[0295] The MAb 67 variable CDR regions were used to create a humanized monoclonal antibody. The humanized VH and VK genes were respectively cloned into vectors containing the human gamma 4 and human kappa constant domains. The pairing of the humanized VH and the humanized VK created the IgG4/kappa MAb 2503. The amino acid sequences of the humanized MAb 67 VH (H2160) and VK (L553) are shown below with the CDR1, CDR2 and CDR3 regions underlined.

Sequence of H2160:

QVQLVQSGAEVKKPGSSVKVSCKASGYSFSDYYMHWVRQAPGQGLEWMGQIN PTTGGASYNQKFKGKATITVDKSTSTAYMELSSLRSEDTAVYYCARYYYGRHFD VWGQGTTVTVSS (SEQ ID NO: 9)

Sequence of L553:

DIVMTQSPDSLAVSLGERATINCKASQSVDYDGDSYMNWYQQKPGQPPKLLIYA ASNLESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQSNEDPYTFGQGTKLEI K (SEQ ID NO: 17)

[0296] Polynucleotides encoding the VH (Human Immunoglobulin gene with human gamma; H2160) and VK (Human Immunoglobulin gene with human kappa; L553) regions of MAb 2503 antibody were cloned into pCMV-Script (Stratagene) vectors and were deposited with the American Type Culture Collection ("ATCC") on May 7, 2009, and given ATCC Deposit Numbers PTA-10004 and PTA-10005, respectively. The ATCC is located at 10801 University Boulevard, Manassas, VA 20110-2209, USA. The ATCC deposits were made pursuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for purposes of patent procedure.

Example 5

Characterization of humanizaed anti-CD100 monoclonal antibody 2503

[0297] Specificity of MAb 2503 was characterized by ELISA and Flow Cytometry. MAb 2503 was tested in an epitope competition assay with MAb 67 and in an affinity determination assay using BIACORE® surface plasmon resonance technology.

[0298] The epitope specificity of MAb 2503 and MAb 67 were determined by competition ELISA. For the ELISA protocol, an ELISA plate was coated with CD100-

Fc. Mouse 67 or humanized 2503 MAbs were titrated. The antibodies were allowed to bind to CD100 and unbound antibody was wash away. Biotinylated MAb 67 was added. The antibodies were allowed to bind to CD100 and unbound antibody was wash away. Biotinylated MAb 67 bound to CD100 was detected using Streptavidin-HRP. The ability of MAb 2503 or MAb 67 to block binding of biotinylated 67 to human CD100 (shown in **Figure 7A**) and mouse CD100 (shown in **Figure 7B**) was analyzed by ELISA.

[0299] Binding affinities of anti-CD100 MAbs were determined using BIACORE® surface plasmon resonance technology. MAb 2503 was shown to only bind CD100 (human, mouse and marmoset) and CD100 expressing cell lines, indicating that MAb 2503 is specific for CD100. MAb 2503 showed an increased affinity for human and mouse CD100 compared to MAb 67. A summary of the affinity characteristics of MAb 2503 and MAb 67 is presented below in **Table 4**.

Table 4. Affinities of anti-CD100 MAbs for human, marmoset and mouse CD100

MAb	Affinity for Human CD100 (nM)*	Affinity for Marmoset CD100 (nM)*	Affinity for Mouse CD100 (nM)*
67	5.1	2.7	1.3
2503	5.4	2.4	1.5

* Affinity was measured using BIACORE® surface plasmon resonance technology

[0300] These assays show that MAb 2503 is CD100 specific and binds to the same epitope as mouse MAb 67. MAb 2503 was shown to bind both mouse and human CD100, thus an advantage of MAb 2503 is that it can be tested for safety and efficacy in mice and also in humans. Furthermore, it was shown that MAb 2305 bound to human, monkey, and mouse CD100.

Example 6

MAb 2503 blocks activity of mouse and human CD100 *in vitro*

[0301] MAb 2503 was tested for the ability to block the function of CD100 using the assays described above in Example 2, including (1) the flow cytometry blocking assay and (2) the cell detachment assay.

CLAIMS

1. An isolated antibody or antigen-binding fragment thereof that can specifically bind to CD100, comprising a heavy chain variable region polypeptide (VH) and a light chain variable region polypeptide (VL), wherein

(a) the VH comprises VH-CDR1, VH-CDR2, and VH-CDR3 amino acid sequences comprising SEQ ID NOs: 6, 7, and 8, respectively and the VL comprises VL-CDR1, VL-CDR2, and VL-CDR3 amino acid sequences comprising SEQ ID NOs: 14, 15, and 16, respectively;

(b) the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO: 9 or SEQ ID NO: 10;

(c) the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO: 17 or SEQ ID NO: 18;

(d) the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO: 9 or SEQ ID NO: 10 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO: 17 or SEQ ID NO: 18; or

(e) the antibody or fragment thereof is a monoclonal antibody that comprises a VH encoded by the polynucleotide deposited as ATCC PTA-10004 and a VL encoded by the polynucleotide deposited as ATCC PTA-10005.

2. The antibody or fragment thereof according to claim 1, further comprising a human constant region.

3. The antibody or fragment thereof according to claim 1 or claim 2, which is selected from the group consisting of a single chain antibody, an Fab fragment, an $F(ab)_2$ fragment, and an Fv fragment.

4. The antibody or fragment thereof according to any one of claims 1 to 3, which inhibits CD100 from binding to a CD100 receptor.

5. The antibody or fragment thereof according to any one of claims 1 to 4, which is humanized, primatized or chimeric.

6. The antibody or fragment thereof according to any one of claims 1 to 5, further comprising a heterologous polypeptide fused thereto.

7. A composition comprising the antibody or fragment thereof according to any one of claims 1 to 6, and a carrier.

8. An isolated polynucleotide comprising

(a) a nucleic acid that encodes an antibody VH, wherein the VH comprises VH-CDR1, VH-CDR2, and VH-CDR3 amino acid sequences comprising SEQ ID NOs: 6, 7, and 8, respectively; and wherein an antibody or antigen-binding fragment thereof comprising the VH specifically binds to CD100,

(b) a nucleic acid which encodes an antibody VH, wherein the amino acid sequence of the VH is at least 90% identical to SEQ ID NO: 9; and wherein an antibody or antigen-binding fragment thereof comprising the VH specifically binds to CD100,

(c) a nucleic acid sequence comprising SEQ ID NO: 19,

(d) a nucleic acid that encodes an antibody VL, wherein the VL comprises VL-CDR1, VL-CDR2, and VL-CDR3 amino acid sequences comprising SEQ ID NOs: 14, 15, and 16, respectively; and wherein an antibody or antigen-binding fragment thereof comprising the VL specifically binds to CD100,

(e) a nucleic acid which encodes an antibody VL, wherein the amino acid sequence of the VL is at least 90% identical to SEQ ID

NO: 17; and wherein an antibody or antigen-binding fragment thereof comprising the VL specifically binds to CD100, or

(f) a nucleic acid sequence comprising SEQ ID NO: 21.

9. A composition comprising an isolated VH-encoding polynucleotide and an isolated VL-encoding polynucleotide, wherein the VH-encoding polynucleotide encodes a VH comprising VH-CDR1, VH-CDR2, and VH-CDR3 amino acid sequences comprising SEQ ID NOs: 6, 7, and 8, respectively; wherein the VL-encoding polynucleotide encodes a VL comprising VL-CDR1, VL-CDR2, and VL-CDR3 amino acid sequences comprising SEQ ID NOs: 14, 15, and 16, respectively; and wherein an antibody or antigen-binding fragment thereof comprising the VH and VL specifically binds CD100.

10. The composition according to claim 9, wherein the VH-encoding polynucleotide and the VL-encoding polynucleotide are contained on a single vector or on separate vectors.

11. A host cell comprising the polynucleotide of claim 8 or the composition of claim 9 or claim 10.

12. A method of producing an antibody or antigen-binding fragment thereof that specifically binds CD100, comprising culturing a host cell comprising the polynucleotide of claim 8 or the composition of claim 10, and recovering the antibody, or fragment thereof.

13. The antibody or antigen-binding fragment thereof of any one of claims 1 to 6, or the composition of claim 7 for use in

(a) neutralizing CD100 in an animal,

(b) the treatment of an autoimmune disease or an inflammatory disease in an animal in need thereof,

(c) the treatment of a cancer in an animal in need thereof, or

(d) inhibiting angiogenesis in an animal in need of cancer treatment.

14. The antibody or antigen-binding fragment, or composition for the use according to claim 13, wherein the autoimmune disease or the inflammatory disease is multiple sclerosis or arthritis.

15. The use of an antibody or antigen-binding fragment thereof of any one of claims 1 to 6, or the use of a composition of claim 7, in the manufacture/preparation of a composition/medicament for the treatment of an autoimmune disease, an inflammatory disease, or cancer.

16. The use according to claim 15, wherein the autoimmune disease or the inflammatory disease is multiple sclerosis or arthritis.

[0295] The MAb 67 variable CDR regions were used to create a humanized monoclonal antibody. The humanized VH and VK genes were respectively cloned into vectors containing the human gamma 4 and human kappa constant domains. The pairing of the humanized VH and the humanized VK created the IgG4/kappa MAb 2503. The amino acid sequences of the humanized MAb 67 VH (H2160) and VK (L553) are shown below with the CDR1, CDR2 and CDR3 regions underlined.

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[0301] MAb 2503 was tested for the ability to block the function of CD100 using the assays described above in Example 2, including (1) the flow cytometry blocking assay and (2) the cell detachment assay.

CLAIMS

1. An isolated antibody or antigen-binding fragment thereof that can specifically bind to CD100, comprising a heavy chain variable region polypeptide (VH) and a light chain variable region polypeptide (VL), wherein

the VH comprises VH-CDR1, VH-CDR2, and VH-CDR3 amino acid sequences comprising SEQ ID NOs: 6, 7, and 8, respectively and the VL comprises VL-CDR1, VL-CDR2, and VL-CDR3 amino acid sequences comprising SEQ ID NOs: 14, 15, and 16, respectively; or

the antibody or fragment thereof is a monoclonal antibody that comprises a VH encoded by the polynucleotide deposited as ATCC PTA-10004 and a VL encoded by the polynucleotide deposited as ATCC PTA-10005.

2. The antibody or fragment thereof according to claim 1, further comprising a human constant region.

3. The antibody or fragment thereof according to claim 1 or claim 2, which is selected from the group consisting of a single chain antibody, an Fab fragment, an $F(ab)_2$ fragment, and an Fv fragment.

4. The antibody or fragment thereof according to any one of claims 1 to 3, wherein the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO: 9 or SEQ ID NO: 10 and the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO: 17 or SEQ ID NO: 18.

5. The antibody or fragment thereof according to any one of claims 1 to 4, which is humanized, primatized or chimeric.

6. The antibody or fragment thereof according to any one of claims 1 to 5, further comprising a heterologous polypeptide fused thereto.

7. A composition comprising the antibody or fragment thereof according to any one of claims 1 to 6, and a carrier.

8. An isolated polynucleotide comprising

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(b) a nucleic acid which encodes an antibody VH, wherein the amino acid sequence of the VH is at least 90% identical to SEQ ID NO: 9; and wherein an antibody or antigen-binding fragment thereof comprising the VH specifically binds to CD100,

(c) a nucleic acid sequence comprising SEQ ID NO: 19,

(d) a nucleic acid that encodes an antibody VL, wherein the VL comprises VL-CDR1, VL-CDR2, and VL-CDR3 amino acid sequences comprising SEQ ID NOs: 14, 15, and 16, respectively; and wherein an antibody or antigen-binding fragment thereof comprising the VL specifically binds to CD100,

(e) a nucleic acid which encodes an antibody VL, wherein the amino acid sequence of the VL is at least 90% identical to SEQ ID NO: 17; and wherein an antibody or antigen-binding fragment thereof comprising the VL specifically binds to CD100, or

(f) a nucleic acid sequence comprising SEQ ID NO: 21.

9. A composition comprising an isolated VH-encoding polynucleotide and an isolated VL-encoding polynucleotide, wherein the VH-encoding polynucleotide encodes a VH comprising VH-CDR1, VH-CDR2, and VH-CDR3 amino acid sequences comprising SEQ ID NOs: 6, 7, and 8, respectively; wherein the VL-encoding polynucleotide encodes a VL comprising VL-CDR1, VL-CDR2, and VL-CDR3 amino acid sequences comprising SEQ ID NOs: 14, 15, and 16, respectively; and wherein an antibody or antigen-binding fragment thereof comprising the VH and VL specifically binds CD100.

10. The composition according to claim 9, wherein the VH-encoding polynucleotide and the VL-encoding polynucleotide are contained on a single vector or on separate vectors.

11. A host cell comprising the polynucleotide of claim 8 or the composition of claim 9 or claim 10.

12. A method of producing an antibody or antigen-binding fragment thereof that specifically binds CD100, comprising culturing a host cell comprising the polynucleotide of claim 8 or the composition of claim 10, and recovering the antibody, or fragment thereof.

13. The antibody or antigen-binding fragment thereof of any one of claims 1 to 6, or the composition of claim 7 for use in

(a) neutralizing CD100 in an animal,

(b) the treatment of an autoimmune disease or an inflammatory disease in an animal in need thereof,

(c) the treatment of a cancer in an animal in need thereof, or

(d) inhibiting angiogenesis in an animal in need of cancer treatment.

14. The antibody or antigen-binding fragment, or composition for the use according to claim 13, wherein the autoimmune disease or the inflammatory disease is multiple sclerosis or arthritis.

15. The use of an antibody or antigen-binding fragment thereof of any one of claims 1 to 6, or the use of a composition of claim 7, in the manufacture/preparation of a composition/medicament for the treatment of an autoimmune disease, an inflammatory disease, or cancer.

16. The use according to claim 15, wherein the autoimmune disease or the inflammatory disease is multiple sclerosis or arthritis.