 3) comprises the amino acid sequence of SEQ ID NO: 4. The sequence is shown in Figure 2.

[0137]

The human Unc93B1 gene (SEQ ID NO: 55) was integrated into retroviral vectors, pMXs (Cell Biolabs, Inc.) tagged with HA×2 added to the C-terminus of the gene using an enzyme, In Fusion (registered trademark) (Takara Bio Inc). The retroviral vectors were transfected into a packaging cell line based on the HEK293 cell line, Plat-E (Cell Biolabs, Inc.) using a transfection reagent, Eugene (registered trademark) 6 (Roche). The culture supernatant was collected 24 hours later and used as a virus suspension. The virus suspension was mixed with a transfection reagent, DOTAP (Roche), the mixture was added to the Ba/F3 cell line expressing human TLR7-Flag-His×6 established above, and high-speed centrifugation was performed at 2000 rpm for 1 hour, to establish a Ba/F3 cell line expressing human TLR7-Flag-His×6/human Unc93B1-HA×2.

[0138]

1)-1-2 Mouse immunization

The Ba/F3 cell line expressing human TLR7-Flag-His×6/human Unc93B1-HA×2 established above in 1)-1-1 was mixed with an adjuvant, TiterMAX (registered trademark) Gold (TiterMax USA, Inc.), and the mixture was administered as an antigen to the sole, the tail base,

and the peritoneal cavity of TLR9-deficient mice with BALB/c background produced by the Institute of Medical Science of the University of Tokyo a total of 3 times per week.

[0139]

At the 4th time, the Ba/F3 cell line expressing human TLR7-Flag-His×6/human Unc93B1-HA×2 suspended in 1×PBS was intraperitoneally administered.

[0140]

The spleen was removed 5 days after the last immunization and used for producing hybridomas.

[0141]

1)-2 Production of hybridomas

The spleen cells after the immunization were mixed with Sp2/o cell line (ATCC), and cell fusion was performed using an HVJ-E cell fusion kit (ISHIHARA SANGYO KAISHA, LTD). In order to select hybridomas, the cells were cultured from the day after the cell fusion operation in RPMI1640 (Life Technologies) containing a HAT (Thermo Fisher Scientific)-containing culture solution (10% FBS), 50-μM 2-Mercaptoethanol (Life Technologies), 50-U/mL Penicillin, and 50-μg/mL Streptomycin (Life Technologies), and the hybridoma colonies that emerged were collected to produce monoclonal hybridomas.

[0142]

1)-3 Screening of antibody binding to human TLR7 by flow cytometry

The culture supernatant was collected from hybridomas forming colonies under a microscope and used for screening. A Ba/F3 cell line expressing hTLR7-Flag-His $\times$ 6/hUnc93B1-HA $\times$ 2 subjected to membrane permeation with 0.1% Saponin (Sigma-Aldrich Co. LLC) and Ba/F3 cells expressing nothing were each stained with the culture supernatant and analyzed by flow cytometry (LSRFortessaTMX-20, Becton, Dickinson and Company), to select hybridomas producing anti-hTLR7 antibody.

[0143]

1)-4 Screening of human TLR7 antibody inhibition of human TLR7 function by flow cytometry

The human TLR7 (variant 2) gene (SEQ ID NO: 3) and the human Unc93B1 D34A mutant-HA $\times$ 2 gene (SEQ ID NO: 86) were introduced into a Ba/F3 cell line (RIKEN, BRC) using a retrovirus. Further, a NF- κ B-green fluorescence protein (GFP) reporter plasmid (STRATAGENE) was transfected into the cells by the electroporation method, so that a Ba/F3 cell line expressing hTLR7/hUnc93B1 D34A mutant-HA $\times$ 2 and capable of analyzing NF- κ B activation was established, and the hybridoma culture supernatant was added to the cell line. The cells were stimulated 4 hours later with 25 ng/ml of a TLR7 ligand, R848 (InvivoGen). As a result of analyzing the inhibitory effects of human TLR7 response by measuring the GFP

fluorescence intensity using flow cytometry 24 hours later, three mouse anti-human TLR7 antibody-producing hybridomas were selected and were named AT01, NB7, and FAN2, respectively.

[0144]

In this description, antibodies produced by hybridomas AT01, NB7, and FAN2 are referred to as AT01 antibody, NB7 antibody, and FAN2 antibody, respectively.

[0145]

1)-5 Determination of isotype of antibody

The isotypes of the antibodies produced by the mouse anti-human TLR7 antibody-producing hybridomas (AT01, NB7, and FAN2) obtained in 1)-4 were each determined using an IsoStrip Mouse Monoclonal Antibody Isotyping Kit (Merck KGaA). As a result, the isotypes of AT01 antibody, NB7 antibody, and FAN2 antibody were each determined to be an IgG1/ κ chain.

[0146]

1)-6 Preparation of mouse anti-human TLR7 antibody

The mouse anti-human TLR7 monoclonal antibody was purified from ascites collected about 10 days after each hybridoma was intraperitoneally administered to ICR-CD1-Foxn1 nude mice (ICR-nu, CHARLES RIVER LABORATORIES JAPAN, INC.) pretreated with pristane (Sigma-Aldrich Co. LLC., currently, Merck KGaA).

[0147]

First, pristane was intraperitoneally administered to ICR-nu, and the mice were bred for 7 days or more. Thereafter, the AT01, NB7, or FAN2-producing hybridoma was cultured in RPMI1640 (Life Technologies) containing 10% FBS, 50- μ M 2-Mercaptoethanol (Life Technologies), 50-U/mL Penicillin, and 50- μ g/mL Streptomycin (Life Technologies), followed by proliferation to a sufficient volume. Thereafter, the hybridoma was suspended in 1 $\times$ PBS and intraperitoneally administered to the ICR-nu.

[0148]

Ascites was collected 10 days later, and the blood cells were sedimented by a high-speed centrifuge, to collect a supernatant. After 10-fold dilution with 1 $\times$ PBS, the ascites was passed through a 0.45 μ m filter (Millipore).

[0149]

The antibody was purified from the aforementioned ascites on a proteinG column (GE Healthcare Japan Corporation) using an AKTAprime plus (GE Healthcare Japan Corporation).

[0150]

Subsequently, buffer replacement with saline was performed together with enrichment using PD-10 (GE Healthcare Japan Corporation), to prepare the antibody at a concentration of 1.0 mg/mL or more.

[0151]

Finally, sterilization with Millex-GV 0.22 µm (Millipore) was performed, to give a purified sample.

[0152]

[Example 2]

In vitro evaluation of mouse anti-human TLR7 antibody

2)-1 Evaluation of binding selectivity of mouse anti-human TLR7 antibody

2)-1-1 Establishment of Ba/F3 cell line expressing mouse TLR7-Flag-His×6

The mouse TLR7 gene (SEQ ID NO: 56) was integrated into retroviral vectors, pMXs (Cell Biolabs, Inc.) tagged with Flag-His×6 added to the C-terminus of the gene using an enzyme, In Fusion (registered trademark) (Takara Bio Inc). The retroviral vectors were transfected into a packaging cell line based on the HEK293 cell line, Plat-E (Cell Biolabs, Inc.) using a transfection reagent, Fugene (registered trademark) 6 (Roche). The culture supernatant was collected 24 hours later and used as a virus suspension. The virus suspension was mixed with a transfection reagent, DOTAP (Roche), the mixture was added to a Ba/F3 cell line (RIKEN, BRC), and high-speed centrifugation was performed at 2000 rpm for 1 hour, to establish a Ba/F3 cell line expressing mouse TLR7-Flag-His×6.

[0153]

The mouse TLR7 encoded by the mouse TLR7 gene (SEQ ID NO: 56) comprises the amino acid sequence of SEQ ID NO: 83. The sequence is shown in Figure 3.

[0154]

2)-1-2 Establishment of Ba/F3 cell line expressing rat TLR7-Flag-His×6

The rat TLR7 gene (SEQ ID NO: 57) was integrated into retroviral vectors, pMXs (Cell Biolabs, Inc.) tagged with Flag-His×6 added to the C-terminus of the gene using an enzyme, In Fusion (registered trademark) (Takara Bio Inc). The retroviral vectors were transfected into a packaging cell line based on the HEK293 cell line, Plat-E (Cell Biolabs, Inc.) using a transfection reagent, Fugene (registered trademark) 6 (Roche). The culture supernatant was collected 24 hours later and used as a virus suspension. The virus suspension was mixed with a transfection reagent, DOTAP (Roche), the mixture was added to a Ba/F3 cell line (RIKEN, BRC), and high-speed centrifugation was performed at 2000 rpm for 1 hour, to establish a Ba/F3 cell line expressing rat TLR7-Flag-His×6.

[0155]

The rat TLR7 encoded by the rat TLR7 gene (SEQ ID NO: 57) comprises the amino acid sequence of SEQ ID NO: 84. The sequence is shown in Figure 4.

[0156]

2)-1-3 Establishment of Ba/F3 cell line expressing monkey TLR7-Flag-His×6

The monkey TLR7 gene (SEQ ID NO: 58) was integrated into retroviral vectors, pMXs (Cell Biolabs, Inc.) tagged with Flag-His×6 added to the C-terminus of the gene using an enzyme, In Fusion (registered trademark) (Takara Bio Inc). The retroviral vectors were transfected into a packaging cell line based on the HEK293 cell line, Plat-E (Cell Biolabs, Inc.) using a transfection reagent, Fugene (registered trademark) 6 (Roche). The culture supernatant was collected 24 hours later and used as a virus suspension. The virus suspension was mixed with a transfection reagent, DOTAP (Roche), the mixture was added to a Ba/F3 cell line (RIKEN, BRC), and high-speed centrifugation was performed at 2000 rpm for 1 hour, to construct a Ba/F3 cell line expressing monkey TLR7-Flag-His×6.

[0157]

The monkey TLR7 encoded by the monkey TLR7 gene (SEQ ID NO: 58) comprises the amino acid sequence of SEQ ID NO: 85. The sequence is shown in Figure 5.

[0158]

2)-1-4 Evaluation of binding selectivity of mouse anti-human TLR7 antibody by flow cytometry

The Ba/F3 cell line expressing human TLR7-Flag-His×6/human Unc93B1-HA×2 established above in 1)-1-1, the Ba/F3 cell line expressing mouse TLR7-Flag-His×6

established above in 2)-1-1, the Ba/F3 cell line expressing rat TLR7-Flag-His×6×2 established above in 2)-1-2, and the Ba/F3 cell line expressing monkey TLR7-Flag-His×6 established above in 2)-1-3 were subjected to membrane permeation with 0.1% Saponin and stained using AT01 antibody, NB7 antibody, and FAN2 antibody, and a secondary antibody, Goat anti-mouse IgG (H+L)-PE (eBioscience). The binding selectivity of each antibody was evaluated by comparing the average fluorescence intensity (MFI) based on flow cytometry analysis.

[0159]

The results indicate that AT01 antibody, NB7 antibody, and FAN2 antibody specifically bound to human TLR7 or monkey TLR7 and did not bind to mouse TLR7 or rat TLR7.

[0160]

2)-2 Evaluation of binding ability of mouse anti-human TLR7 antibody

The Ba/F3 cell line expressing human TLR7-Flag-His×6/human Unc93B1-HA×2 established above in 1)-1-1 was subjected to membrane permeation with 0.1% Saponin and stained using a dilution series of AT01 antibody, NB7 antibody, and FAN2 antibody, and a secondary antibody, Goat anti-mouse IgG (H+L)-PE (eBioscience). The binding activity of each antibody was evaluated by comparing the mean fluorescence intensity (MFI) based on flow cytometry analysis.

[0161]

The results indicate that the antibody having the highest binding activity was in the order of AT01 antibody, NB7 antibody, and FAN2 antibody.

[0162]

2)-3 Inhibitory activity of mouse anti-human TLR7 antibody on cytokine production

Human PBMCs were purchased from Cellular Technology Limited as a frozen product and thawed according to the instructions for use.

[0163]

The PBMCs suspended at a concentration of 2.5×10^6 cells/mL in RPMI1640 (Life Technologies Corp.) containing 10% FBS, 1-mM Sodium Pyruvate (Life Technologies Corp.), 0.1-mM MEM Non-Essential Amino Acids (Life Technologies Corp.), 50- μ M 2-Mercaptoethanol (Life Technologies Corp.), 50-U/mL Penicillin, and 50- μ g/mL Streptomycin (Life Technologies Corp.) were seeded in a 96-well cell culture plate at 100 μ L each, and the mouse anti-human TLR7 antibody, AT01 antibody, NB7 antibody, or FAN2 antibody, or a mouse IgG control antibody (BioLegend, Inc.) was added thereto at 80 μ L/well, followed by pretreatment in an incubator at 37°C for 4 hours.

[0164]

Thereafter, 1-mg/mL CL-264 (InvivoGen) was added thereto at 20 μ L/well, and the mixture was stirred well, followed by culturing at 37°C for about 20 hours under 5%

CO₂. The plate was stirred well and thereafter centrifuged at 1500 rpm for 5 minutes, and the concentration of interleukin-6 (IL-6) contained in the supernatant was measured by the FRET method (Cisbio Bioassay. Inc).

[0165]

Figure 6 shows the results. The mouse anti-human TLR7 antibody suppressed the production of IL-6 from the human PBMCs treated with CL-264 in a concentration dependent manner. The AT01 antibody, NB7 antibody, and FAN2 antibody inhibited the production of IL-6 from the human PBMCs in a concentration dependent manner. The half-inhibitory concentrations (IC₅₀) of AT01 antibody, NB7 antibody and FAN2 antibody were 192 ng/mL, 771 ng/mL, and 8389 ng/mL, respectively, which were calculated from the concentrations of the two values, which are either side of the 50% inhibitory activity, and the inhibitory activity when each of the two concentrations were added.

[0166]

Meanwhile, inhibition was not observed in the mouse IgG control antibody even at a concentration of 10 µg/mL.

[0167]

[Example 3]

Determination of nucleotide sequence and amino acid sequence of cDNA encoding variable region of mouse anti-human TLR7 antibody

3)-1 Synthesis of cDNA

Total RNA was collected from the hybridomas of AT01, NB7, and FAN2 using a TRIzol Reagent (Ambion).

Subsequently, cDNA was synthesized using a PrimeScript 1st Strand cDNA Synthesis Kit (TAKARA).

[0168]

3)-2 Amplification and sequencing of mouse immunoglobulin heavy- and light-chain variable region gene fragments

The nucleotide sequences encoding the variable regions of the antibody contained in the synthesized cDNA were determined by GenScript Corporation.

[0169]

3)-2-1 Mouse anti-human TLR7 antibody (AT01)

The amino acid sequence encoded by the nucleotide sequence of cDNA encoding the heavy-chain variable region of AT01 antibody determined above is set forth in SEQ ID NO: 5 in the Sequence Listing.

[0170]

The amino acid sequence encoded by the nucleotide sequence of cDNA encoding the light-chain variable region of AT01 antibody determined above is set forth in SEQ ID NO: 11 in the Sequence Listing.

[0171]

3)-2-2 Mouse anti-human TLR7 antibody (NB7)

The amino acid sequence encoded by the nucleotide sequence of cDNA encoding the heavy-chain variable region of NB7 antibody determined above is set forth in SEQ ID NO: 7 in the Sequence Listing.

[0172]

The amino acid sequence encoded by the nucleotide sequence of cDNA encoding the light-chain variable region of NB7 antibody determined above is set forth in SEQ ID NO: 13 in the Sequence Listing.

[0173]

3)-2-3 Mouse anti-human TLR7 antibody (FAN2)

The amino acid sequence encoded by the nucleotide sequence of cDNA encoding the heavy-chain variable region of FAN2 antibody determined above is set forth in SEQ ID NO: 9 in the Sequence Listing.

[0174]

The amino acid sequence encoded by the nucleotide sequence of cDNA encoding the light-chain variable region of FAN2 antibody determined above is set forth in SEQ ID NO: 15 in the Sequence Listing.

[0175]

[Example 4]

Production of chimeric anti-human TLR7 antibody

4)-1 Construction of expression vector of chimeric anti-TLR7 antibody

4)-1-1 Construction of chimeric light-chain expression vector, pCMA-LK

A DNA fragment (SEQ ID NO: 59) containing an approximately 5.4-kb fragment obtained by digesting plasmid pCDNA3.3-TOPO/LacZ (Invitrogen Corp.) with restriction enzymes, XbaI and PmeI and a DNA sequence

encoding the human light-chain signal sequence and the human kappa-chain constant region were bound together using an In-Fusion HD PCR cloning kit (Clontech Laboratories, Inc.), to produce pcDNA3.3/LK. pCMA-LK was constructed by removing the neomycin expression unit from pcDNA3.3/LK.

[0176]

4)-1-2 Construction of chimeric IgG1 heavy-chain expression vector, pCMA-G1

A DNA fragment (SEQ ID NO: 60) containing a DNA fragment obtained by digesting pCMA-LK with XbaI and PmeI and removing the light chain signal sequence and the human kappa-chain constant region was bound to a DNA sequence encoding the human heavy-chain signal sequence and the human IgG1 constant region using an In-Fusion HD PCR cloning kit (Clontech Laboratories, Inc.), to construct pCMA-G1.

[0177]

4)-1-3 Construction of AT01 chimeric anti-human TLR7 antibody expression vector

A DNA fragment comprising the nucleotide sequence encoding the heavy-chain variable region of cAT01 antibody represented by nucleotides 36 to 422 in the nucleotide sequence set forth in SEQ ID NO: 61 was synthesized (Geneart AG). The synthesized DNA fragment was inserted into the site where pCMA-G1 was cut with a restriction enzyme, BlnI, using an In-Fusion HD PCR

cloning kit (Clontech Laboratories, Inc.), thereby constructing a cAT01 antibody heavy-chain expression vector. The heavy chain of cAT01 antibody comprises the amino acid sequence of SEQ ID NO: 35 including the signal sequence. The sequence is shown in Figure 7.

[0178]

The DNA fragment (SEQ ID NO: 62) comprising a DNA sequence encoding the light chain of cAT01 antibody was synthesized (Geneart AG). Using the pCMA-LK produced in Example 4)-1-1, a cAT01 antibody light-chain expression vector was constructed in the same manner as above. The light chain of cAT01 antibody comprises the amino acid sequence of SEQ ID NO: 36 including the signal sequence. The sequence is shown in Figure 7.

4)-1-4 Construction of NB7 chimeric anti-human TLR7 antibody expression vector

A DNA fragment comprising the nucleotide sequence encoding the heavy-chain variable region of cNB7 antibody represented by nucleotides 36 to 440 in the nucleotide sequence set forth in SEQ ID NO: 63 was synthesized (Geneart AG). A cNB7 heavy-chain expression vector was constructed in the same manner as in Example 4)-1-3. The heavy chain of cNB7 antibody comprises the amino acid sequence of SEQ ID NO: 37 including the signal sequence. The sequence is shown in Figure 8.

[0179]

A DNA fragment (SEQ ID NO: 64) comprising the DNA sequence encoding the cNB7 light chain was synthesized (Geneart AG). Using the pCMA-LK produced in Example 4)-1-1, a cNB7 light-chain expression vector was constructed in the same manner as in Example 4)-1-3. The light chain of cNB7 antibody comprises the amino acid sequence of SEQ ID NO: 38 including the signal sequence. The sequence is shown in Figure 8.

[0180]

4)-1-5 Construction of FAN2 chimeric anti-human TLR7 antibody expression vector

A DNA fragment comprising the nucleotide sequence encoding the heavy-chain variable region of cFAN2 antibody represented by nucleotides 36 to 437 in the nucleotide sequence set forth in SEQ ID NO: 65 was synthesized (Geneart AG). A cFAN2 heavy-chain expression vector was constructed in the same manner as in Example 4)-1-3. The heavy chain of cFAN2 antibody comprises the amino acid sequence of SEQ ID NO: 39 including the signal sequence. The sequence is shown in Figure 9.

[0181]

A DNA fragment (SEQ ID NO: 66) comprising the DNA sequence encoding the cFAN2 light chain was synthesized (Geneart AG). Using the pCMA-LK produced in Example 4)-1-1, a cFAN2 light-chain expression vector was constructed in the same manner as in Example 4)-1-3. The light chain of cFAN2 antibody comprises the amino acid

sequence of SEQ ID NO: 40 including the signal sequence. The sequence is shown in Figure 9.

[0182]

4)-2 Production of chimeric anti-human TLR7 antibody

4)-2-1 Production of chimeric AT01 antibody

FreeStyle 293F cells (Invitrogen Corp.) were passaged and cultured according to the manual. 1.2×10^9 FreeStyle 293F cells (Invitrogen Corp.) in the logarithmic growth phase were seeded in a 3-L Fernbach Erlenmeyer Flask (Corning Incorporated) and diluted with a FreeStyle293 expression medium (Invitrogen Corp.) at a concentration of to 2.0×10^6 cell/mL. 0.24 mg of the heavy-chain expression vector constructed above in 4)-1-3, 0.36 mg of the light-chain expression vector constructed above in 4)-1-3, and 1.8 mg of Polyethyleneimine (Polyscience #24765) were added to 40 mL of an Opti-Pro SFM medium (Invitrogen Corp.), followed by gentle stirring and further standing for 5 minutes. Thereafter, the mixture was added to the FreeStyle 293F cells. After shaking culture in an incubator at 37°C under 8% CO₂ for 4 hours at 90 rpm, 600 ml of EX-CELL VPRO medium (SAFC Biosciences), 18 ml of GlutaMAX I (Gibco), and 30 mL of Yeastolate Ultrafiltrate (Gibco) were added thereto, followed by shaking culture in an incubator at 37°C under 8% CO₂ at 90 rpm for 7 days, and the culture supernatant obtained was filtered with a Disposable Capsule Filter

(Advantec #CCS-045-E1H), to produce a chimeric AT01 antibody.

[0183]

4)-2-2 Production of chimeric NB7 antibody

Using the heavy-chain expression vector and light-chain expression vector constructed in 4)-1-4 above, a chimeric NB7 antibody was produced by the method of 4)-2-1.

[0184]

4)-2-3 Production of chimeric FAN2 antibody

Using the heavy-chain expression vector and light-chain expression vector constructed in 4)-1-5 above, a chimeric FAN2 antibody was produced by the method of 4)-2-1.

[0185]

4)-3 Purification of chimeric anti-human TLR7 antibody

An antibody of interest was purified from each culture supernatant obtained in Example 4)-2 by a one-step process of rProtein A affinity chromatography. The culture supernatant was applied to a column (GE Healthcare Bioscience) filled with MabSelectSuRe equilibrated with PBS, and thereafter the column was washed with PBS in a volume of twice or more the column volume. Next, elution was carried out with a 2M arginine hydrochloride solution (pH 4.0), to collect a fraction containing the antibody. The fraction was subjected to buffer replacement with PBS(-) by dialysis (Slide-A-Lyzer

Dialysis Cassette, Thermo Scientific). The antibody was concentrated with a Centrifugal UF Filter Device, VIVASPIN20 (fraction molecular weight UF10K, Sartorius AG), to prepare the IgG at a concentration of 10 mg/mL or more. Finally, filtration was carried out with a Minisart-Plus filter (Sartorius AG), to give a purified sample.

[0186]

[Example 5]

In vitro activity of chimeric anti-human TLR7 antibody
5)-1 Antigen-binding activity of chimeric anti-human TLR7 antibody by flow cytometry

A Ba/F3 cell line expressing human TLR7-Flag-His $\times$ 6/hUnc93B1-HA $\times$ 2 was subjected to membrane permeation with 0.1% Saponin and stained using the dilution series of chimeric AT01 antibody, NB7 antibody, and FAN2 antibody at the antibody concentrations obtained in Example 4, and the secondary antibody, Goat anti-human IgG Fc-PE (eBioscience). The binding activity of each antibody was evaluated by comparing the MFI based on flow cytometry analysis. The results indicate that the antibody having the highest binding activity was in the order of chimeric AT01 antibody (cAT01), chimeric NB7 antibody (cNB7), and chimeric FAN2 antibody (cFAN2).

[0187]

5)-2 Inhibitory activity of chimeric anti-human TLR7 antibody on cytokine production

Human PBMCs were purchased from Cellular Technology Limited as a frozen product and thawed according to the instructions for use. The PBMCs suspended at a concentration of 2.5×10^6 cells/mL in RPMI1640 (Life Technologies Corp.) containing 10% FBS, 1 mM Sodium Pyruvate (Life Technologies Corp.), 0.1-mM MEM Non-Essential Amino Acids (Life Technologies Corp.), 50 μ M 2-Mercaptoethanol (Life Technologies Corp.), 50 U/mL Penicillin, and 50 μ g/mL Streptomycin (Life Technologies Corp.) were seeded in a 96-well cell culture plate at 100 μ L each, and the chimeric anti-human TLR7 antibody, cAT01, cNB7, or cFAN2, or a human IgG control antibody (Eureka Therapeutics) was added thereto at 80 μ L/well, followed by pretreatment in an incubator at 37°C for 4 hours. Thereafter, 1-mg/mL CL-264 (InvivoGen) was added thereto at 20 μ L/well, and the mixture was stirred well, followed by culturing at 37°C for about 20 hours under 5% CO₂. The plate was stirred well and thereafter centrifuged at 1500 rpm for 5 minutes, and the concentration of IL-6 contained in the supernatant was measured by the FRET method (Cisbio Bioassay. Inc).

[0188]

Figure 10 shows that the chimeric anti-human TLR7 antibody suppresses the production of IL-6 from the human PBMCs treated with CL-264 depending in a concentration dependent manner. The antibodies cAT01, cNB7, and cFAN2 inhibited the production of IL-6 from the human PBMCs

depending on the concentration added. The half-inhibitory concentrations (IC_{50}) of the antibodies cAT01, cNB7 and cFAN2 were 35 ng/mL, 196 ng/mL, 10000 ng/mL or more, respectively, which were calculated from the concentrations of the two values, which are either side of the 50% inhibitory activity, and the inhibitory activity when each of the two concentrations were added. [0189]

Meanwhile, inhibition was not observed in the human IgG control antibody even at a concentration of 10 μ g/mL. [0190]

[Example 6]

Production of humanized anti-human TLR7 antibody

6)-1 Humanized design of anti-human TLR7 antibody

6)-1-1 Molecular modeling of antibody variable regions

The molecular modeling of the variable regions was performed by a method known as homology modeling (Methods in Enzymology, 203, 121-153, (1991)) using a commercially available protein three-dimensional structure analysis program, BioLuminate (Schrodinger K.K). The template used was a structure registered in the Protein Data Bank (Nuc. Acid Res. 35, D301-D303 (2007)) having high sequence identity to the variable regions of the heavy and light chains.

[0191]

6)-1-2 Design of humanized amino acid sequence

A technique known as CDR grafting (Proc. Natl. Acad. Sci. USA 86, 10029-10033(1989)) was used for humanization. Acceptors having high identity were selected from the consensus sequences defined in Kabat et al. (Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service National Institutes of Health, Bethesda, MD. (1991)) and the human germline sequences registered in IMGT (THE INTERNATIONAL IMMUNOGENETICS INFORMATION SYSTEM (registered trademark), <http://www.imgt.org>), and donor residues to be transferred onto the acceptors were selected by analyzing the three-dimensional models with reference to the standards given by Queen et al. (Proc. Natl. Acad. Sci. USA 86, 10029-10033 (1989)).

[0192]

6)-1-3 Design of humanized amino acid sequence of AT01 antibody

The consensus sequences of human gamma-chain subgroup 1 and kappa-chain subgroup 1 having high identity to the framework regions of cAT01 were selected as the acceptors of cAT01. In the molecular modeling of the variable regions, a known structure (PDB ID:1E4W) was used as a template.

[0193]

6)-1-3-1 Humanization of AT01 antibody heavy chain

(1) The heavy chain designed was named huAT01_H1_IgG1LALA. huAT01_H1_IgG1LALA comprises the amino acid sequence set forth in SEQ ID NO: 45 including

the signal sequence as a heavy-chain full-length amino acid sequence. The sequence is shown in Figure 11. In the sequence, the amino acid sequence at positions 1 to 19 is the signal sequence, the amino acid sequence at positions 20 to 135 is the variable region, and the amino acid sequence at positions 136 to 465 is the constant region. Further, the amino acid sequence at positions 45 to 54 is CDRH1, the amino acid sequence at positions 69 to 78 is CDRH2, and the amino acid sequence at positions 118 to 124 is CDRH3.

[0194]

The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 45 comprises the sequence set forth in SEQ ID NO: 67. Further, the nucleotide sequence encoding the heavy-chain variable region comprises the sequence set forth in SEQ ID NO: 77.

[0195]

(2) The heavy chain designed was named huAT01_H3_IgG1LALA. huAT01_H3_IgG1LALA including the signal sequence as a heavy-chain full-length amino acid sequence comprises the amino acid sequence set forth in SEQ ID NO: 46. The sequence is shown in Figure 12. In the sequence, the amino acid sequence at positions 1 to 19 is the signal sequence, the amino acid sequence at positions 20 to 135 is the variable region, and the amino acid sequence at positions 136 to 465 is the constant region. Further, the amino acid sequence at positions 45

to 54 is CDRH1, the amino acid sequence at positions 69 to 78 is CDRH2, and the amino acid sequence at positions 118 to 124 is CDRH3.

[0196]

The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 46 comprises the sequence set forth in SEQ ID NO: 68. Further, the nucleotide sequence encoding the heavy-chain variable region comprises the sequence set forth in SEQ ID NO: 78.

[0197]

(3) The heavy chain designed was named huAT01_H3_IgG4Pro. huAT01_H3_IgG4Pro including the signal sequence as a heavy-chain full-length amino acid sequence comprises the amino acid sequence set forth in SEQ ID NO: 47. The sequence is shown in Figure 13. In the sequence, the amino acid sequence at positions 1 to 19 is the signal sequence, the amino acid sequence at positions 20 to 135 is the variable region, and the amino acid sequence at positions 136 to 462 is the constant region. Further, the amino acid sequence at positions 45 to 54 is CDRH1, the amino acid sequence at positions 69 to 78 is CDRH2, and the amino acid sequence at positions 118 to 124 is CDRH3.

[0198]

The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 47 comprises the sequence set forth in SEQ ID NO: 69. Further, the nucleotide sequence

encoding the heavy-chain variable region comprises the sequence set forth in SEQ ID NO: 78.

[0199]

Figure 14 shows the comparison of the amino acid sequence (SEQ ID NO: 5) of the variable region of cAT01_H which is the heavy chain of chimeric antibody cAT01 (which will be hereinafter referred to as cAT01_H), the amino acid sequence (SEQ ID NO: 41) of the variable region of the humanized antibody heavy chain, huAT01_H1_IgG1LALA (which will be hereinafter referred to as huAT01_H1), and the amino acid sequence (SEQ ID NO: 42) of the variable region of huAT01_H3_IgG1LALA (which will be hereinafter referred to as huAT01_H3). In the sequences of huAT01_H1 and huAT01_H3, the symbol "." represents the same amino acid residue as that of cAT01_H, and a substituted amino acid residue is shown in the site where the amino acid residue is described.

[0200]

6)-1-3-2 Humanization of AT01 antibody light chain

The light chain designed was named huAT01_L1. huAT01_L1 comprises the amino acid sequence set forth in SEQ ID NO: 48 including the signal sequence as a light-chain full-length amino acid sequence. The sequence is shown in Figure 15. In the sequence, the amino acid sequence at positions 1 to 20 is the signal sequence, the amino acid sequence at positions 21 to 126 is the variable region, and the amino acid sequence at positions

127 to 233 is the constant region. Further, the amino acid sequence at positions 44 to 54 is CDRL1, the amino acid sequence at positions 70 to 76 is CDRL2, and the amino acid sequence at positions 109 to 116 is CDRL3.

[0201]

The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 48 comprises the sequence set forth in SEQ ID NO: 70. Further, the nucleotide sequence encoding the light-chain variable region comprises the sequence set forth in SEQ ID NO: 79.

[0202]

The light chain designed was named huAT01_L2. huAT01_L2 comprises the amino acid sequence set forth in SEQ ID NO: 49 including the signal sequence as a light-chain full-length amino acid sequence. The sequence is shown in Figure 16. In the sequence, the amino acid sequence at positions 1 to 20 is the signal sequence, the amino acid sequence at positions 21 to 126 is the variable region, and the amino acid sequence at positions 127 to 233 is the constant region. Further, the amino acid sequence at positions 44 to 54 is CDRL1, the amino acid sequence at positions 70 to 76 is CDRL2, and the amino acid sequence at positions 109 to 116 is CDRL3.

[0203]

The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 49 is set forth in SEQ ID NO: 71. Further, the nucleotide sequence encoding the light-chain

variable region comprises the sequence set forth in SEQ ID NO: 80.

[0204]

Figure 17 shows the comparison of the amino acid sequence (SEQ ID NO: 11) of the variable region of cAT01_L, which is the light chain of chimeric antibody cAT01 (which will be hereinafter referred to as cAT01_L), the amino acid sequence (SEQ ID NO: 43) of the variable region of humanized antibody light chain huAT01_L1 (which will be hereinafter referred to as huAT01_L1), and the amino acid sequence (SEQ ID NO: 44) of the variable region of huAT01_L2 (which will be hereinafter referred to as huAT01_L2). In the sequences of huAT01_L1 and huAT01_L2, the symbol "." represents the same amino acid residue as that of cAT01_L, and a substituted amino acid residue is shown in the site where the amino acid residue is described.

[0205]

6)-1-4 Design of humanized amino acid sequence of NB7

Human germline sequences IGHV4-30-4*02 and IGHJ6*01 having high identity to the framework regions of cNB7 were selected as the acceptors of cNB7. In the molecular modeling of the variable regions, a known structure (PDB ID:3CDF) was used as a template.

[0206]

6)-1-4-1 Humanization of NB7 antibody heavy chain

(1) The heavy chain designed was named huNB7_H3_IgG1LALA. huNB7_H3_IgG1LALA comprises the amino acid sequence set forth in SEQ ID NO: 52 including the signal sequence as a heavy-chain full-length amino acid sequence. The sequence is shown in Figure 18. In the sequence, the amino acid sequence at positions 1 to 19 is the signal sequence, the amino acid sequence at positions 20 to 141 is the variable region, and the amino acid sequence at positions 142 to 471 is the constant region. Further, the amino acid sequence at positions 45 to 55 is CDRH1, the amino acid sequence at positions 70 to 78 is CDRH2, the amino acid sequence at positions 118 to 130 is CDRH3. [0207]

The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 52 is set forth in SEQ ID NO: 72. Further, the nucleotide sequence encoding the heavy-chain variable region comprises the sequence set forth in SEQ ID NO: 81. [0208]

(2) The heavy chain designed was named huNB7_H3_IgG4Pro. huNB7_H3_IgG4Pro comprises the amino acid sequence set forth in SEQ ID NO: 54 including the signal sequence as a heavy-chain full-length amino acid sequence. The sequence is shown in Figure 19. In the sequence, the amino acid sequence at positions 1 to 19 is the signal sequence, the amino acid sequence at positions 20 to 141 is the variable region, and the amino acid sequence at

positions 142 to 468 is the constant region. Further, the amino acid sequence at positions 45 to 55 is CDRH1, the amino acid sequence at positions 70 to 78 is CDRH2, the amino acid sequence at positions 118 to 130 is CDRH3.

[0209]

The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 54 is set forth in SEQ ID NO: 73. Further, the nucleotide sequence encoding the heavy-chain variable region comprises the sequence set forth in SEQ ID NO: 81.

[0210]

Figure 20 shows the comparison of the amino acid sequence (SEQ ID NO: 7) of the variable region of cNB7_H, which is the heavy chain of chimeric antibody cNB7 (which will be hereinafter referred to as cNB7_H) and the amino acid sequence (SEQ ID NO: 50) of the variable region of humanized antibody heavy chain huNB7_H3_IgG1LALA (which will be hereinafter referred to as huNB7_H3). In huNB7_H3, the symbol "." represents the same amino acid residue as that of cNB7_H, and a substituted amino acid residue is shown in the site where the amino acid residue is described.

[0211]

6)-1-4-2 Humanization of NB7 antibody light chain

The light chain designed was named huNB7_L3. huNB7_L3 comprises the amino acid sequence set forth in SEQ ID NO: 53 including the signal sequence as a light-

chain full-length amino acid sequence. The sequence is shown in Figure 21. In the sequence, the amino acid sequence at positions 1 to 20 is the signal sequence, the amino acid sequence at positions 21 to 126 is the variable region, and the amino acid sequence at positions 127 to 233 is the constant region. Further, the amino acid sequence at positions 44 to 54 is CDRL1, the amino acid sequence at positions 70 to 76 is CDRL2, and the amino acid sequence at positions 109 to 116 is CDRL3.

[0212]

The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 53 is set forth in SEQ ID NO: 74. Further, the nucleotide sequence encoding the light-chain variable region comprises the sequence set forth in SEQ ID NO: 82.

[0213]

Figure 22 shows the comparison of the amino acid sequence (SEQ ID NO: 13) of the variable region of cNB7_L, which is the light chain of chimeric antibody cNB7 (which will be hereinafter referred to as cNB7_L) and the amino acid sequence (SEQ ID NO: 51) of the variable region of humanized antibody light chain huNB7_L3 (which will be hereinafter referred to as huNB7_L3). In huNB7_L3, the symbol "." represents the same amino acid residue as that of cNB7_L, and a substituted amino acid residue is shown in the site where the amino acid residue is described.

[0214]

6)-2 Design of humanized antibody by combining heavy and light chains

6)-2-1 Design of humanized AT01 anti-TLR7 antibody

(1) An antibody comprising huAT01_H1_IgG1LALA consisting of the amino acid sequence at positions 20 to 465 in SEQ ID NO: 45 as the heavy chain and huAT01_L1 consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 48 as the light chain was named "huAT01_H1L1_IgG1LALA antibody" or "huAT01_H1L1_IgG1LALA".

[0215]

(2) An antibody comprising huAT01_H3_IgG1LALA consisting of the amino acid sequence at positions 20 to 465 in SEQ ID NO: 46 as the heavy chain and huAT01_L1 consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 48 as the light chain was named "huAT01_H3L1_IgG1LALA antibody" or "huAT01_H3L1_IgG1LALA".

[0216]

(3) An antibody comprising huAT01_H3_IgG1LALA consisting of the amino acid sequence at positions 20 to 465 in SEQ ID NO: 46 as the heavy chain and huAT01_L2 consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 49 as the light chain was named "huAT01_H3L2_IgG1LALA antibody" or "huAT01_H3L2_IgG1LALA".

[0217]

(4) An antibody comprising huAT01_H3_IgG4Pro consisting of the amino acid sequence at positions 20 to 462 in SEQ ID NO: 47 as the heavy chain and huAT01_L2 consisting of

the amino acid sequence at positions 21 to 233 in SEQ ID NO: 49 as the light chain was named "huAT01_H3L2_IgG4Pro antibody" or "huAT01_H3L2_IgG4Pro".

[0218]

6)-2-2 Design of humanized NB7 anti-TLR7 antibody

(1) An antibody consisting of huNB7_H3_IgG1LALA consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 52 as the heavy chain and huNB7_L3 consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 53 as the light chain was named "huNB7_H3L3_IgG1LALA antibody" or "huNB7_H3L3_IgG1LALA".

[0219]

(2) An antibody consisting of huNB7_H3_IgG4Pro consisting of the amino acid sequence at positions 20 to 468 in SEQ ID NO: 54 as the heavy chain and huNB7_L3 consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 53 as the light chain was named "huNB7_H3L3_IgG4Pro antibody" or "huNB7_H3L3_IgG4Pro".

[0220]

6)-3 Production of humanized anti-human TLR7 antibody

6)-3-1 Construction of humanized IgG1LALA type heavy-chain expression vector, pCMA-G1LALA

pCMA-G1LALA was constructed in the same manner as in Example 4)-1-2 using DNA fragments comprising the nucleotide sequences encoding the amino acid sequences of the human heavy-chain signal sequence and the human IgG1LALA constant region (SEQ ID NO: 75).

[0221]

6)-3-2 Construction of humanized IgG4Pro type heavy-chain expression vector, pCMA-G4Pro

pCMA-G4Pro was constructed in the same manner as in Example 4)-1-2 using DNA fragments comprising the nucleotide sequences encoding the amino acid sequences of the human heavy-chain signal sequence and the human IgG4Pro constant region (SEQ ID NO: 76).

[0222]

6)-3-3 Construction of AT01 humanized heavy-chain expression vector

6)-3-3-1 Construction of huAT01_H1_IgG1LALA expression vector

A DNA fragment represented by nucleotides 36 to 422 in the nucleotide sequence of huAT01_H1_IgG1LALA set forth in SEQ ID NO: 46 was synthesized (Geneart AG). The DNA fragment synthesized was inserted into the site where pCMA-G1LALA was cut with a restriction enzyme, BspI, using an In-Fusion HD PCR cloning kit (Clontech Laboratories, Inc.), thereby constructing a huAT01_H1_IgG1LALA expression vector.

[0223]

6)-3-3-2 Construction of huAT01_H3_IgG1LALA expression vector

A DNA fragment represented by nucleotides 36 to 422 in the nucleotide sequence of huAT01_H3_IgG1LALA set forth in SEQ ID NO: 68 was synthesized (Geneart AG). A

huAT01_H3_IgG1LALA expression vector was constructed in the same manner as in Example 6)-3-3-1.

[0224]

6)-3-3-3 Construction of huAT01_H3_IgG4Pro expression vector

A DNA fragment represented by nucleotides 36 to 422 in the nucleotide sequence of huAT01_H3_IgG4Pro set forth in SEQ ID NO: 69 was synthesized (Geneart AG). The DNA fragment synthesized was inserted into the site where pCMA-G4Pro was cut with a restriction enzyme, BspI, using an In-Fusion HD PCR cloning kit (Clontech Laboratories, Inc.), thereby constructing a huAT01_H3_IgG4Pro expression vector.

[0225]

6)-3-4 Construction of AT01 humanized light-chain expression vector

6)-3-4-1 Construction of huAT01_L1 expression vector

A DNA fragment represented by nucleotides 37 to 399 in the nucleotide sequence of huAT01_L1 set forth in SEQ ID NO: 70 was synthesized (Geneart AG). The DNA fragment synthesized was inserted into the site where pCMA-LK was cut with a restriction enzyme, BspI, using an In-Fusion HD PCR cloning kit (Clontech Laboratories, Inc.), thereby constructing a huAT01_L1 expression vector.

[0226]

6)-3-4-2 Construction of huAT01_L2 expression vector

A DNA fragment represented by nucleotides 37 to 399 in the nucleotide sequence of huAT01_L2 set forth in SEQ ID NO: 71 was synthesized (Geneart AG). A huAT01_L1 expression vector was constructed in the same manner as in Example 6)-3-4-1 using an In-Fusion HD PCR cloning kit (Clontech Laboratories, Inc).

[0227]

6)-3-5 Construction of NB7 humanized heavy-chain expression vector

6)-3-5-1 Construction of huNB7_H3_IgG1LALA expression vector

A DNA fragment represented by nucleotides 36 to 440 in the nucleotide sequence of huNB7_H3_IgG1LALA set forth in SEQ ID NO: 72 was synthesized (Geneart AG). A huNB7_H3_IgG1LALA expression vector was constructed in the same manner as in Example 6)-3-3-1.

[0228]

6)-3-5-2 Construction of huNB7_H3_IgG4Pro expression vector

A DNA fragment represented by nucleotides 36 to 440 in the nucleotide sequence of huNB7_H3_IgG4Pro set forth in SEQ ID NO: 73 was synthesized (Geneart AG). A huNB7_H3_IgG4Pro expression vector was constructed in the same manner as in Example 6)-3-3-3.

[0229]

6)-3-6 Construction of NB7 humanized light-chain expression vector

6)-3-6-1 Construction of huNB7_L3 expression vector

A DNA fragment represented by nucleotides 37 to 399 in the nucleotide sequence of huNB7_L3 set forth in SEQ ID NO: 74 was synthesized (Geneart AG). A huNB7_L3 expression vector was constructed in the same manner as in Example 6)-3-4-1 using an In-Fusion HD PCR cloning kit (Clontech Laboratories, Inc).

[0230]

6)-3-7 Preparation of humanized antibodies

6)-3-7-1 Production of humanized antibodies

Humanized antibodies of huAT01_H1L1_IgG1LALA, huAT01_H3L1_IgG1LALA, huAT01_H3L2_IgG1LALA, huAT01_H3L2_IgG4Pro, huNB7_H3L3_IgG1LALA, and huNB7_H3L3_IgG4Pro were produced by the combination of the heavy and light chains designed in Example 6)-2, using the expression vectors constructed in Examples 6)-3-1 to 6)-3-6, in the same manner as in Example 4)-2.

[0231]

6)-3-7-2 Purification of humanized antibodies

The culture supernatant obtained in Example 6)-3-7-1 was purified by a two-step process of rProtein A affinity chromatography and ceramic hydroxyapatite.

[0232]

The culture supernatant was applied to a column (GE Healthcare Bioscience) filled with MabSelectSuRe equilibrated with PBS, and thereafter the column was washed with PBS in a volume of twice or more the column

volume. Next, each antibody was eluted with a 2M arginine hydrochloride solution (pH 4.0).

[0233]

The fraction containing the antibody was subjected to buffer replacement with PBS by dialysis (Slide-A-Lyzer Dialysis Cassette, Thermo Scientific), diluted 5-fold with a 5 mM sodium phosphate/50-mM MES/pH 7.0 buffer, and applied to a ceramic hydroxyapatite column (Japan Bio-Rad Laboratories, Inc., Bio-Scale CHT Type-1 Hydroxyapatite Column) equilibrated with a 5-mM NaPi/50-mM MES/30 mM NaCl/pH 7.0 buffer.

[0234]

Linear concentration gradient elution with sodium chloride was performed, and a fraction containing the antibody was collected. The fraction was subjected to buffer replacement with HBSor (25 mM histidine/5% sorbitol, pH 6.0) by dialysis (Slide-A-Lyzer Dialysis Cassette, Thermo Scientific). The antibody was concentrated with a Centrifugal UF Filter Device, VIVASPIN20 (fraction molecular weight UF10K, Sartorius AG), to prepare the IgG at a concentration of 50 mg/mL. Finally, filtration was carried out with a Minisart-Plus filter (Sartorius AG), to give a purified sample.

[0235]

[Example 7]

In vitro activity of humanized anti-human TLR7 antibody

7)-1 Inhibitory activity of humanized anti-human TLR7 antibody on cytokine production

Human PBMCs were purchased from Cellular Technology Limited as a frozen product and thawed according to the instructions for use.

[0236]

The PBMCs suspended at a concentration of 2.5×10^6 cells/mL in RPMI1640 (Life Technologies Corp.) containing 10% FBS, 1 mM Sodium Pyruvate (Life Technologies Corp.), 0.1 mM MEM Non-Essential Amino Acids (Life Technologies Corp.), 50 μ M 2-Mercaptoethanol (Life Technologies Corp.), 50 U/mL Penicillin, and 50- μ g/mL Streptomycin (Life Technologies Corp.) were seeded in a 96-well cell culture plate at 100 μ L each, and the humanized anti-human TLR7 antibody, huAT01_H1L1_IgG1LALA, huAT01_H3L1_IgG1LALA, huAT01_H3L2_IgG1LALA, huAT01_H3L2_IgG4Pro, huNB7_H3L3_IgG1LALA, or huNB7_H3L3_IgG4Pro, or a human IgG control antibody (Eureka Therapeutics) was added thereto at 80 μ L/well, followed by pretreatment in an incubator at 37°C for 4 hours.

[0237]

Thereafter, 1 mg/mL CL-264 (InvivoGen) was added thereto at 20 μ L/well, and the mixture was stirred well, followed by culturing at 37°C for about 20 hours under 5% CO₂.

[0238]

The plate was stirred well and thereafter centrifuged at 1500 rpm for 5 minutes, and the concentration of IL-6 contained in the supernatant was measured by the FRET method (Cisbio Bioassay. Inc).

[0239]

Figure 23 shows that the humanized anti-human TLR7 antibody suppresses the production of IL-6 from the human PBMCs treated with CL-264 in a concentration dependent manner.

[0240]

The antibodies huAT01_H1L1_IgG1LALA, huAT01_H3L1_IgG1LALA, huAT01_H3L2_IgG1LALA, huAT01_H3L2_IgG4Pro, huNB7_H3L3_IgG1LALA, and huNB7_H3L3_IgG4Pro inhibited the production of IL-6 from the human PBMCs in a concentration dependent manner.

[0241]

The half-inhibitory concentrations (IC_{50}) of the antibodies huAT01_H1L1_IgG1LALA, huAT01_H3L1_IgG1LALA, huAT01_H3L2_IgG1LALA, huAT01_H3L2_IgG4Pro, huNB7_H3L3_IgG1LALA and huNB7_H3L3_IgG4Pro were 74 ng/mL, 138 ng/mL, 38 ng/mL, 24 ng/mL, 307 ng/mL and 31 ng/mL, respectively, which were calculated from the concentrations of the two values, which are either side of the 50% inhibitory activity, and the inhibitory activity when each of the two concentrations were added.

[0242]

[Example 8]

Confirmation of antigen binding to humanized anti-human TLR7 antibody

8)-1 Preparation of HEK293 cells expressing human TLR7 (variant 1)-Flag

Lenti-X (registered trademark) HEK293 T cells (Clontech Laboratories, Inc.) at a concentration of 4.5×10^6 cells/flask were seeded in a 75-cm² flask (Sumitomo Bakelite Co., Ltd.) and cultured overnight in a DMEM medium (FUJIFILM Wako Pure Chemical Corporation) containing 10% FBS, 50 U/mL Penicillin, and 50 µg/mL Streptomycin (Life Technologies Corp.) under conditions of 37°C and 5% CO₂.

[0243]

On the next day, plasmids expressing pcDNA3.1-human TLR7 (variant 1)-Flag, produced by GenScript Corporation, were transfected into Lenti-X (registered trademark) HEK293 T cells (Clontech Laboratories, Inc.) using a transfection reagent, Lipofectamine (registered trademark) 2000 (Life Technologies Corp.), and the cells were further cultured overnight under conditions of 37°C and 5% CO₂.

[0244]

On the next day, the expression plasmid-introduced cells were treated with TrypLE Express (Life Technologies Corporation), and the cells after the treatment were washed with a Flow Cytometry Staining Buffer (Life Technologies Corporation) and thereafter suspended in a

Flow Cytometry Staining Buffer (Life Technologies Corporation). The cell suspension obtained was used in flow cytometry analysis.

[0245]

It was confirmed that human TLR7 was expressed in the cells transfected with the plasmids expressing pcDNA3.1-human TLR7 (variant 1)-Flag, by flow cytometry (Miltenyi Biotec K.K.) using an anti-Flag-PE antibody (BioLegend, Inc.) or an isotype control antibody (BioLegend, Inc.) as a negative control.

[0246]

The amino acid sequence of human TLR7 (variant 1) and a polynucleotide sequence encoding the amino acid sequence are set forth in SEQ ID NOs: 2 and 1 in the Sequence Listing, respectively.

[0247]

8)-2 Preparation of HEK293 cells forcibly expressing human TLR7 (variant 2)-Flag

Lenti-X (registered trademark) HEK293 T cells (Clontech Laboratories, Inc.) at a concentration of 4.5×10^6 cells/flask were seeded in a 75-cm² flask (Sumitomo Bakelite Co., Ltd.) and cultured overnight in a DMEM medium (FUJIFILM Wako Pure Chemical Corporation) containing 10% FBS, 50 U/mL Penicillin, and 50-μg/mL Streptomycin (Life Technologies Corp.) under conditions of 37°C and 5% CO₂.

[0248]

On the next day, plasmids expressing pcDNA3.1-human TLR7 (variant 2)-Flag, produced by GenScript Biotech Corporation, were transfected into Lenti-X (registered trademark) HEK293 T cells (Clontech Laboratories, Inc.) using a transfection reagent, Lipofectamine (registered trademark) 2000 (Life Technologies Corp.), and the cells were further cultured overnight under conditions of 37°C and 5% CO₂.

[0249]

On the next day, the expression vector-transfected cells were treated with TrypLE Express (Life Technologies Corporation), and the cells after the treatment were washed with a Flow Cytometry Staining Buffer (Life Technologies Corporation) and thereafter suspended in a Flow Cytometry Staining Buffer (Life Technologies Corporation). The cell suspension obtained was used in flow cytometry analysis.

[0250]

It was confirmed that human TLR7 was expressed in the cells transfected with the plasmids expressing pcDNA3.1-human TLR7 (variant 2)-Flag, by flow cytometry (Miltenyi Biotec K.K.) using an anti-Flag-PE antibody (BioLegend, Inc.) or an isotype control antibody (BioLegend, Inc.) as a negative control.

[0251]

8)-3 Preparation of HEK293 cells expressing monkey TLR7-Flag

Lenti-X (registered trademark) HEK293 T cells (Clontech Laboratories, Inc.) at a concentration of 4.5×10^6 cells/flask were seeded in a 75-cm² flask (Sumitomo Bakelite Co., Ltd.) and cultured overnight in a DMEM medium (FUJIFILM Wako Pure Chemical Corporation) containing 10% FBS, 50 U/mL Penicillin, and 50-μg/mL Streptomycin (Life Technologies Corp.) under conditions of 37°C and 5% CO₂.

[0252]

On the next day, plasmids expressing pcDNA3.1-monkey TLR7-Flag, produced by GenScript Corporation, were transfected into Lenti-X (registered trademark) HEK293 T cells (Clontech Laboratories, Inc.) using a transfection reagent, Lipofectamine (registered trademark) 2000 (Life Technologies Corp.), and the cells were further cultured overnight under conditions of 37°C and 5% CO₂.

[0253]

On the next day, the expression vector-transfected cells were treated with TrypLE Express (Life Technologies Corporation), and the cells after the treatment were washed with a Flow Cytometry Staining Buffer (Life Technologies Corporation) and thereafter suspended in a Flow Cytometry Staining Buffer (Life Technologies Corporation). The cell suspension obtained was used in flow cytometry analysis.

[0254]

It was confirmed that monkey TLR7 was expressed in the cells transfected with the plasmids expressing pcDNA3.1-monkey TLR7-Flag, by flow cytometry (Miltenyi Biotec K.K.), using an anti-Flag-PE antibody (BioLegend, Inc.) or an isotype control antibody (BioLegend, Inc.) as a negative control.

[0255]

8)-4 Preparation of HEK293 cells expressing mouse TLR7-Flag

Lenti-X (registered trademark) HEK293 T cells (Clontech Laboratories, Inc.) at a concentration of 4.5×10^6 cells/flask were seeded in a 75-cm² flask (Sumitomo Bakelite Co., Ltd.) and cultured overnight in a DMEM medium (FUJIFILM Wako Pure Chemical Corporation) containing 10%FBS, 50-U/mL Penicillin, and 50-μg/mL Streptomycin (Life Technologies Corp.) under conditions of 37°C and 5% CO₂.

[0256]

On the next day, plasmids expressing pcDNA3.1-mouse TLR7-Flag, produced by GenScript Corporation, were transfected into Lenti-X (registered trademark) HEK293 T cells (Clontech Laboratories, Inc.) using a transfection reagent, Lipofectamine (registered trademark) 2000 (Life Technologies Corp.), and the cells were further cultured overnight under conditions of 37°C and 5% CO₂.

[0257]

On the next day, the expression vector-transfected cells were treated with TrypLE Express (Life Technologies Corporation), and the cells after the treatment were washed with a Flow Cytometry Staining Buffer (Life Technologies Corporation) and thereafter suspended in a Flow Cytometry Staining Buffer (Life Technologies Corporation). The cell suspension obtained was used in flow cytometry analysis.

[0258]

It was confirmed that mouse TLR7 was expressed in the cells transfected with the plasmids expressing pcDNA3.1-mouse TLR7-Flag by flow cytometry (Miltenyi Biotec K.K.), using an anti-Flag-PE antibody (BioLegend, Inc.) or an isotype control antibody (BioLegend, Inc.) as a negative control.

[0259]

8)-5 Preparation of HEK293 cells expressing rat TLR7-Flag

Lenti-X (registered trademark) HEK293 T cells (Clontech Laboratories, Inc.) at a concentration of 4.5×10^6 cells/flask were seeded in a 75-cm² flask (Sumitomo Bakelite Co., Ltd.) and cultured overnight in a DMEM medium (FUJIFILM Wako Pure Chemical Corporation) containing 10% FBS, 50 U/mL Penicillin, and 50-μg/mL Streptomycin (Life Technologies Corp.) under conditions of 37°C and 5% CO₂.

[0260]

On the next day, plasmids expressing pcDNA3.1-rat TLR7-Flag, produced by GenScript Corporation, were transfected into Lenti-X (registered trademark) HEK293 T cells (Clontech Laboratories, Inc.) using a transfection reagent, Lipofectamine (registered trademark) 2000 (Life Technologies Corp.), and the cells were further cultured overnight under conditions of 37°C and 5% CO₂.

[0261]

On the next day, the expression vector-transfected cells were treated with TrypLE Express (Life Technologies Corporation), and the cells after the treatment were washed with a Flow Cytometry Staining Buffer (Life Technologies Corporation) and thereafter suspended in a Flow Cytometry Staining Buffer (Life Technologies Corporation). The cell suspension obtained was used in flow cytometry analysis.

[0262]

It was confirmed that rat TLR7 was expressed in the cells transfected with the plasmids expressing pcDNA3.1-rat TLR7-Flag by flow cytometry (Miltenyi Biotec K.K.), using an anti-Flag-PE antibody (BioLegend, Inc.) or an isotype control antibody (BioLegend, Inc.) as a negative control.

[0263]

8)-6 Evaluation of binding selectivity of humanized anti-human TLR7 antibody by flow cytometry

The HEK293 cells expressing human TLR7 (variant 1)-Flag prepared in 8)-1, the HEK293 cells expressing human TLR7 (variant 2)-Flag prepared in 8)-2, the HEK293 cells expressing monkey TLR7-Flag prepared in 8)-3, the HEK293 cells expressing mouse TLR7-Flag prepared in 8)-4, and the HEK293 cells expressing rat TLR7-Flag prepared in 8)-5 were immobilized/subjected to membrane permeation with a Fixation and Permeabilization Solution (Becton, Dickinson and Company) and thereafter stained using huAT01_H3L2_IgG1LALA, which is one of the humanized anti-human TLR7 antibodies, and Alexa Fluor (registered trademark) 647 AffiniPure Goat anti-human IgG + IgM (H+L) (Jackson ImmunoResearch Inc.), which is the secondary antibody.

[0264]

The binding of the humanized anti-human TLR7 antibody to human TLR7 was detected based on flow cytometry (Miltenyi Biotec K.K.), and the data was analyzed using software (Flowjo) which analyzes the data of flow cytometry.

[0265]

The results indicate that huAT01_H3L2_IgG1LALA, which is one of the humanized anti-human TLR7 antibodies, bound specifically to human TLR7 (variant 1), human TLR7 (variant 2), or monkey TLR7, and did not bind to mouse TLR7 or rat TLR7. Figures 25 to 29 show the results.

Industrial Applicability

[0266]

The humanized anti-TLR7 antibody of the present invention has a human TLR7 function-inhibitory activity-suppressing action and thus can be an agent for treating or preventing immune inflammation-related diseases and the like.

Sequence Listing

[0267]

SEQ ID NO: 1: Nucleotide sequence encoding human TLR7
(variant 1)

SEQ ID NO: 2: Amino acid sequence of human TLR7 (variant
1)

SEQ ID NO: 3: Nucleotide sequence encoding human TLR7
(variant 2)

SEQ ID NO: 4: Amino acid sequence of human TLR7 (variant
2)

SEQ ID NO: 5: Amino acid sequence of heavy-chain variable
region of AT01 antibody

SEQ ID NO: 6: Nucleotide sequence encoding heavy-chain
variable region of cAT01 antibody

SEQ ID NO: 7: Amino acid sequence of heavy-chain variable
region of NB7 antibody

SEQ ID NO: 8: Nucleotide sequence encoding heavy-chain
variable region of cNB7 antibody

SEQ ID NO: 9: Amino acid sequence of heavy-chain variable region of FAN2 antibody

SEQ ID NO: 10: Nucleotide sequence encoding heavy-chain variable region of cFAN2 antibody

SEQ ID NO: 11: Amino acid sequence of light-chain variable region of AT01 antibody

SEQ ID NO: 12: Nucleotide sequence encoding light-chain variable region of cAT01 antibody

SEQ ID NO: 13: Amino acid sequence of light-chain variable region of NB7 antibody

SEQ ID NO: 14: Nucleotide sequence encoding light-chain variable region of cNB7 antibody

SEQ ID NO: 15: Amino acid sequence of light-chain variable region of FAN2 antibody

SEQ ID NO: 16: Nucleotide sequence encoding light-chain variable region of cFAN2 antibody

SEQ ID NO: 17: Amino acid sequence of CDRH1 of AT01 antibody

SEQ ID NO: 18: Amino acid sequence of CDRH2 of AT01 antibody

SEQ ID NO: 19: Amino acid sequence of CDRH3 of AT01 antibody

SEQ ID NO: 20: Amino acid sequence of CDRL1 of AT01 antibody

SEQ ID NO: 21: Amino acid sequence of CDRL2 of AT01 antibody

SEQ ID NO: 22: Amino acid sequence of CDRL3 of AT01 antibody

SEQ ID NO: 23: Amino acid sequence of CDRH1 of NB7 antibody

SEQ ID NO: 24: Amino acid sequence of CDRH2 of NB7 antibody

SEQ ID NO: 25: Amino acid sequence of CDRH3 of NB7 antibody

SEQ ID NO: 26: Amino acid sequence of CDRL1 of NB7 antibody

SEQ ID NO: 27: Amino acid sequence of CDRL2 of NB7 antibody

SEQ ID NO: 28: Amino acid sequence of CDRL3 of NB7 antibody

SEQ ID NO: 29: Amino acid sequence of CDRH1 of FAN2 antibody

SEQ ID NO: 30: Amino acid sequence of CDRH2 of FAN2 antibody

SEQ ID NO: 31: Amino acid sequence of CDRH3 of FAN2 antibody

SEQ ID NO: 32: Amino acid sequence of CDRL1 of FAN2 antibody

SEQ ID NO: 33: Amino acid sequence of CDRL2 of FAN2 antibody

SEQ ID NO: 34: Amino acid sequence of CDRL3 of FAN2 antibody

SEQ ID NO: 35: Amino acid sequence of heavy chain of AT01 chimeric anti-human TLR7 antibody (cAT01)

SEQ ID NO: 36: Amino acid sequence of light chain of AT01 chimeric anti-human TLR7 antibody (cAT01)

SEQ ID NO: 37: Amino acid sequence of heavy chain of NB7 chimeric anti-human TLR7 antibody (cNB7)

SEQ ID NO: 38: Amino acid sequence of light chain of NB7 chimeric anti-human TLR7 antibody (cNB7)

SEQ ID NO: 39: Amino acid sequence of heavy chain of FAN2 chimeric anti-human TLR7 antibody (cFAN2)

SEQ ID NO: 40: Amino acid sequence of light chain of FAN2 chimeric anti-human TLR7 antibody (cFAN2)

SEQ ID NO: 41: Amino acid sequence of heavy-chain variable region of huAT01_H1_IgG1LALA

SEQ ID NO: 42: Amino acid sequence of heavy-chain variable region of huAT01_H3_IgG1LALA

SEQ ID NO: 43: Amino acid sequence of light-chain variable region of huAT01_L1

SEQ ID NO: 44: Amino acid sequence of light-chain variable region of huAT01_L2

SEQ ID NO: 45: Amino acid sequence of huAT01_H1_IgG1LALA

SEQ ID NO: 46: Amino acid sequence of huAT01_H3_IgG1LALA

SEQ ID NO: 47: Amino acid sequence of huAT01_H3_IgG4Pro

SEQ ID NO: 48: Amino acid sequence of huAT01_L1

SEQ ID NO: 49: Amino acid sequence of huAT01_L2

SEQ ID NO: 50: Amino acid sequence of heavy-chain variable region of huNB7_H3_IgG1LALA

SEQ ID NO: 51: Amino acid sequence of light-chain variable region of huNB7_L3

SEQ ID NO: 52: Amino acid sequence of huNB7_H3_IgG1LALA

SEQ ID NO: 53: Amino acid sequence of huNB7_L3

SEQ ID NO: 54: Amino acid sequence of huNB7_H3_IgG4Pro

SEQ ID NO: 55: Nucleotide sequence of human Unc93B1 gene

SEQ ID NO: 56: Nucleotide sequence of mouse TLR7 gene

SEQ ID NO: 57: Nucleotide sequence of rat TLR7 gene

SEQ ID NO: 58: Nucleotide sequence of monkey TLR7 gene

SEQ ID NO: 59: Nucleotide sequences of DNA fragments comprising nucleotide sequences encoding human light-chain signal sequence and human kappa-chain constant region

SEQ ID NO: 60: Nucleotide sequences of DNA fragments comprising nucleotide sequences encoding human heavy-chain signal sequence and human IgG1 constant region

SEQ ID NO: 61: Nucleotide sequence encoding heavy chain of cAT01 antibody

SEQ ID NO: 62: Nucleotide sequence of DNA fragment comprising nucleotide sequence encoding light chain of cAT01 antibody

SEQ ID NO: 63: Nucleotide sequence encoding heavy chain of cNB7 antibody

SEQ ID NO: 64: Nucleotide sequence of DNA fragment comprising nucleotide sequence encoding light chain of cNB7 antibody

SEQ ID NO: 65: Nucleotide sequence encoding heavy chain of cFAN2 antibody

SEQ ID NO: 66: Nucleotide sequence of DNA fragment comprising nucleotide sequence encoding cFAN2 antibody light chain

SEQ ID NO: 67: Nucleotide sequence encoding huAT01_H1_IgG1LALA

SEQ ID NO: 68: Nucleotide sequence encoding huAT01_H3_IgG1LALA

SEQ ID NO: 69: Nucleotide sequence encoding huAT01_H3_IgG4Pro

SEQ ID NO: 70: Nucleotide sequence encoding huAT01_L1

SEQ ID NO: 71: Nucleotide sequence encoding huAT01_L2

SEQ ID NO: 72: Nucleotide sequence encoding huNB7_H3_IgG1LALA

SEQ ID NO: 73: Nucleotide sequence encoding huNB7_H3_IgG4Pro

SEQ ID NO: 74: Nucleotide sequence encoding huNB7_L3

SEQ ID NO: 75: Nucleotide sequences of DNA fragments comprising nucleotide sequences encoding human heavy-chain signal sequence and human IgG1LALA constant region

SEQ ID NO: 76: Nucleotide sequences of DNA fragments comprising nucleotide sequences encoding human heavy-chain signal sequence and human IgG4Pro constant region

SEQ ID NO: 77: Nucleotide sequence encoding heavy-chain variable region of huAT01_H1

SEQ ID NO: 78: Nucleotide sequence encoding heavy-chain variable region of huAT01_H3

SEQ ID NO: 79: Nucleotide sequence encoding light-chain variable region of huAT01_L1

SEQ ID NO: 80: Nucleotide sequence encoding light-chain variable region of huAT01_L2

SEQ ID NO: 81: Nucleotide sequence encoding heavy-chain variable region of huNB7_H3

SEQ ID NO: 82: Nucleotide sequence encoding light-chain variable region of huNB7_L3

SEQ ID NO: 83: Amino acid sequence of mouse TLR7

SEQ ID NO: 84: Amino acid sequence of rat TLR7

SEQ ID NO: 85: Amino acid sequence of monkey TLR7

SEQ ID NO: 86: Nucleotide sequence of human Unc93B1 D34A mutant-HA×2 gene

Claims

[Claim 1]

An antibody or an antigen-binding fragment of the antibody having the properties of:

(a) specifically binding to human TLR7 or monkey TLR7 and not binding to mouse TLR7 or rat TLR7;

(b) inhibiting a function of human TLR7 or monkey TLR7; and

(c) comprising CDRH1 consisting of the amino acid sequence set forth in SEQ ID NO: 17, CDRH2 consisting of the amino acid sequence set forth in SEQ ID NO: 18 and CDRH3 consisting of the amino acid sequence set forth in SEQ ID NO: 19, as complementarity determining regions in the heavy chain, and CDRL1 consisting of the amino acid sequence set forth in SEQ ID NO: 20, CDRL2 consisting of the amino acid sequence set forth in SEQ ID NO: 21 and CDRL3 consisting of the amino acid sequence set forth in SEQ ID NO: 22, as complementarity determining regions in the light chain.

[Claim 2]

The antibody or the antigen-binding fragment of the antibody according to claim 1, wherein the human TLR7 is a molecule consisting of the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4, the monkey TLR7 is a molecule consisting of the amino acid sequence set forth in SEQ ID NO: 85, the mouse TLR7 is a molecule consisting of the

amino acid sequence set forth in SEQ ID NO: 83, or the rat TLR7 is a molecule consisting of the amino acid sequence set forth in SEQ ID NO: 84.

[Claim 3]

The antibody or the antigen-binding fragment of the antibody according to claim 1 or 2, having a competitive inhibitory activity for binding to human TLR7 with:

an antibody comprising a heavy chain with a heavy-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 5 and a light chain with a light-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 11.

[Claim 4]

The antibody or the antigen-binding fragment of the antibody according to any one of claims 1 to 3, comprising a heavy-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 5 and a light-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 11.

[Claim 5]

The antibody or the antigen-binding fragment of the antibody according to any one of claims 1 to 4, wherein the antibody comprises a human-derived constant region.

[Claim 6]

The antibody or the antigen-binding fragment of the antibody according to claim 5, comprising a heavy chain consisting of an amino acid sequence at positions 20 to 465

in the amino acid sequence set forth in SEQ ID NO: 35 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 36.

[Claim 7]

The antibody or the antigen-binding fragment of the antibody according to any one of claims 1 to 3, being humanized.

[Claim 8]

The antibody or the antigen-binding fragment of the antibody according to claim 7, comprising:

a heavy-chain variable region consisting of an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence set forth in SEQ ID NO: 41;

(b) the amino acid sequence set forth in SEQ ID NO: 42;

(c) an amino acid sequence having a homology of at least 95% or more to the sequence (a) or (b); and

(d) an amino acid sequence derived from the sequence (a) or (b) by deletion, substitution or addition of 1 to 10 amino acids; and

a light-chain variable region consisting of an amino acid sequence selected from the group consisting of:

(e) the amino acid sequence set forth in SEQ ID NO: 43;

(f) the amino acid sequence set forth in SEQ ID NO: 44;

(g) an amino acid sequence having a homology of at least 95% or more to the sequence (e) or (f); and

(h) an amino acid sequence derived from the sequence (e) or (f) by deletion, substitution or addition of 1 to 10 amino acids.

[Claim 9]

The antibody or the antigen-binding fragment of the antibody according to claim 8, comprising:

(a) a heavy-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 41 and a light-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 43;

(b) a heavy-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 41 and a light-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 44;

(c) a heavy-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 42 and a light-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 43; or

(d) a heavy-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 42 and a light-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 44.

[Claim 10]

The antibody or the antigen-binding fragment of the antibody according to claim 9, comprising a heavy-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 42 and a light-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 44.

[Claim 11]

The antibody or the antigen-binding fragment of the antibody according to claim 9, comprising:

(a) a heavy chain consisting of an amino acid sequence at positions 20 to 465 in the amino acid sequence set forth in SEQ ID NO: 45 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 48;

(b) a heavy chain consisting of an amino acid sequence at positions 20 to 465 in the amino acid sequence set forth in SEQ ID NO: 45 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 49;

(c) a heavy chain consisting of an amino acid sequence at positions 20 to 465 in the amino acid sequence set forth in SEQ ID NO: 46 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 48;

(d) a heavy chain consisting of an amino acid sequence at positions 20 to 465 in the amino acid sequence set forth in SEQ ID NO: 46 and a light chain consisting of an amino

acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 49;

(e) a heavy chain consisting of an amino acid sequence at positions 20 to 462 in the amino acid sequence set forth in SEQ ID NO: 47 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 48; or

(f) a heavy chain consisting of an amino acid sequence at positions 20 to 462 in the amino acid sequence set forth in SEQ ID NO: 47 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 49.

[Claim 12]

The antibody or the antigen-binding fragment of the antibody according to claim 11, comprising a heavy chain consisting of an amino acid sequence at positions 20 to 465 in the amino acid sequence set forth in SEQ ID NO: 46 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 49.

[Claim 13]

An antibody or an antigen-binding fragment of the antibody having the properties of:

- (a) specifically binding to human TLR7,
- (b) inhibiting a function of human TLR7, and
- (c) comprising CDRH1 consisting of the amino acid sequence set forth in SEQ ID NO: 17, CDRH2 consisting of

the amino acid sequence set forth in SEQ ID NO: 18 and CDRH3 consisting of the amino acid sequence set forth in SEQ ID NO: 19, as complementarity determining regions in the heavy chain, and CDRL1 consisting of the amino acid sequence set forth in SEQ ID NO: 20, CDRL2 consisting of the amino acid sequence set forth in SEQ ID NO: 21 and CDRL3 consisting of the amino acid sequence set forth in SEQ ID NO: 22, as complementarity determining regions in the light chain.

[Claim 14]

The antibody or the antigen-binding fragment of the antibody according to claim 13, comprising a heavy-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 5 and a light-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 11.

[Claim 15]

The antibody or the antigen-binding fragment of the antibody according to claim 13 or 14, wherein the antibody comprises a human-derived constant region.

[Claim 16]

The antibody or the antigen-binding fragment of the antibody according to claim 15, comprising a heavy chain consisting of an amino acid sequence at positions 20 to 465 in the amino acid sequence set forth in SEQ ID NO: 35 and a light chain consisting of an amino acid sequence at

positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 36.

[Claim 17]

The antibody or the antigen-binding fragment of the antibody according to claim 13 being humanized.

[Claim 18]

The antibody or the antigen-binding fragment of the antibody according to claim 17, comprising:

a heavy-chain variable region consisting of an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence set forth in SEQ ID NO: 41;

(b) the amino acid sequence set forth in SEQ ID NO: 42;

(c) an amino acid sequence having a homology of at least 95% or more to the sequence (a) or (b); and

(d) an amino acid sequence derived from the sequence (a) or (b) by deletion, substitution or addition of 1 to 10 amino acids; and

a light-chain variable region consisting of an amino acid sequence selected from the group consisting of:

(e) the amino acid sequence set forth in SEQ ID NO: 43;

(f) the amino acid sequence set forth in SEQ ID NO: 44;

(g) an amino acid sequence having a homology of at least 95% or more to the sequence (e) or (f); and

(h) an amino acid sequence derived from the sequence (e) or (f) by deletion, substitution or addition of 1 to 10 amino acids.

[Claim 19]

The antibody or the antigen-binding fragment of the antibody according to claim 18, comprising:

(a) a heavy-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 41 and a light-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 43;

(b) a heavy-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 41 and a light-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 44;

(c) a heavy-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 42 and a light-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 43; or

(d) a heavy-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 42 and a light-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 44.

[Claim 20]

The antibody or the antigen-binding fragment of the antibody according to claim 19, comprising a heavy-chain variable region consisting of the amino acid sequence set forth in SEQ ID NO: 42 and a light-chain variable region

consisting of the amino acid sequence set forth in SEQ ID NO: 44.

[Claim 21]

The antibody or the antigen-binding fragment of the antibody according to claim 19, comprising:

(a) a heavy chain consisting of an amino acid sequence at positions 20 to 465 in the amino acid sequence set forth in SEQ ID NO: 45 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 48;

(b) a heavy chain consisting of an amino acid sequence at positions 20 to 465 in the amino acid sequence set forth in SEQ ID NO: 45 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 49;

(c) a heavy chain consisting of an amino acid sequence at positions 20 to 465 in the amino acid sequence set forth in SEQ ID NO: 46 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 48;

(d) a heavy chain consisting of an amino acid sequence at positions 20 to 465 in the amino acid sequence set forth in SEQ ID NO: 46 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 49;

(e) a heavy chain consisting of an amino acid sequence at positions 20 to 462 in the amino acid sequence set forth

in SEQ ID NO: 47 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 48; or

(f) a heavy chain consisting of an amino acid sequence at positions 20 to 462 in the amino acid sequence set forth in SEQ ID NO: 47 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 49.

[Claim 22]

The antibody or the antigen-binding fragment of the antibody according to claim 21, comprising a heavy chain consisting of an amino acid sequence at positions 20 to 465 in the amino acid sequence set forth in SEQ ID NO: 46 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 49.

[Claim 23]

An antibody or an antigen-binding fragment of the antibody comprising a heavy chain consisting of an amino acid sequence at positions 20 to 465 in the amino acid sequence set forth in SEQ ID NO: 45 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 48.

[Claim 24]

An antibody or an antigen-binding fragment of the antibody comprising a heavy chain consisting of an amino acid sequence at positions 20 to 465 in the amino acid

sequence set forth in SEQ ID NO: 46 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 48.

[Claim 25]

An antibody or an antigen-binding fragment of the antibody comprising a heavy chain consisting of an amino acid sequence at positions 20 to 465 in the amino acid sequence set forth in SEQ ID NO: 46 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 49.

[Claim 26]

An antibody or an antigen-binding fragment of the antibody comprising a heavy chain consisting of an amino acid sequence at positions 20 to 462 in the amino acid sequence set forth in SEQ ID NO: 47 and a light chain consisting of an amino acid sequence at positions 21 to 233 in the amino acid sequence set forth in SEQ ID NO: 49.

[Claim 27]

The antibody or the antigen-binding fragment of the antibody according to any one of claims 23 to 26, having the properties of:

- (a) specifically binding to human TLR7; and
- (b) inhibiting a function of human TLR7.

[Claim 28]

The antigen-binding fragment of the antibody according to any one of claims 1 to 27, wherein the antigen-binding

fragment is selected from the group consisting of Fab, F(ab)₂, Fab' and Fv.

[Claim 29]

A polynucleotide comprising a polynucleotide sequence encoding the antibody or the antigen-binding fragment of the antibody according to any one of claims 1 to 28.

[Claim 30]

The polynucleotide according to claim 29, comprising:

a polynucleotide sequence encoding CDRH1 consisting of the amino acid sequence set forth in SEQ ID NO: 17, a polynucleotide sequence encoding CDRH2 consisting of the amino acid sequence set forth in SEQ ID NO: 18 and a polynucleotide sequence encoding CDRH3 consisting of the amino acid sequence set forth in SEQ ID NO: 19, and a polynucleotide sequence encoding CDRL1 consisting of the amino acid sequence set forth in SEQ ID NO: 20, a polynucleotide sequence encoding CDRL2 consisting of the amino acid sequence set forth in SEQ ID NO: 21 and a polynucleotide sequence encoding CDRL3 consisting of the amino acid sequence set forth in SEQ ID NO: 22.

[Claim 31]

A polynucleotide comprising a polynucleotide sequence encoding the antibody or the antigen-binding fragment of the antibody according to any one of claims 7 to 12 and 17 to 22, comprising:

a polynucleotide sequence selected from the group consisting of:

(a) a polynucleotide sequence encoding the heavy-chain variable region set forth in SEQ ID NO: 77;

(b) a polynucleotide sequence encoding the heavy-chain variable region set forth in SEQ ID NO: 78; and

(c) a polynucleotide sequence of a polynucleotide which hybridizes with a polynucleotide consisting of a polynucleotide sequence complementary to the polynucleotide sequence (a) or (b) under stringent conditions; and

a polynucleotide sequence selected from the group consisting of:

(d) a polynucleotide sequence encoding the light-chain variable region set forth in SEQ ID NO: 79;

(e) a polynucleotide sequence encoding the light-chain variable region set forth in SEQ ID NO: 80; and

(f) a polynucleotide sequence of a polynucleotide which hybridizes with a polynucleotide consisting of a polynucleotide sequence complementary to the polynucleotide sequence (d) or (e) under stringent conditions.

[Claim 32]

The polynucleotide according to claim 31, comprising:

(a) a polynucleotide sequence encoding the heavy-chain variable region set forth in SEQ ID NO: 77 and a polynucleotide sequence encoding the light-chain variable region set forth in SEQ ID NO: 79;

(b) a polynucleotide sequence encoding the heavy-chain variable region set forth in SEQ ID NO: 77 and a

polynucleotide sequence encoding the light-chain variable region set forth in SEQ ID NO: 80;

(c) a polynucleotide sequence encoding the heavy-chain variable region set forth in SEQ ID NO: 78 and a polynucleotide sequence encoding the light-chain variable region set forth in SEQ ID NO: 79; or

(d) a polynucleotide sequence encoding the heavy-chain variable region set forth in SEQ ID NO: 78 and a polynucleotide sequence encoding the light-chain variable region set forth in SEQ ID NO: 80.

[Claim 33]

An expression vector comprising the polynucleotide according to any one of claims 29 to 32.

[Claim 34]

A host cell transformed with the expression vector according to claim 33.

[Claim 35]

The host cell according to claim 34, wherein the host cell is a eukaryotic cell.

[Claim 36]

A method for producing an antibody or an antigen-binding fragment of the antibody, comprising the steps of:
culturing the host cell according to claim 34 or 35;
and

collecting the antibody or the fragment of interest from the culture obtained in the above step.

[Claim 37]

An antibody or an antigen-binding fragment of the antibody obtained by the production method according to claim 36.

[Claim 38]

The antibody according to any one of claims 1 to 27, comprising one or more modifications selected from the group consisting of glycosylation to N-linkage, glycosylation to O-linkage, N-terminal processing, C-terminal processing, deamidation, isomerization of aspartic acid, oxidation of methionine, addition of a methionine residue to N-terminal, amidation of a proline residue, and deletion of one or two amino acids at the carboxyl terminus of the heavy chain.

[Claim 39]

The antibody according to claim 38, comprising two heavy chains consisting of one kind of or a combination of any two kinds of heavy chains selected from the group consisting of the full length and deletion variants with one or two amino acids deleted at the carboxyl terminus.

[Claim 40]

The antibody according to claim 39, wherein one amino acid is deleted at the carboxyl terminus in each of the two heavy chains.

[Claim 41]

The antibody according to any one of claims 38 to 40, wherein a proline residue at the carboxyl terminus of the heavy chain is further amidated.

[Claim 42]

A pharmaceutical composition for treating and/or preventing a disease, comprising at least one of the antibodies or the antigen-binding fragments of the antibodies according to claims 1 to 28 and 37 to 41 or the expression vector according to claim 33 as an active ingredient.

[Claim 43]

The pharmaceutical composition according to claim 42, for treating a disease caused by a function of human TLR7, wherein the disease caused by a function of human TLR7 is an immune inflammation-related disease, an allergic disease, an infection or a cancer.

[Claim 44]

The pharmaceutical composition according to claim 43, wherein the immune inflammation-related disease is systemic lupus erythematosus, rheumatoid arthritis, juvenile idiopathic arthritis, adult-onset Still's disease, ankylosing spondylitis, systemic scleroderma, polymyositis, dermatomyositis, psoriatic arthritis, osteoarthritis, mixed connective tissue disease or muscular dystrophy.

[Claim 45]

The pharmaceutical composition according to claim 43 or 44, wherein the immune inflammation-related disease is systemic lupus erythematosus.

[Claim 46]

The pharmaceutical composition according to any one of claims 42 to 45, to be used in combination with an immunosuppressant, an anti-inflammatory drug, an antiallergic agent, an anti-infective agent or an anticancer agent.

[Claim 47]

The antibody or antigen-binding fragment according to claims 1 to 28 and 37 to 41 for use in the treatment of a disease caused by a function of human TLR7, wherein an effective amount of at least one of the antibodies or the antigen-binding fragment of the antibodies is to be administered to an individual in need thereof, and wherein the disease caused by a function of human TLR7 is an immune inflammation-related disease, an allergic disease, an infection or a cancer.

[Claim 48]

Use of the antibody or antigen-binding fragment according to claims 1 to 28 and 37 to 41 in the manufacture of a medicament for the treatment of a disease caused by a function of human TLR7, wherein an effective amount of at least one of the antibodies or the antigen-binding fragment of the antibodies is to be administered together with an immunosuppressant, an anti-inflammatory drug, an antiallergic agent, an anti-infective agent or an anticancer agent, simultaneously, separately or sequentially to an individual in need thereof, and wherein the disease caused by a function of human TLR7 is an immune

inflammation-related disease, an allergic disease, an infection or a cancer.

[Claim 49]

The use according to claim 48, wherein the immune inflammation-related disease is systemic lupus erythematosus, rheumatoid arthritis, juvenile idiopathic arthritis, adult-onset Still's disease, ankylosing spondylitis, systemic scleroderma, polymyositis, dermatomyositis, psoriatic arthritis, osteoarthritis, mixed connective tissue disease or muscular dystrophy.

[Claim 50]

The use according to claim 48, wherein the immune inflammation-related disease is systemic lupus erythematosus.