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(54) Title: ANTI-MASP-1 ANTIBODIES AND METHODS OF USE

(57) Abstract: The present invention provides anti-MASP-1 (mannan-binding lectin (MBL)-associated serine protease 1) antibodies and methods of using the same. In some embodiments, an anti-MASP-1 antibody of the invention binds to MASP-1 but does not bind to MASP-3. The invention also provides nucleic acids comprising a nucleotide sequence encoding an anti-MASP-1 antibody of the present invention. The invention further provides pharmaceutical compositions comprising an anti-MASP-1 antibody of the present invention and a pharmaceutically acceptable carrier.



Description

Title of Invention: ANTI-MASP-1 ANTIBODIES AND METHODS OF USE

Technical Field

[0001] The present invention relates to anti-MASP-1 antibodies and methods of using the same.

Background Art

[0002] The complement system plays a crucial role in the innate immune response (NPL 1). It is widely accepted that the complement system can be activated through three distinct pathways: the classical pathway (CP), lectin pathway (LP), and alternative pathway (AP). Activation of these pathways leads to a number of immunological effector functions including recruitment of inflammatory cells, augmentation of phagocytosis, and formation of membrane attack complex (MAC) on the surface of the target cell.

[0003] The first step of the LP is the binding of pattern-recognition molecules (PRMs), such as mannan-binding lectin (MBL), collectin-10, collectin-11, and the ficolins (ficolin-1, ficolin-2, and ficolin-3), to patterns of ligands, e.g., surface-linked carbohydrates or acetyl groups on pathogens or damaged self-tissue (NPL 2). The PRMs are subsequently associated with three MBL-associated serine proteases (MASP-1, MASP-2, and MASP-3). MASP-2 was originally considered to auto activate and be the only active protease in the LP. However, many recent reports show that MASP-1 activates MASP-2 and is essential for the LP. When PRM-MASP complexes bind to target cells, MASP-1 will first autoactivate and then it activates MASP-2 and MASP-3. The activated MASP-2 cleaves C4 into C4a, C4b, and C4c, while activated MASP-1 and MASP-2 cleave C2 into C2a and C2b. The assembly of the C4a and C2b fragments results in the formation of the C3 convertase C4bC2a, to subsequently activate the complement system. In addition to its complement activating property, MASP-1 mediates coagulation and also contributes to a more powerful inflammatory reaction through bradykinin release, and endothelial and platelet activation (NPL 3).

[0004] While a properly functioning LP provides a robust defense against infectious microbes and maintenance of tissue homeostasis, inappropriate regulation of the LP has been implicated in the pathogenesis of a variety of disorders including, e.g., thrombosis (NPL 4); IgA nephropathy (NPL 5); and delayed graft function in transplanted kidneys (NPL 6). Therefore, MASP-1 inhibitory agents would serve effective therapeutic options in the treatment of diseases caused by the LP activation.

[0005] MASP-1 and MASP-3 are the alternative splicing variants of the MASP-1 gene.

They contain five identical domains (CUB1-EGF-CUB2-CCP1-CCP2) prolonged by different serine protease domains at the C-terminal end. Mutations in MASP-1 gene were found to be responsible for 3MC syndrome, which is characterized by hearing loss and distinctive facial features including hypertelorism, blepharophimosis, and highly arched eyebrows. The disruption of MASP-3 with normal MASP-1 was found to be sufficient to cause 3MC syndrome (NPL 7). Thus, there is a need to develop therapeutically effective MASP-1 specific inhibitors to provide robust efficacy and to prevent adverse effects for patients with thrombosis and other lectin pathway-mediated disorders.

Citation List

Non Patent Literature

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- [NPL 3] Peter G, et.al., Immunological Reviews 274, 74-97, 2016
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- [NPL 5] Liu L.L., et.al., Clinical & Experimental Immunology, 174, 152-160, 2013
- [NPL 6] Giuseppe C, et.al., American Journal of Pathology, 176 (4), 1648-1659, 2010
- [NPL 7] Tahir A, et.al., Orphanet Journal of Rare Diseases, 10, 128, 2015

Summary of Invention

Technical Problem

- [0007] An objective of the invention is to provide anti-MASP-1 antibodies and methods of using the same.

Solution to Problem

- [0008] The invention provides anti-MASP-1 antibodies and methods of using the same.
- [0009] In some embodiments, an anti-MASP-1 antibody of the present invention specifically binds to MASP-1. In some embodiments, an anti-MASP-1 antibody of the present invention binds to MASP-1 but does not or does not substantially bind to MASP-3. In further embodiments, an anti-MASP-1 antibody of the present invention binds to the serine protease domain of MASP-1.
- [0010] In some embodiments, an anti-MASP-1 antibody of the present invention inhibits activity of MASP-1. In some embodiments, an anti-MASP-1 antibody of the present invention does not inhibit activity of MASP-3. In certain embodiments, the activity of MASP-1 is complement lectin-pathway activation mediated by MASP-1, and the activity MASP-3 is complement lectin-pathway activation mediated by MASP-3.
- [0011] In some embodiments, an anti-MASP-1 antibody of the present invention is selected from the group consisting of:

- (a) an antibody comprising the HVR-H1 sequence of SEQ ID NO: 32, the HVR-H2 sequence of SEQ ID NO: 33, the HVR-H3 sequence of SEQ ID NO: 34, the HVR-L1 sequence of SEQ ID NO: 35, the HVR-L2 sequence of SEQ ID NO: 36, and the HVR-L3 sequence of SEQ ID NO: 37;
- (b) an antibody comprising the HVR-H1 sequence of SEQ ID NO: 38, the HVR-H2 sequence of SEQ ID NO: 39, the HVR-H3 sequence of SEQ ID NO: 40, the HVR-L1 sequence of SEQ ID NO: 41, the HVR-L2 sequence of SEQ ID NO: 42, and the HVR-L3 sequence of SEQ ID NO: 43;
- (c) an antibody comprising the HVR-H1 sequence of SEQ ID NO: 44, the HVR-H2 sequence of SEQ ID NO: 45, the HVR-H3 sequence of SEQ ID NO: 46, the HVR-L1 sequence of SEQ ID NO: 47, the HVR-L2 sequence of SEQ ID NO: 48, and the HVR-L3 sequence of SEQ ID NO: 49;
- (d) an antibody comprising the HVR-H1 sequence of SEQ ID NO: 50, the HVR-H2 sequence of SEQ ID NO: 51, the HVR-H3 sequence of SEQ ID NO: 52, the HVR-L1 sequence of SEQ ID NO: 53, the HVR-L2 sequence of SEQ ID NO: 54, and the HVR-L3 sequence of SEQ ID NO: 55;
- (e) an antibody comprising the HVR-H1 sequence of SEQ ID NO: 56, the HVR-H2 sequence of SEQ ID NO: 57, the HVR-H3 sequence of SEQ ID NO: 58, the HVR-L1 sequence of SEQ ID NO: 59, the HVR-L2 sequence of SEQ ID NO: 60, and the HVR-L3 sequence of SEQ ID NO: 61; and
- (f) an antibody comprising the HVR-H1 sequence of SEQ ID NO: 62, the HVR-H2 sequence of SEQ ID NO: 63, the HVR-H3 sequence of SEQ ID NO: 64, the HVR-L1 sequence of SEQ ID NO: 65, the HVR-L2 sequence of SEQ ID NO: 66, and the HVR-L3 sequence of SEQ ID NO: 67

[0012] In some embodiments, an anti-MASP-1 antibody of the present invention binds to the same epitope as a reference antibody which is selected from the group consisting of:

- (a) an antibody comprising the HVR-H1 sequence of SEQ ID NO: 32, the HVR-H2 sequence of SEQ ID NO: 33, the HVR-H3 sequence of SEQ ID NO: 34, the HVR-L1 sequence of SEQ ID NO: 35, the HVR-L2 sequence of SEQ ID NO: 36, and the HVR-L3 sequence of SEQ ID NO: 37;
- (b) an antibody comprising the HVR-H1 sequence of SEQ ID NO: 38, the HVR-H2 sequence of SEQ ID NO: 39, the HVR-H3 sequence of SEQ ID NO: 40, the HVR-L1 sequence of SEQ ID NO: 41, the HVR-L2 sequence of SEQ ID NO: 42, and the HVR-L3 sequence of SEQ ID NO: 43;
- (c) an antibody comprising the HVR-H1 sequence of SEQ ID NO: 44, the HVR-H2 sequence of SEQ ID NO: 45, the HVR-H3 sequence of SEQ ID NO: 46, the HVR-L1 sequence of SEQ ID NO: 47, the HVR-L2 sequence of SEQ ID NO: 48, and the HVR-

L3 sequence of SEQ ID NO: 49;

(d) an antibody comprising the HVR-H1 sequence of SEQ ID NO: 50, the HVR-H2 sequence of SEQ ID NO: 51, the HVR-H3 sequence of SEQ ID NO: 52, the HVR-L1 sequence of SEQ ID NO: 53, the HVR-L2 sequence of SEQ ID NO: 54, and the HVR-L3 sequence of SEQ ID NO: 55;

(e) an antibody comprising the HVR-H1 sequence of SEQ ID NO: 56, the HVR-H2 sequence of SEQ ID NO: 57, the HVR-H3 sequence of SEQ ID NO: 58, the HVR-L1 sequence of SEQ ID NO: 59, the HVR-L2 sequence of SEQ ID NO: 60, and the HVR-L3 sequence of SEQ ID NO: 61; and

(f) an antibody comprising the HVR-H1 sequence of SEQ ID NO: 62, the HVR-H2 sequence of SEQ ID NO: 63, the HVR-H3 sequence of SEQ ID NO: 64, the HVR-L1 sequence of SEQ ID NO: 65, the HVR-L2 sequence of SEQ ID NO: 66, and the HVR-L3 sequence of SEQ ID NO: 67

[0013] In some embodiments, an anti-MASP-1 antibody of the present invention is capable of binding to, and inhibiting activity of, human MASP-1, cynomolgus monkey MASP-1, and/or mouse MASP-1.

[0014] In some embodiments, an anti-MASP-1 antibody of the present invention inhibits C3 and/or C4 activation with an IC 50 of less than 0.35 micro g/mL.

[0015] In some embodiments, an anti-MASP-1 antibody of the present invention is a monoclonal antibody. In further embodiments, an anti-MASP-1 antibody of the present invention is a human, humanized, or chimeric antibody. In certain embodiments, an anti-MASP-1 antibody of the present invention is an antibody fragment that binds to MASP-1.

[0016] The invention also provides nucleic acids comprising a nucleotide sequence encoding an anti-MASP-1 antibody of the present invention. The invention also provides recombinant vectors comprising a nucleic acid of the present invention. The invention also provides recombinant cells comprising a nucleic acid or a recombinant vector of the present invention. The invention also provides methods of producing an antibody comprising culturing a recombinant cell of the present invention so that the antibody is produced.

[0017] The invention also provides pharmaceutical compositions comprising an anti-MASP-1 antibody of the present invention and a pharmaceutically acceptable carrier.

[0018] Anti-MASP-1 antibodies of the present invention may be for use as a medicament. Anti-MASP-1 antibodies of the present invention may be for use in treating a disease caused by complement lectin-pathway activation. In certain embodiments, the disease caused by the complement lectin-pathway activation is thrombotic microangiopathy (TMA). In certain embodiments, the subject is a human.

[0019] In some embodiments, anti-MASP-1 antibodies of the present invention may be for

use in inhibiting complement lectin-pathway activation mediated by MASP-1. In some embodiments, anti-MASP-1 antibodies of the present invention may be for use in inhibiting C3 and/or C4 activation.

[0020] The invention also provides methods of treating a subject having a disease caused by complement lectin-pathway activation. In some embodiments, a method of the present invention comprises administering to the subject an effective amount of an antibody of the present invention. In certain embodiments, the disease caused by the complement lectin-pathway activation is thrombotic microangiopathy (TMA). In certain embodiments, the subject is a human.

[0021] The invention also provides methods of inhibiting complement lectin-pathway activation mediated by MASP-1 in a subject. In some embodiments, a method of the present invention comprises administering to the subject an effective amount of an antibody of the present invention. The invention also provides methods of inhibiting C3 and/or C4 activation in a subject. In some embodiments, a method of the present invention comprises administering to the subject an effective amount of an antibody of the present invention.

[0022] Anti-MASP-1 antibodies of the present invention may be used in the manufacture of a medicament. In some embodiments, the medicament is for treating a disease caused by complement lectin-pathway activation. In certain embodiments, the disease caused by complement lectin-pathway activation is thrombotic microangiopathy (TMA).

[0023] The invention also provides methods of inhibiting C3 and/or C4 activation in serum. In some embodiments, a method of the present invention comprises contacting an antibody of the present invention with mannan and a biological sample containing MBL (mannose-binding lectin), MASP-1, C2 and C4 under conditions permissive for binding of the antibody to MASP-1. In some embodiments, the biological sample is a human serum, cynomolgus monkey serum, or mouse serum.

[0024] The invention also provides methods of detecting the presence of MASP-1. In some embodiments, a method of the present invention comprises:

(a) contacting a biological sample with an antibody of the present invention under conditions permissive for binding of the antibody to MASP-1; and

(b) detecting whether a complex is formed between the antibody and MASP-1.

[0025] The invention also provides methods for producing an anti-MASP-1 antibody which inhibits MASP-1 activity but does not inhibit MASP-3 activity. In some embodiments, an method of the present invention comprises:

(a) (i) contacting a biological sample comprising mannan and serum with a test antibody, wherein the serum contains MASP-1 but does not contain MASP-3, and

(ii) measuring inhibition of C3 and/or C4 activation;

(b) (i) contacting a biological sample comprising mannan and serum with a test

antibody, wherein the serum contains MASP-3 but does not contain MASP-1, and
(ii) measuring inhibition of C3 and/or C4 activation;
(c) selecting the test antibody which inhibits MASP-1 activity but does not inhibit MASP-3 activity, if the test antibody inhibits C3 and/or C4 activation in step (a) but does not inhibit C3 and/or C4 activation in step (b);
(d) obtaining amino acid sequence information of the anti-MASP-1 antibody selected in step (c); and
(e) introducing a gene encoding the amino acid sequence obtained in step (d) into a host cell.

[0026] More specifically, the present invention provides the following:

- [1] An anti-MASP-1 antibody which specifically binds to MASP-1.
- [2] An anti-MASP-1 antibody which binds to MASP-1 but does not bind to MASP-3.
- [3] An anti-MASP-1 antibody which binds to the serine protease domain of MASP-1.
- [4] The anti-MASP-1 antibody according to any one of [1] to [3], wherein the antibody inhibits MASP-1 activity.
- [5] The anti-MASP-1 antibody according to any one of [1] to [4], wherein the antibody does not inhibit MASP-3 activity.
- [6] The anti-MASP-1 antibody according to [5], wherein the MASP-1 activity is MASP-1 mediated complement lectin-pathway activation and the MASP-3 activity is MASP-3 mediated complement lectin-pathway activation.
- [7] The anti-MASP-1 antibody according to any one of [1] to [6], wherein the antibody competes for binding to MASP-1 with an antibody selected from the group consisting of:
 - (a) an antibody comprising the HVR-H1 sequence of SEQ ID NO: 32, the HVR-H2 sequence of SEQ ID NO: 33, the HVR-H3 sequence of SEQ ID NO: 34, the HVR-L1 sequence of SEQ ID NO: 35, the HVR-L2 sequence of SEQ ID NO: 36, and the HVR-L3 sequence of SEQ ID NO: 37;
 - (b) an antibody comprising the HVR-H1 sequence of SEQ ID NO: 38, the HVR-H2 sequence of SEQ ID NO: 39, the HVR-H3 sequence of SEQ ID NO: 40, the HVR-L1 sequence of SEQ ID NO: 41, the HVR-L2 sequence of SEQ ID NO: 42, and the HVR-L3 sequence of SEQ ID NO: 43;
 - (c) an antibody comprising the HVR-H1 sequence of SEQ ID NO: 44, the HVR-H2 sequence of SEQ ID NO: 45, the HVR-H3 sequence of SEQ ID NO: 46, the HVR-L1 sequence of SEQ ID NO: 47, the HVR-L2 sequence of SEQ ID NO: 48, and the HVR-L3 sequence of SEQ ID NO: 49;
 - (d) an antibody comprising the HVR-H1 sequence of SEQ ID NO: 50, the HVR-H2 sequence of SEQ ID NO: 51, the HVR-H3 sequence of SEQ ID NO: 52, the HVR-L1 sequence of SEQ ID NO: 53, the HVR-L2 sequence of SEQ ID NO: 54, and the HVR-

L3 sequence of SEQ ID NO: 55;

(e) an antibody comprising the HVR-H1 sequence of SEQ ID NO: 56, the HVR-H2 sequence of SEQ ID NO: 57, the HVR-H3 sequence of SEQ ID NO: 58, the HVR-L1 sequence of SEQ ID NO: 59, the HVR-L2 sequence of SEQ ID NO: 60, and the HVR-L3 sequence of SEQ ID NO: 61; and

(f) an antibody comprising the HVR-H1 sequence of SEQ ID NO: 62, the HVR-H2 sequence of SEQ ID NO: 63, the HVR-H3 sequence of SEQ ID NO: 64, the HVR-L1 sequence of SEQ ID NO: 65, the HVR-L2 sequence of SEQ ID NO: 66, and the HVR-L3 sequence of SEQ ID NO: 67.

[8] The anti-MASP-1 antibody according to any one of [1] to [7], wherein the antibody binds to the same epitope to which a reference antibody binds, wherein the reference antibody is selected from the group consisting of:

(a) an antibody comprising the HVR-H1 sequence of SEQ ID NO: 32, the HVR-H2 sequence of SEQ ID NO: 33, the HVR-H3 sequence of SEQ ID NO: 34, the HVR-L1 sequence of SEQ ID NO: 35, the HVR-L2 sequence of SEQ ID NO: 36, and the HVR-L3 sequence of SEQ ID NO: 37;

(b) an antibody comprising the HVR-H1 sequence of SEQ ID NO: 38, the HVR-H2 sequence of SEQ ID NO: 39, the HVR-H3 sequence of SEQ ID NO: 40, the HVR-L1 sequence of SEQ ID NO: 41, the HVR-L2 sequence of SEQ ID NO: 42, and the HVR-L3 sequence of SEQ ID NO: 43;

(c) an antibody comprising the HVR-H1 sequence of SEQ ID NO: 44, the HVR-H2 sequence of SEQ ID NO: 45, the HVR-H3 sequence of SEQ ID NO: 46, the HVR-L1 sequence of SEQ ID NO: 47, the HVR-L2 sequence of SEQ ID NO: 48, and the HVR-L3 sequence of SEQ ID NO: 49;

(d) an antibody comprising the HVR-H1 sequence of SEQ ID NO: 50, the HVR-H2 sequence of SEQ ID NO: 51, the HVR-H3 sequence of SEQ ID NO: 52, the HVR-L1 sequence of SEQ ID NO: 53, the HVR-L2 sequence of SEQ ID NO: 54, and the HVR-L3 sequence of SEQ ID NO: 55;

(e) an antibody comprising the HVR-H1 sequence of SEQ ID NO: 56, the HVR-H2 sequence of SEQ ID NO: 57, the HVR-H3 sequence of SEQ ID NO: 58, the HVR-L1 sequence of SEQ ID NO: 59, the HVR-L2 sequence of SEQ ID NO: 60, and the HVR-L3 sequence of SEQ ID NO: 61; and

(f) an antibody comprising the HVR-H1 sequence of SEQ ID NO: 62, the HVR-H2 sequence of SEQ ID NO: 63, the HVR-H3 sequence of SEQ ID NO: 64, the HVR-L1 sequence of SEQ ID NO: 65, the HVR-L2 sequence of SEQ ID NO: 66, and the HVR-L3 sequence of SEQ ID NO: 67

[9] The anti-MASP-1 antibody according to any one of [1] to [8], wherein the antibody is capable of binding to, and inhibiting activity of, human MASP-1, cynomolgus

monkey MASP-1, and/or mouse MASP-1.

[10] The anti-MASP-1 antibody according to any one of [1] to [9], wherein the antibody inhibits C3 and/or C4 activation with an IC₅₀ of less than 0.35 micro g/mL.

[11] The anti-MASP-1 antibody according to any one of [1] to [10], wherein the antibody is a monoclonal antibody.

[12] The anti-MASP-1 antibody according to any one of [1] to [11], wherein the antibody is a human, humanized or chimeric antibody.

[13] The anti-MASP-1 antibody according to any one of [1] to [11], wherein the antibody is an antibody fragment that binds to MASP-1.

[14] A nucleic acid comprising a nucleotide sequence encoding the antibody of any one of [1] to [13].

[15] A recombinant vector comprising the nucleic acid of [14].

[16] A recombinant cell comprising the nucleic acid of [14] or the recombinant vector of [15].

[17] A method of producing an antibody comprising culturing the recombinant cell of [16] so that the antibody is produced.

[18] A pharmaceutical composition comprising the antibody according to any one of [1] to [13] and a pharmaceutically acceptable carrier.

[19] A pharmaceutical composition for use in treating a disease caused by the complement lectin-pathway activation in a subject comprising the antibody according to any one of [1] to [13].

[20] The pharmaceutical composition according to [19], wherein the disease caused by the complement lectin-pathway activation is TMA.

[21] The pharmaceutical composition according to [19] or [20], wherein the subject is a human.

[22] The antibody according to any one of [1] to [13] for use in a method of treating TMA.

[23] A method of treating a subject having a disease caused by the complement lectin-pathway activation comprising administering to the subject an effective amount of the antibody according to any one of [1] to [13].

[24] The method according to [23], wherein the disease caused by the complement lectin-pathway activation is TMA.

[25] The method according to [23] or [24], wherein the subject is a human.

[26] Use of the antibody according to any one of [1] to [13] in the manufacture of a medicament of treating a disease caused by the complement lectin-pathway activation.

[27] The use according to [26], wherein the disease caused by the complement lectin-pathway activation is TMA.

[28] A method of inhibiting C3 and/or C4 activation in serum, comprising contacting

the antibody according to any one of [1] to [13] with mannan and a biological sample containing MBL (mannan-binding lectin), MASP-1, C2 and C4 under conditions permissive for binding of the antibody to MASP-1.

[29] The method according to [28], wherein the biological sample is human serum, cynomolgus monkey serum or mouse serum.

[30] A method of detecting the presence of MASP-1 comprising:

- (a) contacting a biological sample with the antibody according to any one of [1] to [13] under conditions permissive for binding of the antibody to MASP-1; and
- (b) detecting whether a complex is formed between the antibody and MASP-1.

[31] A method for producing an anti-MASP-1 antibody which inhibits MASP-1 activity but does not inhibit MASP-3 activity, comprising:

- (a) (i) contacting a biological sample comprising mannan and serum with a test antibody, wherein the serum contains MASP-1 but does not contain MASP-3, and
- (ii) measuring inhibition of C3 and/or C4 activation;
- (b) (i) contacting a biological sample comprising mannan and serum with a test antibody, wherein the serum contains MASP-3 but does not contain MASP-1, and
- (ii) measuring inhibition of C3 and/or C4 activation;
- (c) selecting the test antibody which inhibits MASP-1 activity but does not inhibit MASP-3 activity, if the test antibody inhibits C3 and/or C4 activation in step (a) but does not inhibit C3 and/or C4 activation in step (b);
- (d) obtaining amino acid sequence information of the anti-MASP-1 antibody selected in step (c); and
- (e) introducing a gene encoding the amino acid sequence obtained in step (d) into a host cell.

[32] A pharmaceutical composition for use in inhibiting complement lectin-pathway activation mediated by MASP-1, comprising the antibody according to any one of [1] to [13].

[33] A pharmaceutical composition for use in inhibiting C3 and/or C4 activation, comprising the antibody according to any one of [1] to [13].

[34] A method of inhibiting complement lectin-pathway activation mediated by MASP-1 in a subject, comprising administering to the subject an effective amount of the antibody according to any one of [1] to [13].

[35] A method of inhibiting C3 and/or C4 activation in a subject, comprising administering to the subject an effective amount of the antibody according to any one of [1] to [13].

Brief Description of Drawings

[0027] [fig.1]Figure 1 shows comparison of the amino acid sequences of the SP domain of

human MASP-1 (SEQ ID NO: 7) and the SP domain of human MASP-3 (SEQ ID NO: 11).

[fig.2]Figure 2 shows comparison of the amino acid sequences of the SP domains of human MASP-1 (SEQ ID NO: 7), monkey MASP-1 (SEQ ID NO: 8) and mouse MASP-1 (SEQ ID NO: 9).

[fig.3]Figure 3 shows inhibition of C3c or C4c deposition by anti-MASP-1 monoclonal antibodies.

[fig.4]Figure 4 shows the result of cynomolgus monkey C3c deposition assay using an anti-MASP-1 chimeric mAb (MAS0141aa) after 2-week incubation in PBS at 40 degrees C.

[fig.5]Figure 5 illustrates the inhibitory effect of anti-MASP-1 monoclonal antibodies on MASP-1 enzymatic activity with a fluorogenic peptide.

[fig.6]Figure 6 illustrates the in vivo inhibitory effect of anti-MASP-1 monoclonal antibody MAS0154-mF18 on the lectin pathway in mice.

Description of Embodiments

[0028] I. DEFINITIONS

An "acceptor human framework" for the purposes herein is a framework comprising the amino acid sequence of a light chain variable domain (VL) framework or a heavy chain variable domain (VH) framework derived from a human immunoglobulin framework or a human consensus framework, as defined below. An acceptor human framework "derived from" a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain amino acid sequence changes. In some embodiments, the number of amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. In some embodiments, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

[0029] "Affinity" refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (Kd). Affinity can be measured by common methods known in the art, including those described herein. Specific illustrative and exemplary embodiments for measuring binding affinity are described in the following.

[0030] An "affinity matured" antibody refers to an antibody with one or more alterations in

one or more hypervariable regions (HVRs), compared to a parent antibody which does not possess such alterations, such alterations resulting in an improvement in the affinity of the antibody for antigen.

- [0031] The terms "anti- MASP-1 antibody" and "an antibody that binds to MASP-1" refer to an antibody that is capable of binding MASP-1 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting MASP-1. In one embodiment, the extent of binding of an anti-MASP-1 antibody to an unrelated, non-MASP-1 protein is less than about 10% of the binding of the antibody to MASP-1 as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to MASP-1 has a dissociation constant (K_d) of 1 micro M or less, 100 nM or less, 10 nM or less, 1 nM or less, 0.1 nM or less, 0.01 nM or less, or 0.001 nM or less (e.g. 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M, e.g., from 10^{-9} M to 10^{-13} M). In certain embodiments, an anti-MASP-1 antibody binds to an epitope of MASP-1 that is conserved among MASP-1 from different species.
- [0032] The term "antibody" herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity.
- [0033] Without intending to be bound by any theory, an antibody binds to its antigen with amino acids in more than one HVR.
- [0034] An "antibody fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')₂; diabodies; linear antibodies; single-chain antibody molecules (e.g. scFv); and multispecific antibodies formed from antibody fragments.
- [0035] An "antibody that binds to the same epitope" as a reference antibody refers to an antibody that blocks binding of the reference antibody to its antigen in a competition assay by 50% or more, and conversely, the reference antibody blocks binding of the antibody to its antigen in a competition assay by 50% or more. An exemplary competition assay is provided herein.
- [0036] A "blocking" antibody or an "antagonist" antibody is one which significantly inhibits (either partially or completely) a biological activity of the antigen it binds.
- [0037] The term "chimeric" antibody refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species.
- [0038] The "class" of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes),

e.g., IgG₁, IgG₂, IgG₃, IgG₄, IgA₁, and IgA₂. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively.

- [0039] The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents a cellular function and/or causes cell death or destruction. Cytotoxic agents include, but are not limited to, radioactive isotopes (e.g., ²¹¹At, ¹³¹I, ¹²⁵I, ⁹⁰Y, ¹⁸⁶Re, ¹⁸⁸Re, ¹⁵³Sm, ²¹²Bi, ³²P, ²¹²Pb and radioactive isotopes of Lu); chemotherapeutic agents or drugs (e.g., methotrexate, adriamycin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents); growth inhibitory agents; enzymes and fragments thereof such as nucleolytic enzymes; antibiotics; toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof; and the various antitumor or anticancer agents disclosed below.
- [0040] "Effector functions" refer to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g. B cell receptor); and B cell activation.
- [0041] An "effective amount" of an agent, e.g., a pharmaceutical formulation, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result.
- [0042] The term "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. In one embodiment, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) or glycine-lysine (residues 446-447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991.
- [0043] The term "Fc region-comprising antibody" refers to an antibody that comprises an Fc region. The C-terminal lysine (residue 447 according to the EU numbering system) or C-terminal glycine-lysine (residues 446-447) of the Fc region may be removed, for example, during purification of the antibody or by recombinant engineering of the nucleic acid encoding the antibody. Accordingly, a composition comprising an antibody having an Fc region according to this invention can comprise an antibody

with G446-K447, with G446 and without K447, with all G446-K447 removed, or a mixture of three types of antibodies described above.

[0044] "Framework" or "FR" refers to variable domain residues other than hypervariable region (HVR) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following sequence in VH (or VL):
FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

[0045] The terms "full length antibody," "intact antibody," and "whole antibody" are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or having heavy chains that contain an Fc region as defined herein.

[0046] The terms "host cell," "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

[0047] A "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

[0048] A "human consensus framework" is a framework which represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat et al., Sequences of Proteins of Immunological Interest, Fifth Edition, NIH Publication 91-3242, Bethesda MD (1991), vols. 1-3. In one embodiment, for the VL, the subgroup is subgroup kappa I as in Kabat et al., supra. In one embodiment, for the VH, the subgroup is subgroup III as in Kabat et al., supra.

[0049] A "humanized" antibody refers to a chimeric antibody comprising amino acid residues from non-human HVRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (e.g., CDRs) correspond to those of a non-human antibody, and all or substantially all of the

FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A "humanized form" of an antibody, e.g., a non-human antibody, refers to an antibody that has undergone humanization.

- [0050] The term "hypervariable region" or "HVR" as used herein refers to each of the regions of an antibody variable domain which are hypervariable in sequence ("complementarity determining regions" or "CDRs") and/or form structurally defined loops ("hypervariable loops") and/or contain the antigen-contacting residues ("antigen contacts"). Generally, antibodies comprise six HVRs: three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). Exemplary HVRs herein include:
- (a) hypervariable loops occurring at amino acid residues 26-32 (L1), 50-52 (L2), 91-96 (L3), 26-32 (H1), 53-55 (H2), and 96-101 (H3) (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987));
 - (b) CDRs occurring at amino acid residues 24-34 (L1), 50-56 (L2), 89-97 (L3), 31-35b (H1), 50-65 (H2), and 95-102 (H3) (Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991));
 - (c) antigen contacts occurring at amino acid residues 27c-36 (L1), 46-55 (L2), 89-96 (L3), 30-35b (H1), 47-58 (H2), and 93-101 (H3) (MacCallum et al. *J. Mol. Biol.* 262: 732-745 (1996)); and
 - (d) combinations of (a), (b), and/or (c), including HVR amino acid residues 46-56 (L2), 47-56 (L2), 48-56 (L2), 49-56 (L2), 26-35 (H1), 26-35b (H1), 49-65 (H2), 93-102 (H3), and 94-102 (H3).
- [0051] In one embodiment, HVR residues comprise those identified in the heavy chain variable regions or light chain variable regions provided in Table 6.
- [0052] Unless otherwise indicated, HVR residues and other residues in the variable domain (e.g., FR residues) are numbered herein according to Kabat et al., *supra*.
- [0053] An "immunoconjugate" is an antibody conjugated to one or more heterologous molecule(s), including but not limited to a cytotoxic agent.
- [0054] An "individual" or "subject" is a mammal. Mammals include, but are not limited to, domesticated animals (e.g., cows, sheep, cats, dogs, and horses), primates (e.g., humans and non-human primates such as monkeys), rabbits, and rodents (e.g., mice and rats). In certain embodiments, the individual or subject is a human.
- [0055] An "isolated" antibody is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (e.g., ion exchange or reverse phase HPLC). For review of methods for assessment of antibody

purity, see, e.g., Flatman et al., J. Chromatogr. B 848:79-87 (2007).

- [0056] An "isolated" nucleic acid refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.
- [0057] "Isolated nucleic acid encoding an anti-MASP-1 antibody" refers to one or more nucleic acid molecules encoding antibody heavy and light chains (or fragments thereof), including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.
- [0058] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies composing the population are identical and/or bind the same epitope, except for possible variant antibodies, e.g., containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, the modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein.
- [0059] A "naked antibody" refers to an antibody that is not conjugated to a heterologous moiety (e.g., a cytotoxic moiety) or radiolabel. The naked antibody may be present in a pharmaceutical formulation.
- [0060] "Native antibodies" refer to naturally occurring immunoglobulin molecules with varying structures. For example, native IgG antibodies are heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light chains and two identical heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CH1, CH2, and CH3). Similarly, from N- to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a light chain variable domain, followed by a constant light

(CL) domain. The light chain of an antibody may be assigned to one of two types, called kappa and lambda, based on the amino acid sequence of its constant domain.

[0061] The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

[0062] "Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, Megalign (DNASTAR) software, or GENETYX (registered trademark) (Genetyx Co., Ltd.). Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

[0063] The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, California, or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary. In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

100 times the fraction X/Y

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid

sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

[0064] The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

[0065] A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

[0066] "Polynucleotide" or "nucleic acid" as used interchangeably herein, refers to polymers of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase or by a synthetic reaction. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and their analogs. A sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may comprise modification(s) made after synthesis, such as conjugation to a label. Other types of modifications include, for example, "caps," substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoramidates, carbamates, etc.) and with charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), those containing pendant moieties, such as, for example, proteins (e.g., nucleases, toxins, antibodies, signal peptides, poly-L-lysine, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those containing chelators (e.g., metals, radioactive metals, boron, oxidative metals, etc.), those containing alkylators, those with modified linkages (e.g., alpha anomeric nucleic acids, etc.), as well as unmodified forms of the polynucleotides(s). Further, any of the hydroxyl groups ordinarily present in the sugars may be replaced, for example, by phosphonate groups, phosphate groups, protected by standard protecting groups, or activated to prepare additional linkages to additional nucleotides, or may be conjugated to solid or semi-solid supports. The 5' and 3' terminal OH can be phosphorylated or substituted with amines or organic capping group moieties of from 1 to 20 carbon atoms. Other hydroxyls may also be derivatized to standard protecting groups. Polynucleotides can also contain analogous forms of ribose or deoxyribose sugars that are generally known in the art, including, for example, 2'-O-methyl-, 2'-O-allyl-, 2'-fluoro- or 2'-azido-ribose, carbocyclic sugar analogs, alpha-anomeric sugars, epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars, furanose sugars, sedo-

heptuloses, acyclic analogs, and basic nucleoside analogs such as methyl riboside. One or more phosphodiester linkages may be replaced by alternative linking groups. These alternative linking groups include, but are not limited to, embodiments wherein phosphate is replaced by P(O)S ("thioate"), P(S)S ("dithioate"), (O)NR₂ ("amidate"), P(O)R, P(O)OR', CO, or CH₂ ("formacetal"), in which each R or R' is independently H or substituted or unsubstituted alkyl (1-20 C) optionally containing an ether (-O-) linkage, aryl, alkenyl, cycloalkyl, cycloalkenyl or araldyl. Not all linkages in a polynucleotide need be identical. The preceding description applies to all polynucleotides referred to herein, including RNA and DNA.

[0067] The phrase "substantially reduced," "substantially inhibit" or "substantially different," as used herein, refers to a sufficiently high degree of difference between two numeric values (generally one associated with a molecule and the other associated with a reference/comparator molecule) such that one of skill in the art would consider the difference between the two values to be of statistical significance within the context of the biological characteristic measured by said values (e.g., K_d values).

[0068] The term "substantially similar" or "substantially the same," as used herein, refers to a sufficiently high degree of similarity between two numeric values (for example, one associated with an antibody of the invention and the other associated with a reference/comparator antibody), such that one of skill in the art would consider the difference between the two values to be of little or no biological and/or statistical significance within the context of the biological characteristic measured by said values (e.g., K_d values).

[0069] The term "MASP-1," as used herein, refers to any native MASP-1 from any vertebrate source, including mammals such as primates (e.g. humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses "full-length" unprocessed MASP-1 as well as any form of MASP-1 that results from processing in the cell. The term also encompasses naturally occurring variants of MASP-1, e.g., splice variants or allelic variants. The amino acid sequence of an exemplary human MASP-1 is shown in SEQ ID NO: 1, and the nucleotide sequence encoding the exemplary human MASP-1 is shown in SEQ ID NO: 2. The amino acid sequences of an exemplary monkey MASP-1 and mouse MASP-1 are shown in SEQ ID NO: 3 and 4, respectively.

[0070] Accession numbers for exemplary MASP-1 are as follows:

Human MASP-1 protein: NCBI RefSeq NP_001870

Human MASP-1 nucleotide: NCBI RefSeq NM_001879

Mouse MASP-1 protein: NCBI RefSeq NP_032581

Mouse MASP-1 nucleotide: NCBI RefSeq NM_008555

[0071] The term "MASP-3," as used herein, refers to any native MASP-3 from any

vertebrate source, including mammals such as primates (e.g. humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses "full-length" unprocessed MASP-3 as well as any form of MASP-3 that results from processing in the cell. The term also encompasses naturally occurring variants of MASP-3, e.g., splice variants or allelic variants. The amino acid sequence of an exemplary human MASP-3 is shown in SEQ ID NO: 5.

- [0072] NCBI RefSeq accession numbers for exemplary MASP-3 are as follows:
 Human MASP-3 protein: Genbank AAK84071.1
 Human MASP-3 nucleotide: GenBank AF284421.1
 MonkeyMASP-3 protein: GenBank AAI06947
 Mouse MASP-3 protein: GenBank BAB69688
- [0073] MASP-1 and MASP-3 are alternative splicing variants of the MASP-1 gene. MASP-1 and MASP-3 have an identical heavy/alpha chain comprising, from the N-terminus, CUB1 (C1r/s, Uegf, Bone morphogenetic protein 1), EGF (epidermal growth factor), CUB2 (C1r/s, Uegf, Bone morphogenetic protein 2), CCP1 (complement control protein 1) and CCP2 (complement control protein 2). MASP-1 and MASP-3 have a different light/beta chain at the C-terminal end that comprises the SP (serine protease) domain containing a cleavage site that separates the heavy and light chain upon MASP activation.
- [0074] For MASP-1, amino acid residues 1 to 19 of SEQ ID NO: 1 constitute a signal peptide. The amino acid sequence of the heavy/alpha chain of an exemplary human MASP-1 is SEQ ID NO: 6, which corresponds to amino acid residues 20 to 448 of SEQ ID NO: 1. The amino acid sequences of the CUB1 domain, EGF domain, CUB2 domain, CCP1 domain and CCP2 domain of an exemplary human MASP-1 are amino acid residues 20 to 138, 139 to 182, 185 to 297, 299 to 364, and 365 to 434 of SEQ ID NO: 1, respectively. The amino acid sequence of the SP domain of an exemplary human MASP-1 is SEQ ID NO: 7, which corresponds to amino acid residues 449 to 699 of SEQ ID NO: 1. The amino acid sequence of the SP domain of an exemplary monkey MASP-1 is SEQ ID NO: 8, which corresponds to amino acid residues 449 to 699 of SEQ ID NO: 3. The amino acid sequence of the SP domain of an exemplary mouse MASP-1 is SEQ ID NO: 9, which corresponds to amino acid residues 454 to 704 of SEQ ID NO: 4.
- [0075] For MASP-3, the amino acid sequence of the heavy/alpha chain of an exemplary human MASP-3 is SEQ ID NO: 10, which corresponds to amino acid residues 20 to 449 of SEQ ID NO: 5. For MASP-3, amino acid residues 1 to 19 of SEQ ID NO: 5 constitute a signal peptide. The amino acid sequences of the CUB1 domain, EGF domain, CUB2 domain, CCP1 domain and CCP2 domain of an exemplary human MASP-3 are amino acid residues 20 to 138, 139 to 182, 185 to 297, 299 to 364, and

365 to 434 of SEQ ID NO: 5, respectively. The amino acid sequence of the SP domain of an exemplary human MASP-3 is SEQ ID NO: 11, which corresponds to amino acid residues 450 to 728 of SEQ ID NO: 5.

- [0076] The term "MASP-2," as used herein, refers to any native MASP-2 from any vertebrate source, including mammals such as primates (e.g. humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses "full-length" unprocessed MASP-2 as well as any form of MASP-2 that results from processing in the cell. The term also encompasses naturally occurring variants of MASP-2, e.g., splice variants or allelic variants. The amino acid sequence of an exemplary human MASP-2 is shown in SEQ ID NO: 12.
- [0077] Accession numbers for exemplary MASP-2 are as follows:
 Human MASP-2 protein: NCBI RefSeq NP_006601
 Human MASP-2 nucleotide: NCBI RefSeq NM_006610
 Mouse MASP-2 protein: NCBI RefSeq NP_001003893, XP_358353
 Mouse MASP-2 nucleotide: NCBI RefSeq NM_001003893 XM_358353
- [0078] The amino acid sequence of the heavy/alpha chain of an exemplary human MASP-2 is SEQ ID NO: 13, which corresponds to amino acid residues 16 to 444 of SEQ ID NO: 12. The amino acid sequence of the SP domain of an exemplary human MASP-2 is SEQ ID NO: 14, which corresponds to amino acid residues 445 to 686 of SEQ ID NO: 12.
- [0079] Figure 1 shows comparison of the amino acid sequences of the SP domain of human MASP-1 (SEQ ID NO: 7) and the SP domain of human MASP-3 (SEQ ID NO: 11). Figure 2 shows comparison of the amino acid sequences of the SP domains of human MASP-1 (SEQ ID NO: 7), monkey MASP-1 (SEQ ID NO: 8) and mouse MASP-1 (SEQ ID NO: 9). Identical amino acids are boxed.
- [0080] The percent identities between the heavy/alpha and light/beta chains of the human MASP-1, MASP-2 and MASP-3 proteins are provided in Table 1 below. The percent amino acid sequence identities were determined by using ALIGN.

[0081] [Table 1]

	% identity between heavy/alpha chains			% identity between Light/beta chains		
	hMASP-1	hMASP-2	hMASP-3	hMASP-1	hMASP-2	hMASP-3
hMASP-1	100%	47.0%	97.9%	100%	35.5%	32.3%
hMASP-2	47.0%	100%	46.4%	35.5%	100%	35.1%
hMASP-3	97.9%	46.4%	100%	32.3%	35.1%	100%

- [0082] The percent identities between the SP domains of the human MASP-1, monkey MASP-1, mouse MASP-1 proteins are provided in Table 2 below. The percent amino acid sequence identities were determined by using ALIGN.

[0083]

[Table 2]

	% identity between SP domains		
	Human MASP-1	Monkey MASP-2	Mouse MASP-3
Human MASP-1	100%	96.8%	83.3%
Monkey MASP-2	96.8%	100%	82.1%
Mouse MASP-3	83.3%	82.1%	100%

[0084] As used herein, "treatment" (and grammatical variations thereof such as "treat" or "treating") refers to clinical intervention in an attempt to alter the natural course of the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, antibodies of the invention are used to delay development of a disease or to slow the progression of a disease.

[0085] The term "variable region" or "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). (See, e.g., Kindt et al. Kuby Immunology, 6th ed., W.H. Freeman and Co., page 91 (2007).) A single VH or VL domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a VH or VL domain from an antibody that binds the antigen to screen a library of complementary VL or VH domains, respectively. See, e.g., Portolano et al., J. Immunol. 150:880-887 (1993); Clarkson et al., Nature 352:624-628 (1991).

[0086] The term "vector," as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors."

[0087] II. COMPOSITIONS AND METHODS

In one aspect, the invention is based, in part, on anti-MASP-1 antibodies and uses thereof. In certain embodiments, antibodies that bind to MASP-1 are provided. Antibodies of the invention are useful, e.g., for the diagnosis or treatment of a disease caused by the complement lectin-pathway activation, preferably thrombotic microan-

giopathy (TMA), membranous nephropathy, age-related macular degeneration (AMD), myocardial infarctions, and/or stroke.

[0088] A. Exemplary Anti-MASP-1 Antibodies

In one aspect, the invention provides isolated antibodies that bind to MASP-1. In certain embodiments, an anti-MASP-1 antibody:

- specifically binds to MASP-1
- binds to an activated or inactivated form of MASP-1
- binds to MASP-1 but does not or does not substantially bind to MASP-3
- binds to the serine protease domain of MASP-1
- inhibits activity of MASP-1 but does not inhibit activity of MASP-3
- binds to the same epitope as any anti-MASP-1 antibody described herein
- which is capable of binding to, and inhibiting activity of, human MASP-1, cynomolgus monkey MASP-1, and/or mouse MASP-1
- inhibits C3 and/or C4 activation with an IC₅₀ of less than 0.35 micro g/mL.

[0089] In one aspect, the invention provides an anti-MASP-1 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 32; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 33; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 34; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 35; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 36; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 37.

[0090] In one aspect, the invention provides an anti-MASP-1 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 38; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 39; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 40; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 41; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 42; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 43.

[0091] In one aspect, the invention provides an anti-MASP-1 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 44; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 45; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 46; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 47; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 48; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 49.

[0092] In one aspect, the invention provides an anti-MASP-1 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 50; (b) HVR-H2 comprising the amino acid

sequence of SEQ ID NO: 51; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 52; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 53; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 54; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 55.

[0093] In one aspect, the invention provides an anti-MASP-1 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 56; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 57; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 58; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 59; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 60; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 61.

[0094] In one aspect, the invention provides an anti-MASP-1 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 62; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 63; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 64; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 65; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 66; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 67.

[0095] In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 32, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 33, and (iii) HVR-H3 comprising an amino acid sequence of SEQ ID NO: 34; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 35, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 36, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 37.

[0096] In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 38, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 39, and (iii) HVR-H3 comprising an amino acid sequence of SEQ ID NO: 40; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 41, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 42, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 43.

[0097] In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i)

HVR-H1 comprising the amino acid sequence of SEQ ID NO: 44, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 45, and (iii) HVR-H3 comprising an amino acid sequence of SEQ ID NO: 46; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 47, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 48, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 49.

[0098] In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 50, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 51, and (iii) HVR-H3 comprising an amino acid sequence of SEQ ID NO: 52; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 53, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 54, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 55.

[0099] In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 56, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 57, and (iii) HVR-H3 comprising an amino acid sequence of SEQ ID NO: 58; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 59, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 60, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 61.

[0100] In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 62, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 63, and (iii) HVR-H3 comprising an amino acid sequence of SEQ ID NO: 64; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 65, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 66, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 67.

[0101] In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 32; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 33; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 34; (d) HVR-L1 comprising the amino acid sequence of SEQ

ID NO: 35; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 36; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO: 37.

[0102] In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 38; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 39; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 40; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 41; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 42; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO: 43.

[0103] In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 44; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 45; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 46; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 47; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 48; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO: 49.

[0104] In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 50; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 51; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 52; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 53; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 54; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO: 55.

[0105] In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 56; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 57; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 58; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 59; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 60; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO: 61.

[0106] In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 62; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 63; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 64; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 65; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 66; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO: 67.

[0107] In any of the above embodiments, an anti-MASP-1 antibody is humanized. In one embodiment, an anti-MASP-1 antibody comprises HVRs as in any of the above embodiments, and further comprises an acceptor human framework, e.g. a human immunoglobulin framework or a human consensus framework.

[0108] In another aspect, an anti-MASP-1 antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%,

98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 20, 22, 24, 26, 28, or 30. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-MASP-1 antibody comprising that sequence retains the ability to bind to MASP-1. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 20, 22, 24, 26, 28, or 30. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-MASP-1 antibody comprises the VH sequence in SEQ ID NO: 20, 22, 24, 26, 28, or 30, including post-translational modifications of that sequence. . Post-translational modifications include but are not limited to a modification of glutamine or glutamate in N-terminal of heavy chain or light chain to pyroglutamic acid by pyroglutamylation.

[0109] In another aspect, an anti-MASP-1 antibody is provided, wherein the antibody comprises a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 21, 23, 25, 27, 29, or 31. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-PRO antibody comprising that sequence retains the ability to bind to PRO. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 21, 23, 25, 27, 29, or 31. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-MASP-1 antibody comprises the VL sequence in SEQ ID NO: 21, 23, 25, 27, 29, or 31, including post-translational modifications of that sequence. Post-translational modifications include but are not limited to a modification of glutamine or glutamate in N-terminal of heavy chain or light chain to pyroglutamic acid by pyroglutamylation.

[0110] In another aspect, an anti-MASP-1 antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above.

[0111] In a further aspect, the invention provides an antibody that binds to the same epitope as an anti-MASP-1 antibody provided herein. For example, in certain embodiments, an antibody is provided that binds to the same epitope as:

an anti-MASP-1 antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 32; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 33; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 34; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 35; (e) HVR-L2

comprising the amino acid sequence of SEQ ID NO: 36; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO: 37,

an anti-MASP-1 antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 38; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 39; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 40; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 41; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 42; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO: 43,

an anti-MASP-1 antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 44; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 45; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 46; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 47; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 48; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO: 49,

an anti-MASP-1 antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 50; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 51; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 52; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 53; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 54; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO: 55,

an anti-MASP-1 antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 56; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 57; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 58; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 59; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 60; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO: 61, or

an anti-MASP-1 antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 62; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 63; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 64; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 65; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 66; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO: 67.

[0112] In a further aspect, the invention provides an antibody that binds to the same epitope as an anti-MASP-1 antibody provided herein. For example, in certain embodiments, an antibody is provided that binds to the same epitope as:

an anti-MASP-1 antibody comprising a VH sequence of SEQ ID NO: 20 and a VL sequence of SEQ ID NO: 21,

an anti-MASP-1 antibody comprising a VH sequence of SEQ ID NO: 22 and a VL

sequence of SEQ ID NO: 23,
an anti-MASP-1 antibody comprising a VH sequence of SEQ ID NO: 24 and a VL sequence of SEQ ID NO: 25,
an anti-MASP-1 antibody comprising a VH sequence of SEQ ID NO: 26 and a VL sequence of SEQ ID NO: 27,
an anti-MASP-1 antibody comprising a VH sequence of SEQ ID NO: 28 and a VL sequence of SEQ ID NO: 29, or
an anti-MASP-1 antibody comprising a VH sequence of SEQ ID NO: 30 and a VL sequence of SEQ ID NO: 31.

[0113] In certain embodiments, an antibody that binds to an epitope within the serine protease (SP) domain of human MASP-1 (SEQ ID NO: 7) is provided.

[0114] In a further aspect of the invention, an anti-MASP-1 antibody according to any of the above embodiments is a monoclonal antibody, including a chimeric, humanized or human antibody. In one embodiment, an anti-MASP-1 antibody is an antibody fragment, e.g., a Fv, Fab, Fab', scFv, diabody, or F(ab')₂ fragment. In another embodiment, the antibody is a full length antibody, e.g., an intact IgG1, IgG2 or IgG4 antibody or other antibody class or isotype as defined herein.

[0115] In a further aspect, an anti-MASP-1 antibody according to any of the above embodiments may incorporate any of the features, singly or in combination, as described in Sections 1-13 below:

[0116] 1. Antibody Which Binds to MASP-1 but Does not Bind to MASP-3

In certain embodiments, an antibody that specifically binds to MASP-1 is provided. In another embodiments, an anti-MASP-1 antibody binds to MASP-1 but does not bind to MASP-3. In another embodiments, an anti-MASP-1 antibody binds to MASP-1 but does not substantially bind to MASP-3.

[0117] Examples of monoclonal antibodies which bind to MASP-1 are described below in Table 3. All monoclonal antibodies which bind to MASP-1 in Table 3 also bind to MASP-3.

[0118]

[Table 3]

Antigen	Antibody Type	Reference
hMASP-1 (full-length)	Mouse mAbs ; mAb 1E2 and 2B11 (both Abs recognize the heavy chain common to both MASP-1 and MASP-3)	Terai I. et al., <i>Clin Exp Immunol</i> 110:317-323 (1997); mAb 1E2: Commercially available from Hycult Biotech Cat#HM2092 mAb 2B11: Commercially available from Hycult Biotech: Cat#HM2093
hMASP-1 (full-length)	Mouse mAb 4C2	Endo M. et al., <i>Nephrol Dial Transplant</i> 13: 1984-1990 (1998); mAb 4C2 is reported as mouse mAb directed against MASP-1/3 heavy chain in Roos A., et al., <i>J Am Soc Nephrol.</i> 2006 Jun;17(6):1724-34.
hMASP-1 (full-length)	MASP-1 chicken Abs; mAb D14 and 1E10 (both Abs recognize the heavy chain common to both MASP-1 and MASP-3)	WO2013/180834 (Example 15), and WO2013/192240 (Example 15); MASP-1 specific antibody is not actually obtained.

[0119] In certain aspects, an anti-MASP-1 antibody does not bind, or does not substantially bind to MASP-3. In certain embodiments, an anti-MASP-1 antibody binds to MASP-1 with higher affinity than to MASP-3. In certain embodiments, the antibodies of the present invention bind to MASP-1 with at least 2, 3, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 400, 1000, 10000, 100000 or more times higher affinity than to MASP-3 when compared, e.g., on the basis of dissociation constant (K_d). In certain embodiments, the binding of the antibody to the MASP-3 protein is less than about 50%, 40%, 30%, 20% or 10% of the binding of the antibody to MASP-1 as measured, e.g., by a radioimmunoassay (RIA) or Enzyme-Linked ImmunoSorbent Assay (ELISA).

[0120] As described herein, the inventors have unexpectedly discovered monoclonal antibodies which bind to MASP-1 but do not bind to MASP-3. The inventors have also confirmed that the monoclonal antibodies which bind to MASP-1 but do not bind to MASP-3 have inhibition activity against MASP-1. WO2013/180834 discloses selection methods for MASP-1 antigen binding (Example 15 of WO2013/180834), the methods comprising parallel screens for binders to MASP-1 and MASP-3 using (i) the heavy/alpha chain which is shared between MASP-1 and MASP-3 and (ii) the distinct light/beta chains comprising a serine protease domain. However, no monoclonal antibodies which specifically bind to MASP-1 have been actually obtained. It has been reported that the catalytic activity of MASP-1 suppresses its expression through rapid

auto-activation and auto-degradation (Protein Expr Purif Vol 88, Issue 2, April 2013, pp173-182). Also, MASP-1 is known to be able to auto-activate and, owing to its high activity, MASP-1 furthermore seems prone to auto-degradation. Because the light/beta-chain (the serine protease domain) of MASP-1 is a part of the MASP-1 catalytic region (CCP1-CCP2-SP domains), it has been difficult to purify or produce a stable MASP-1 SP domain and to screen antibodies for binding to the SP domain of MASP-1.

[0121] 2. Antibody Which Binds to the SP (serine protease) Domain of MASP-1

In certain embodiments, an antibody that binds to an epitope within the serine protease (SP) domain of human MASP-1 (SEQ ID NO: 7) is provided.

[0122] MASP-1 and MASP-3 are alternative splicing variants of the MASP-1 gene. They contain an identical heavy/alpha chain (CUB1-EGF-CUB2-CCP1-CCP2 domains) prolonged by a different light/beta chain (SP domain) at the C-terminal end. Therefore, in certain embodiments, it is expected that an antibody which binds to MASP-1 but does not bind to MASP-3 binds or specifically binds to the SP domain of MASP-1.

[0123] In certain embodiments, an anti-MASP-1 antibody is an antibody which cross-reacts with human MASP-1, monkey MASP-1 and mouse MASP-1. In certain embodiments, an anti-MASP-1 antibody binds to an epitope of MASP-1 that is conserved among MASP-1 SP domains from human, monkey and mouse. In further embodiments, it is expected that an antibody which cross-reacts with human MASP-1, monkey MASP-1 and mouse MASP-1 binds to an epitope of MASP-1 that is conserved among MASP-1 SP domains from human, monkey and mouse. An exemplary amino acid sequence that is conserved among MASP-1 SP domains from human, monkey and mouse is amino acid residues 1 to 8, 13 to 34, 39 to 51, 60 to 69, 71 to 75, 100 to 104, 135 to 151, 153 to 161, 172 to 178, 180 to 204, 211 to 216, 223 to 236, or 239 to 245 of SEQ ID NO: 7. (Figure 2)

[0124] 3. Antibody Which Binds to an Activated or Inactivated Form of MASP-1

In certain embodiments, an anti-MASP-1 antibody is an antibody that binds to the activated form of MASP-1. MASP-1 contains a heavy/alpha chain (CUB1-EGF-CUB2-CCP1-CCP2 domains) prolonged by a light/beta chain (SP domain) at the C-terminal end. MASP-1 is activated by cleavage in the activation peptide, separating the heavy/alpha chain and light/beta chain. The two chains are then held together by an interchain cysteine bridge (disulphide bridge). In certain embodiments, an anti-MASP-1 antibody inhibits more than 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90% of MASP-1 enzymatic activity.

[0125] In certain embodiments, an anti-MASP-1 antibody is an antibody that binds to the activated form of MASP-1 but does not bind to the inactivated form of MASP-1. In certain embodiments, an anti-MASP-1 antibody is an antibody that binds to the in-

activated form of MASP-1 but does not bind to the activated form of MASP-1. In certain embodiments, an anti-MASP-1 antibody is an antibody that binds to both the activated and inactivated forms of MASP-1.

[0126] 4. Antibody Which Inhibits LP (lectin pathway) of Complement Activation

In certain embodiments, an anti-MASP-1 antibody is an antibody that inhibits MASP-1 activity. In further embodiments, the MASP-1 activity is MASP-1-mediated complement lectin-pathway activation. Without intending to be bound by any theory, MASP-1 functions as a component of the lectin pathway of complement activation. Upon binding, MASP-1 will first autoactivate, then it activates MASP-2 and MASP-3. The activated MASP-2 cleaves C4 into C4a, C4b, and C4c, while activated MASP-1 and MASP-2 cleave C2 into C2a, and C2b. The assembly of the C4a and C2b fragments results in the formation of the C3 convertase C4bC2a. The C4bC2a cleaves C3 into C3a and C3b, followed by cleavage of C3b into C3c and C3d by Factor I. In certain embodiments, an anti-MASP-1 antibody binds to MASP-1 and inhibits MASP-1 activity to activate MASP-2 and MASP-3. In further embodiments, an anti-MASP-1 antibody binds to MASP-1 and inhibits activation of the lectin pathway.

[0127] In certain embodiments, an anti-MASP-1 antibody inhibits activation of the lectin pathway with an IC 50 of less than 0.5micro g/mL, 0.45 micro g/mL, 0.40 micro g/mL, 0.35 micro g/mL, 0.34 micro g/mL, 0.33 micro g/mL, 0.32 micro g/mL, 0.31 micro g/mL, 0.3 micro g/mL, 0.29 micro g/mL, 0.28 micro g/mL, 0.27 micro g/mL, 0.26 micro g/mL, 0.25 micro g/mL, 0.24 micro g/mL, 0.23 micro g/mL, 0.22 micro g/mL, 0.21 micro g/mL, 0.2 micro g/mL, 0.19 micro g/mL, 0.18 micro g/mL, 0.17 micro g/mL, 0.16 micro g/mL, 0.15 micro g/mL, 0.14 micro g/mL, 0.13 micro g/mL or 0.12micro g/mL, preferably less than 0.35 micro g/mL, 0.34 micro g/mL, 0.33 micro g/mL, 0.32 micro g/mL, 0.31 micro g/mL or 0.3 micro g/mL, more preferably less than 0.35 micro g/mL.

[0128] 5. Antibody Which Inhibits MASP-1 Activity but Does not Inhibit MASP-3 Activity

In certain embodiments, an anti-MASP-1 antibody is an antibody that inhibits MASP-1 activity but does not directly inhibit MASP-3 activity. An antibody that directly inhibits MASP-3 is an antibody which binds to MASP-3 and inhibits its activity, while an antibody that indirectly inhibits MASP-3 is an antibody which binds to a molecule other than MASP3 and inhibits MASP-3 activity (e.g., an anti-MASP-1 antibody that inhibits MASP-3 activation). It has been reported that MASP-1 activates MASP-3, which converts the alternative pathway activation enzyme factor D from its zymogen form into its enzymatically active form (J. Immunol. 187:3751-58 (2011)). Methods to assess the activation of factor D are already known in the art (e.g., J Immunol January 15, 2016, 196 (2) 857-865)

[0129] 6. Stable Anti-MASP-1 Antibody

In certain embodiments, an anti-MASP-1 antibody is stable after being incubated in PBS at 40 degrees C for 1 week, 2 weeks or more. The term "stable" as used herein means that the anti-MASP-1 antibody after incubation has MASP-1 inhibition activity which is substantially similar to the anti-MASP-1 antibody before incubation. The term "stable" as used herein means that the anti-MASP-1 antibody after incubation has more than 50%, 60%, 70%, 80% or 90% of MASP-1 inhibition activity of the anti-MASP-1 antibody before incubation.

[0130] 7. Antibody Affinity

In certain embodiments, an antibody provided herein has a dissociation constant (Kd) of 1 micro M or less, 100 nM or less, 10 nM or less, 1 nM or less, 0.1 nM or less, 0.01 nM or less, or 0.001 nM or less (e.g. 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M, e.g., from 10^{-9} M to 10^{-13} M).

[0131] In one embodiment, Kd is measured by a radiolabeled antigen binding assay (RIA). In one embodiment, an RIA is performed with the Fab version of an antibody of interest and its antigen. For example, solution binding affinity of Fabs for antigen is measured by equilibrating Fab with a minimal concentration of (^{125} I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (see, e.g., Chen et al., J. Mol. Biol. 293:865-881(1999)). To establish conditions for the assay, MICROTITER (registered trademark) multi-well plates (Thermo Scientific) are coated overnight with 5 micro g/ml of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23 degrees C). In a non-adsorbent plate (Nunc #269620), 100 pM or 26 pM [^{125} I]-antigen are mixed with serial dilutions of a Fab of interest (e.g., consistent with assessment of the anti-VEGF antibody, Fab-12, in Presta et al., Cancer Res. 57:4593-4599 (1997)). The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period (e.g., about 65 hours) to ensure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature (e.g., for one hour). The solution is then removed and the plate washed eight times with 0.1% polysorbate 20 (TWEEN-20 (registered trademark)) in PBS. When the plates have dried, 150 micro l/well of scintillant (MICROSCINT-20™; Packard) is added, and the plates are counted on a TOPCOUNT™ gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

[0132] According to another embodiment, Kd is measured using a BIACORE (registered trademark) surface plasmon resonance assay. For example, an assay using a BIACORE (registered trademark)-2000 or a BIACORE(registered trademark)-3000 (BIAcore,

Inc., Piscataway, NJ) is performed at 25 degrees C with immobilized antigen CM5 chips at ~10 response units (RU). In one embodiment, carboxymethylated dextran biosensor chips (CM5, BIAcore, Inc.) are activated with N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5 micro g/ml (~0.2 micro M) before injection at a flow rate of 5 micro l/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% polysorbate 20 (TWEEN-20™) surfactant (PBST) at 25 degrees C at a flow rate of approximately 25 micro l/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIAcore (registered trademark) Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (K_d) is calculated as the ratio k_{off}/k_{on} . See, e.g., Chen et al., J. Mol. Biol. 293:865-881 (1999). If the on-rate exceeds $10^6 \text{ M}^{-1} \text{ s}^{-1}$ by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25 degrees C of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophotometer (Aviv Instruments) or a 8000-series SLM-AMINCO™ spectrophotometer (ThermoSpectronic) with a stirred cuvette.

[0133] 8. Antibody Fragments

In certain embodiments, an antibody provided herein is an antibody fragment. Antibody fragments include, but are not limited to, Fab, Fab', Fab'-SH, F(ab')₂, Fv, and scFv fragments, and other fragments described below. For a review of certain antibody fragments, see Hudson et al. Nat. Med. 9:129-134 (2003). For a review of scFv fragments, see, e.g., Pluckthun, in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenberg and Moore eds., (Springer-Verlag, New York), pp. 269-315 (1994); see also WO 93/16185; and U.S. Patent Nos. 5,571,894 and 5,587,458. For discussion of Fab and F(ab')₂ fragments comprising salvage receptor binding epitope residues and having increased in vivo half-life, see U.S. Patent No. 5,869,046.

[0134] Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific. See, for example, EP 404,097; WO 1993/01161; Hudson et al., Nat. Med. 9:129-134 (2003); and Hollinger et al., Proc. Natl. Acad. Sci. USA 90: 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., Nat. Med. 9:129-134 (2003).

- [0135] Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; see, e.g., U.S. Patent No. 6,248,516 B1).
- [0136] Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (e.g. E. coli or phage), as described herein.
- [0137] 9. Chimeric and Humanized Antibodies
- In certain embodiments, an antibody provided herein is a chimeric antibody. Certain chimeric antibodies are described, e.g., in U.S. Patent No. 4,816,567; and Morrison et al., Proc. Natl. Acad. Sci. USA, 81:6851-6855 (1984)). In one example, a chimeric antibody comprises a non-human variable region (e.g., a variable region derived from a mouse, rat, hamster, rabbit, or non-human primate, such as a monkey) and a human constant region. In a further example, a chimeric antibody is a "class switched" antibody in which the class or subclass has been changed from that of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof.
- [0138] In certain embodiments, a chimeric antibody is a humanized antibody. Typically, a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which HVRs, e.g., CDRs, (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (e.g., the antibody from which the HVR residues are derived), e.g., to restore or improve antibody specificity or affinity.
- [0139] Humanized antibodies and methods of making them are reviewed, e.g., in Almagro and Fransson, Front. Biosci. 13:1619-1633 (2008), and are further described, e.g., in Riechmann et al., Nature 332:323-329 (1988); Queen et al., Proc. Nat'l Acad. Sci. USA 86:10029-10033 (1989); US Patent Nos. 5, 821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri et al., Methods 36:25-34 (2005) (describing specificity determining region (SDR) grafting); Padlan, Mol. Immunol. 28:489-498 (1991) (describing "resurfacing"); Dall'Acqua et al., Methods 36:43-60 (2005) (describing "FR shuffling"); and Osbourn et al., Methods 36:61-68 (2005) and Klimka et al., Br. J. Cancer, 83:252-260 (2000) (describing the "guided selection" approach to FR shuffling).
- [0140] Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the "best-fit" method (see, e.g., Sims et al.

J. Immunol. 151:2296 (1993)); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (see, e.g., Carter et al. Proc. Natl. Acad. Sci. USA, 89:4285 (1992); and Presta et al. J. Immunol., 151:2623 (1993)); human mature (somatically mutated) framework regions or human germline framework regions (see, e.g., Almagro and Fransson, Front. Biosci. 13:1619-1633 (2008)); and framework regions derived from screening FR libraries (see, e.g., Baca et al., J. Biol. Chem. 272:10678-10684 (1997) and Rosok et al., J. Biol. Chem. 271:22611-22618 (1996)).

[0141] 10. Human Antibodies

In certain embodiments, an antibody provided herein is a human antibody. Human antibodies can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, Curr. Opin. Pharmacol. 5: 368-74 (2001) and Lonberg, Curr. Opin. Immunol. 20:450-459 (2008).

[0142] Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic mice, the endogenous immunoglobulin loci have generally been inactivated. For review of methods for obtaining human antibodies from transgenic animals, see Lonberg, Nat. Biotech. 23:1117-1125 (2005). See also, e.g., U.S. Patent Nos. 6,075,181 and 6,150,584 describing XENOMOUSE™ technology; U.S. Patent No. 5,770,429 describing HUMAB (registered trademark) technology; U.S. Patent No. 7,041,870 describing K-M MOUSE (registered trademark) technology, and U.S. Patent Application Publication No. US 2007/0061900, describing VELOCIMOUSE (registered trademark) technology). Human variable regions from intact antibodies generated by such animals may be further modified, e.g., by combining with a different human constant region.

[0143] Human antibodies can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described. (See, e.g., Kozbor J. Immunol., 133: 3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner et al., J. Immunol., 147: 86 (1991).) Human antibodies generated via human B-cell hybridoma technology are also described in Li et al., Proc. Natl. Acad. Sci. USA, 103:3557-3562 (2006). Additional methods include those described, for example, in U.S. Patent No. 7,189,826 (describing production of monoclonal human IgM antibodies from hybridoma cell lines) and Ni, Xiandai Mianyixue, 26(4):265-268 (2006) (describing human-human hy-

bridomas). Human hybridoma technology (Trioma technology) is also described in Vollmers and Brandlein, *Histology and Histopathology*, 20(3):927-937 (2005) and Vollmers and Brandlein, *Methods and Findings in Experimental and Clinical Pharmacology*, 27(3):185-91 (2005).

[0144] Human antibodies may also be generated by isolating Fv clone variable domain sequences selected from human-derived phage display libraries. Such variable domain sequences may then be combined with a desired human constant domain. Techniques for selecting human antibodies from antibody libraries are described below.

[0145] 11. Library-Derived Antibodies

Antibodies of the invention may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, e.g., in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, 2001) and further described, e.g., in the McCafferty et al., *Nature* 348:552-554; Clackson et al., *Nature* 352: 624-628 (1991); Marks et al., *J. Mol. Biol.* 222: 581-597 (1992); Marks and Bradbury, in *Methods in Molecular Biology* 248:161-175 (Lo, ed., Human Press, Totowa, NJ, 2003); Sidhu et al., *J. Mol. Biol.* 338(2): 299-310 (2004); Lee et al., *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee et al., *J. Immunol. Methods* 284(1-2): 119-132(2004).

[0146] In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter et al., *Ann. Rev. Immunol.*, 12: 433-455 (1994). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (e.g., from human) to provide a single source of antibodies to a wide range of non-self and also self antigens without any immunization as described by Griffiths et al., *EMBO J*, 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement in vitro, as described by Hoogenboom and Winter, *J. Mol. Biol.*, 227: 381-388 (1992). Patent publications describing human antibody phage libraries include, for example: US Patent No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

[0147] Antibodies or antibody fragments isolated from human antibody libraries are considered human antibodies or human antibody fragments herein.

[0148] 12. Multispecific Antibodies

In certain embodiments, an antibody provided herein is a multispecific antibody, e.g. a bispecific antibody. Multispecific antibodies are monoclonal antibodies that have binding specificities for at least two different sites. In certain embodiments, one of the binding specificities is for MASP-1 and the other is for any other antigen. In certain embodiments, bispecific antibodies may bind to two different epitopes of MASP-1. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express MASP-1. Bispecific antibodies can be prepared as full length antibodies or antibody fragments.

[0149] Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (see Milstein and Cuello, *Nature* 305: 537 (1983)), WO 93/08829, and Traunecker et al., *EMBO J.* 10: 3655 (1991)), and "knob-in-hole" engineering (see, e.g., U.S. Patent No. 5,731,168). Multi-specific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004A1); cross-linking two or more antibodies or fragments (see, e.g., US Patent No. 4,676,980, and Brennan et al., *Science*, 229: 81 (1985)); using leucine zippers to produce bi-specific antibodies (see, e.g., Kostelny et al., *J. Immunol.*, 148(5):1547-1553 (1992)); using "diabody" technology for making bispecific antibody fragments (see, e.g., Hollinger et al., *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993)); and using single-chain Fv (scFv) dimers (see, e.g. Gruber et al., *J. Immunol.*, 152:5368 (1994)); and preparing trispecific antibodies as described, e.g., in Tutt et al. *J. Immunol.* 147: 60 (1991).

[0150] Engineered antibodies with three or more functional antigen binding sites, including "Octopus antibodies," are also included herein (see, e.g. US 2006/0025576A1).

[0151] The antibody or fragment herein also includes a "Dual Acting Fab" or "DAF" comprising an antigen binding site that binds to MASP-1 as well as another, different antigen (see, US 2008/0069820, for example).

[0152] 13. Antibody Variants

In certain embodiments, amino acid sequence variants of the antibodies provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any com-

bination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, e.g., antigen-binding.

[0153] a) Substitution, Insertion, and Deletion Variants

In certain embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs and FRs. Conservative substitutions are shown in Table 4 under the heading of "preferred substitutions." More substantial changes are provided in Table 4 under the heading of "exemplary substitutions," and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, e.g., retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

[0154]

[Table 4]

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

[0155] Amino acids may be grouped according to common side-chain properties:

- (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;
- (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;
- (3) acidic: Asp, Glu;
- (4) basic: His, Lys, Arg;
- (5) residues that influence chain orientation: Gly, Pro;
- (6) aromatic: Trp, Tyr, Phe.

[0156] Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[0157] One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (e.g. a humanized or human antibody). Generally,

the resulting variant(s) selected for further study will have modifications (e.g., improvements) in certain biological properties (e.g., increased affinity, reduced immunogenicity) relative to the parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, e.g., using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more HVR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (e.g. binding affinity).

[0158] Alterations (e.g., substitutions) may be made in HVRs, e.g., to improve antibody affinity. Such alterations may be made in HVR "hotspots," i.e., residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (see, e.g., Chowdhury, *Methods Mol. Biol.* 207:179-196 (2008)), and/or residues that contact antigen, with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, e.g., in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, (2001).) In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (e.g., error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (e.g., 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, e.g., using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

[0159] In certain embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example, conservative alterations (e.g., conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations may, for example, be outside of antigen contacting residues in the HVRs. In certain embodiments of the variant VH and VL sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

[0160] A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) *Science*, 244:1081-1085. In this method, a residue or group of target residues (e.g., charged residues such as arg, asp, his, lys, and glu) are identified and replaced by a neutral or negatively charged amino acid (e.g., alanine or polyalanine) to determine whether the interaction of the antibody with antigen is

affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex may be analyzed to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

[0161] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion of an enzyme (e.g. for ADEPT) or a polypeptide which increases the plasma half-life of the antibody to the N- or C-terminus of the antibody.

[0162] b) Glycosylation variants

In certain embodiments, an antibody provided herein is altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

[0163] Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. See, e.g., Wright et al. TIBTECH 15:26-32 (1997). The oligosaccharide may include various carbohydrates, e.g., mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an antibody of the invention may be made in order to create antibody variants with certain improved properties.

[0164] In one embodiment, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (e. g. complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (EU numbering of Fc region residues); however, Asn297 may also be located about +/- 3 amino acids upstream or downstream of position 297,

i.e., between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. See, e.g., US Patent Publication Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to "defucosylated" or "fucose-deficient" antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki et al. J. Mol. Biol. 336:1239-1249 (2004); Yamane-Ohnuki et al. Biotech. Bioeng. 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. Arch. Biochem. Biophys. 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams et al., especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, FUT8, knockout CHO cells (see, e.g., Yamane-Ohnuki et al. Biotech. Bioeng. 87: 614 (2004); Kanda, Y. et al., Biotechnol. Bioeng., 94(4):680-688 (2006); and WO2003/085107).

[0165] Antibodies variants are further provided with bisected oligosaccharides, e.g., in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, e.g., in WO 2003/011878 (Jean-Mairet et al.); US Patent No. 6,602,684 (Umana et al.); and US 2005/0123546 (Umana et al.). Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, e.g., in WO 1997/30087 (Patel et al.); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

[0166] c) Fc region variants

In certain embodiments, one or more amino acid modifications may be introduced into the Fc region of an antibody provided herein, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (e.g., a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (e.g. a substitution) at one or more amino acid positions.

[0167] In certain embodiments, the invention contemplates an antibody variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half life of the antibody in vivo is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. In vitro and/or in vivo cytotoxicity assays can be conducted to confirm the reduction/

depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks Fc gamma R binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express Fc gamma RIII only, whereas monocytes express Fc gamma RI, Fc gamma RII and Fc gamma RIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991). Non-limiting examples of in vitro assays to assess ADCC activity of a molecule of interest is described in U.S. Patent No. 5,500,362 (see, e.g. Hellstrom, I. et al. *Proc. Nat'l Acad. Sci. USA* 83:7059-7063 (1986)) and Hellstrom, I et al., *Proc. Nat'l Acad. Sci. USA* 82:1499-1502 (1985); 5,821,337 (see Bruggemann, M. et al., *J. Exp. Med.* 166:1351-1361 (1987)). Alternatively, non-radioactive assays methods may be employed (see, for example, ACT1™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA; and CytoTox 96 (registered trademark) non-radioactive cytotoxicity assay (Promega, Madison, WI).

Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g., in an animal model such as that disclosed in Clynes et al. *Proc. Nat'l Acad. Sci. USA* 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. See, e.g., C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro et al., *J. Immunol. Methods* 202:163 (1996); Cragg, M.S. et al., *Blood* 101:1045-1052 (2003); and Cragg, M.S. and M.J. Glennie, *Blood* 103:2738-2743 (2004)). FcRn binding and in vivo clearance/half life determinations can also be performed using methods known in the art (see, e.g., Petkova, S.B. et al., *Int'l. Immunol.* 18(12):1759-1769 (2006)).

- [0168] Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581).
- [0169] Certain antibody variants with increased or decreased binding to FcRs are described. (See, e.g., U.S. Patent No. 6,737,056; WO 2004/056312, and Shields et al., *J. Biol. Chem.* 9(2): 6591-6604 (2001).)
- [0170] In certain embodiments, an antibody variant comprises an Fc region with one or more amino acid substitutions which improve ADCC, e.g., substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues).
- [0171] In some embodiments, alterations are made in the Fc region that result in altered (i.e.,

either increased or decreased) C1q binding and/or Complement Dependent Cytotoxicity (CDC), e.g., as described in US Patent No. 6,194,551, WO 99/51642, and Idusogie et al. J. Immunol. 164: 4178-4184 (2000).

[0172] Antibodies with increased half lives and increased binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., J. Immunol. 117:587 (1976) and Kim et al., J. Immunol. 24:249 (1994)), are described in US2005/0014934A1 (Hinton et al.). Those antibodies comprise an Fc region with one or more substitutions therein which increase binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, e.g., substitution of Fc region residue 434 (US Patent No. 7,371,826).

[0173] See also Duncan & Winter, Nature 322:738-40 (1988); U.S. Patent No. 5,648,260; U.S. Patent No. 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

[0174] d) Cysteine engineered antibody variants

In certain embodiments, it may be desirable to create cysteine engineered antibodies, e.g., "thioMAbs," in which one or more residues of an antibody are substituted with cysteine residues. In particular embodiments, the substituted residues occur at accessible sites of the antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate, as described further herein. In certain embodiments, any one or more of the following residues may be substituted with cysteine: V205 (Kabat numbering) of the light chain; A118 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc region. Cysteine engineered antibodies may be generated as described, e.g., in U.S. Patent No. 7,521,541.

[0175] e) Antibody Derivatives

In certain embodiments, an antibody provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, polypropylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated

polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

[0176] In another embodiment, conjugates of an antibody and nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In one embodiment, the nonproteinaceous moiety is a carbon nanotube (Kam et al., Proc. Natl. Acad. Sci. USA 102: 11600-11605 (2005)). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a temperature at which cells proximal to the antibody-nonproteinaceous moiety are killed.

[0177] B. Recombinant Methods and Compositions

Antibodies may be produced using recombinant methods and compositions, e.g., as described in U.S. Patent No. 4,816,567. In one embodiment, isolated nucleic acid encoding an anti-MASP-1 antibody described herein is provided. Such nucleic acid may encode an amino acid sequence comprising the VL and/or an amino acid sequence comprising the VH of the antibody (e.g., the light and/or heavy chains of the antibody). In a further embodiment, one or more vectors (e.g., expression vectors) comprising such nucleic acid are provided. In a further embodiment, a host cell comprising such nucleic acid is provided. In one such embodiment, a host cell comprises (e.g., has been transformed with): (1) a vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and an amino acid sequence comprising the VH of the antibody, or (2) a first vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and a second vector comprising a nucleic acid that encodes an amino acid sequence comprising the VH of the antibody. In one embodiment, the host cell is eukaryotic, e.g. a Chinese Hamster Ovary (CHO) cell or lymphoid cell (e.g., Y0, NS0, Sp2/0 cell). In one embodiment, a method of making an anti-MASP-1 antibody is provided, wherein the method comprises culturing a host cell comprising a nucleic acid encoding the antibody, as provided above, under conditions suitable for expression of the antibody, and optionally recovering the antibody from the host cell (or host cell culture medium).

[0178] For recombinant production of an anti-MASP-1 antibody, nucleic acid encoding an antibody, e.g., as described above, is isolated and inserted into one or more vectors for

further cloning and/or expression in a host cell. Such nucleic acid may be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody).

- [0179] Suitable host cells for cloning or expression of antibody-encoding vectors include prokaryotic or eukaryotic cells described herein. For example, antibodies may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of antibody fragments and polypeptides in bacteria, see, e.g., U.S. Patent Nos. 5,648,237, 5,789,199, and 5,840,523. (See also Charlton, *Methods in Molecular Biology*, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, NJ, 2003), pp. 245-254, describing expression of antibody fragments in *E. coli*.) After expression, the antibody may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.
- [0180] In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been "humanized," resulting in the production of an antibody with a partially or fully human glycosylation pattern. See Gerngross, *Nat. Biotech.* 22:1409-1414 (2004), and Li et al., *Nat. Biotech.* 24:210-215 (2006).
- [0181] Suitable host cells for the expression of glycosylated antibody are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of *Spodoptera frugiperda* cells.
- [0182] Plant cell cultures can also be utilized as hosts. See, e.g., US Patent Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTIBODIES™ technology for producing antibodies in transgenic plants).
- [0183] Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293 cells as described, e.g., in Graham et al., *J. Gen Virol.* 36:59 (1977)); baby hamster kidney cells (BHK); mouse sertoli cells (TM4 cells as described, e.g., in Mather, *Biol. Reprod.* 23:243-251 (1980)); monkey kidney cells (CV1); African green monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK); buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); TRI cells, as described, e.g., in Mather et al., *Annals N.Y. Acad. Sci.* 383:44-68 (1982); MRC 5 cells; and FS4 cells. Other useful mammalian host cell lines

include Chinese hamster ovary (CHO) cells, including DHFR⁺ CHO cells (Urlaub et al., Proc. Natl. Acad. Sci. USA 77:4216 (1980)); and myeloma cell lines such as Y0, NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, see, e.g., Yazaki and Wu, *Methods in Molecular Biology*, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, NJ), pp. 255-268 (2003).

[0184] C. Assays

Anti-MASP-1 antibodies provided herein may be identified, screened for, or characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

[0185] 1. Binding assays and other assays

In one aspect, an antibody of the invention is tested for its antigen binding activity, e.g., by known methods such as ELISA, Western blot, etc.

[0186] In another aspect, competition assays may be used to identify an antibody that competes for binding to MASP-1 with any anti-MASP-1 antibody described herein. In certain embodiments, such a competing antibody binds to the same epitope (e.g., a linear or a conformational epitope) that is bound by an anti-MASP-1 antibody described herein. Detailed exemplary methods for mapping an epitope to which an antibody binds are provided in Morris (1996) "Epitope Mapping Protocols," in *Methods in Molecular Biology* vol. 66 (Humana Press, Totowa, NJ).

[0187] In an exemplary competition assay, immobilized MASP-1 is incubated in a solution comprising a first labeled antibody that binds to MASP-1 and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to MASP-1. The second antibody may be present in a hybridoma supernatant. As a control, immobilized MASP-1 is incubated in a solution comprising the first labeled antibody but not the second unlabeled antibody. After incubation under conditions permissive for binding of the first antibody to MASP-1, excess unbound antibody is removed, and the amount of label associated with immobilized MASP-1 is measured. If the amount of label associated with immobilized MASP-1 is substantially reduced in the test sample relative to the control sample, then that indicates that the second antibody is competing with the first antibody for binding to MASP-1. See Harlow and Lane (1988) *Antibodies: A Laboratory Manual* ch.14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

[0188] 2. Activity assays

In one aspect, assays are provided for identifying anti-MASP-1 antibodies thereof having biological activity. Biological activity may include, e.g., an inhibitory activity against MASP-1. Antibodies having such biological activity in vivo and/or in vitro are also provided.

[0189] In certain embodiments, an antibody of the invention is tested for such biological

activity.

[0190] In certain embodiments, an antibody of the invention is tested for its cross-reactivity against MASP-1 and MASP-3 by, for example, known binding assays such as ELISA, radioimmunoassay (RIA), Western blot, BIACORE (registered trademark), etc. In certain embodiments, an antibody that binds to MASP-1 with at least 2, 3, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 400, 1000, 10000, 100000 or more times higher affinity than to MASP-3 when compared, e.g., on the basis of dissociation constant (Kd), is selected. In certain embodiments, an antibody whose binding to MASP-3 is less than about 50%, 40%, 30%, 20% or 10% of the binding of the antibody to MASP-1 as measured, e.g., by a radioimmunoassay (RIA) or Enzyme-Linked ImmunoSorbent Assay (ELISA), is selected.

[0191] In certain embodiments, an antibody of the invention is tested for its inhibitory activity on the lectin pathway of the complement system. The inhibitory activity of a test antibody against the lectin pathway of complement activation can be determined by, for example, detecting and/or measuring deposition of a complement component (e.g., C4c, C3c, or such) generated through the lectin pathway. Such detection methods are known, for example, as described in Petersen et al., *Journal of Immunological Methods* 257: 107-116 (2001). In an exemplary detection method, a test antibody is contacted with normal serum in vitro and incubated in a mannan-coated plate, and the deposition of C4c or C3c is detected and/or measured by conventional methods such as ELISA. Alternatively, a test antibody is contacted with normal serum in vivo by administering it to an animal. Blood is collected from the animal and centrifuged to separate serum, and the separated serum is incubated in a mannan-coated plate to detect and/or measure C4c or C3c deposition as described above. If the C4c or C3c deposition is reduced by the test antibody as compared to a negative control, the test antibody is determined to have inhibitory activity on the lectin pathway of complement activation. In certain embodiments, an antibody of the invention may inhibit the lectin pathway of complement activation by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% or greater as compared to a negative control as measured in the assay. Based on results of the assay, IC50 values may be calculated using appropriate analysis software such as GraphPad Prism (registered trademark).

[0192] In certain embodiments, an antibody of the invention is tested for its inhibitory activity on the enzymatic activity of the active form of MASP-1. The enzymatic activity of the active form of MASP-1 includes, but is not limited to, endopeptidase activity. The inhibitory activity of a test antibody against the enzymatic activity of the active form of MASP-1 can be detected and/or measured by, for example, a peptide cleavage assay as described in, e.g., Degn et al., *Protein Expression and Purification*

88: 173-182 (2013). In an exemplary method, an artificial fluorogenic peptide substrate such as Boc-VPR-AMC is used as a substrate for MASP-1. The peptide substrate is allowed to react with MASP-1 in the presence of a test antibody, and cleavage of the peptide is measured as relative fluorescence intensity. If the fluorescence is reduced in the presence of the test antibody as compared to that in the absence of the test antibody, the test antibody is determined to have inhibitory activity on the enzymatic activity of MASP-1. In certain embodiments, an antibody of the invention may inhibit the enzymatic activity of the active form of MASP-1 by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% or greater as compared to a negative control as measured in the assay.

[0193] D. Immunoconjugates

The invention also provides immunoconjugates comprising an anti-MASP-1 antibody herein conjugated to one or more cytotoxic agents, such as chemotherapeutic agents or drugs, growth inhibitory agents, toxins (e.g., protein toxins, enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), or radioactive isotopes.

[0194] In one embodiment, an immunoconjugate is an antibody-drug conjugate (ADC) in which an antibody is conjugated to one or more drugs, including but not limited to a maytansinoid (see U.S. Patent Nos. 5,208,020, 5,416,064 and European Patent EP 0 425 235 B1); an auristatin such as monomethylauristatin drug moieties DE and DF (MMAE and MMAF) (see U.S. Patent Nos. 5,635,483 and 5,780,588, and 7,498,298); a dolastatin; a calicheamicin or derivative thereof (see U.S. Patent Nos. 5,712,374, 5,714,586, 5,739,116, 5,767,285, 5,770,701, 5,770,710, 5,773,001, and 5,877,296; Hinman et al., *Cancer Res.* 53:3336-3342 (1993); and Lode et al., *Cancer Res.* 58:2925-2928 (1998)); an anthracycline such as daunomycin or doxorubicin (see Kratz et al., *Current Med. Chem.* 13:477-523 (2006); Jeffrey et al., *Bioorganic & Med. Chem. Letters* 16:358-362 (2006); Torgov et al., *Bioconj. Chem.* 16:717-721 (2005); Nagy et al., *Proc. Natl. Acad. Sci. USA* 97:829-834 (2000); Dubowchik et al., *Bioorg. & Med. Chem. Letters* 12:1529-1532 (2002); King et al., *J. Med. Chem.* 45:4336-4343 (2002); and U.S. Patent No. 6,630,579); methotrexate; vindesine; a taxane such as docetaxel, paclitaxel, larotaxel, tesetaxel, and ortataxel; a trichothecene; and CC1065.

[0195] In another embodiment, an immunoconjugate comprises an antibody as described herein conjugated to an enzymatically active toxin or fragment thereof, including but not limited to diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, *Phytolacca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, croton, saponaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin,

enomycin, and the tricothecenes.

- [0196] In another embodiment, an immunoconjugate comprises an antibody as described herein conjugated to a radioactive atom to form a radioconjugate. A variety of radioactive isotopes are available for the production of radioconjugates. Examples include ^{211}At , ^{131}I , ^{125}I , ^{90}Y , ^{186}Re , ^{188}Re , ^{153}Sm , ^{212}Bi , ^{32}P , ^{212}Pb and radioactive isotopes of Lu. When the radioconjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example Tc-99m or ^{123}I , or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, MRI), such as iodine-123 again, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron.
- [0197] Conjugates of an antibody and cytotoxic agent may be made using a variety of bi-functional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., Science 238:1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionuclide to the antibody. See WO94/11026. The linker may be a "cleavable linker" facilitating release of a cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari et al., Cancer Res. 52:127-131 (1992); U.S. Patent No. 5,208,020) may be used.
- [0198] The immunoconjugates or ADCs herein expressly contemplate, but are not limited to such conjugates prepared with cross-linker reagents including, but not limited to, BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (e.g., from Pierce Biotechnology, Inc., Rockford, IL., U.S.A).
- [0199] E. Methods and Compositions for Diagnostics and Detection
- In certain embodiments, any of the anti-MASP-1 antibodies provided herein is useful for detecting the presence of MASP-1 in a biological sample. The term "detecting" as used herein encompasses quantitative or qualitative detection. In certain embodiments, a biological sample comprises a cell or tissue, such as serum, whole blood, plasma,

biopsy sample, tissue sample, cell suspension, saliva, sputum, oral fluid, cerebrospinal fluid, amniotic fluid, ascites fluid, milk, colostrum, mammary gland secretion, lymph, urine, sweat, lacrimal fluid, gastric fluid, synovial fluid, peritoneal fluid, ocular lens fluid or mucus.

[0200] In one embodiment, an anti-MASP-1 antibody for use in a method of diagnosis or detection is provided. In a further aspect, a method of detecting the presence of MASP-1 in a biological sample is provided. In certain embodiments, the method comprises contacting the biological sample with an anti-MASP-1 antibody as described herein under conditions permissive for binding of the anti-MASP-1 antibody to MASP-1, and detecting whether a complex is formed between the anti-MASP-1 antibody and MASP-1. Such method may be an in vitro or in vivo method. In one embodiment, an anti-MASP-1 antibody is used to select subjects eligible for therapy with an anti-MASP-1 antibody, e.g. where MASP-1 is a biomarker for selection of patients.

[0201] Exemplary disorders that may be diagnosed using an antibody of the invention include diseases caused by the complement lectin-pathway activation, such as thrombotic microangiopathy (TMA), membranous nephropathy, age-related macular degeneration (AMD), myocardial infarctions, and stroke.

[0202] In certain embodiments, labeled anti-MASP-1 antibodies are provided. Labels include, but are not limited to, labels or moieties that are detected directly (such as fluorescent, chromophoric, electron-dense, chemiluminescent, and radioactive labels), as well as moieties, such as enzymes or ligands, that are detected indirectly, e.g., through an enzymatic reaction or molecular interaction. Exemplary labels include, but are not limited to, the radioisotopes ^{32}P , ^{14}C , ^{125}I , ^3H , and ^{131}I , fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luciferases, e.g., firefly luciferase and bacterial luciferase (U.S. Patent No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase, beta-galactosidase, glucoamylase, lysozyme, saccharide oxidases, e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, those coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals, and the like.

[0203] F. Pharmaceutical Formulations

Pharmaceutical formulations of an anti-MASP-1 antibody as described herein are prepared by mixing such antibody having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at

the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include interstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX (registered trademark), Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Patent Publication Nos. 2005/0260186 and 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

- [0204] Exemplary lyophilized antibody formulations are described in US Patent No. 6,267,958. Aqueous antibody formulations include those described in US Patent No. 6,171,586 and WO2006/044908, the latter formulations including a histidine-acetate buffer.
- [0205] The formulation herein may also contain more than one active ingredients as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended.
- [0206] Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).
- [0207] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers

containing the antibody, which matrices are in the form of shaped articles, e.g. films, or microcapsules.

[0208] The formulations to be used for in vivo administration are generally sterile. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes.

[0209] G. Therapeutic Methods and Compositions

Any of the anti-MASP-1 antibodies provided herein may be used in therapeutic methods.

[0210] In one aspect, an anti-MASP-1 antibody for use as a medicament is provided. In further aspects, an anti-MASP-1 antibody for use in treating a disease caused by complement lectin-pathway activation is provided. In certain embodiments, an anti-MASP-1 antibody for use in a method of treatment is provided. In certain embodiments, the invention provides an anti-MASP-1 antibody for use in a method of treating an individual having a disease caused by complement lectin-pathway activation comprising administering to the individual an effective amount of the anti-MASP-1 antibody. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below. In further embodiments, the invention provides an anti-MASP-1 antibody for use in inhibiting complement lectin-pathway activation mediated by MASP-1. In certain embodiments, the invention provides an anti-MASP-1 antibody for use in a method of inhibiting complement lectin-pathway activation mediated by MASP-1 in an individual comprising administering to the individual an effective amount of the anti-MASP-1 antibody to inhibit complement lectin-pathway activation mediated by MASP-1. An "individual" according to any of the above embodiments is preferably a human. In further embodiments, the invention provides an anti-MASP-1 antibody for use in inhibiting C3 and/or C4 activation. In certain embodiments, the invention provides an anti-MASP-1 antibody for use in a method of inhibiting C3 and/or C4 activation in an individual comprising administering to the individual an effective amount of the anti-MASP-1 antibody to inhibit C3 and/or C4 activation. An "individual" according to any of the above embodiments is preferably a human.

[0211] In a further aspect, the invention provides for the use of an anti-MASP-1 antibody in the manufacture or preparation of a medicament. In one embodiment, the medicament is for treatment of a disease caused by complement lectin-pathway activation. In a further embodiment, the medicament is for use in a method of treating a disease caused by complement lectin-pathway activation comprising administering to an individual having a disease caused by complement lectin-pathway activation an effective amount of the medicament. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below. In a further embodiment, the medicament is for in-

hibiting complement lectin-pathway activation mediated by MASP-1. In a further embodiment, the medicament is for use in a method of inhibiting complement lectin-pathway activation mediated by MASP-1 in an individual comprising administering to the individual an amount effective of the medicament to inhibit complement lectin-pathway activation mediated by MASP-1. An "individual" according to any of the above embodiments may be a human. In a further embodiment, the medicament is for C3 and/or C4 activation. In a further embodiment, the medicament is for use in a method of C3 and/or C4 activation in an individual comprising administering to the individual an amount effective of the medicament to inhibit C3 and/or C4 activation. An "individual" according to any of the above embodiments may be a human.

- [0212] In a further aspect, the invention provides a method for treating a disease caused by complement lectin-pathway activation. In one embodiment, the method comprises administering to an individual having such a disease caused by complement lectin-pathway activation an effective amount of an anti-MASP-1 antibody. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, as described below. An "individual" according to any of the above embodiments may be a human.
- [0213] In a further aspect, the invention provides a method for inhibiting complement lectin-pathway activation mediated by MASP-1 in an individual. In one embodiment, the method comprises administering to the individual an effective amount of an anti-MASP-1 antibody to inhibit complement lectin-pathway activation mediated by MASP-1. In one embodiment, an "individual" is a human.
- [0214] In a further aspect, the invention provides a method for inhibiting C3 and/or C4 activation in an individual. In one embodiment, the method comprises administering to the individual an effective amount of an anti-MASP-1 antibody to inhibit C3 and/or C4 activation. In one embodiment, an "individual" is a human.
- [0215] In a further aspect, the invention provides pharmaceutical formulations comprising any of the anti-MASP-1 antibodies provided herein, e.g., for use in any of the above therapeutic methods. In one embodiment, a pharmaceutical formulation comprises any of the anti-MASP-1 antibodies provided herein and a pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical formulation comprises any of the anti-MASP-1 antibodies provided herein and at least one additional therapeutic agent, e.g., as described below.
- [0216] Antibodies of the invention can be used either alone or in combination with other agents in a therapy. For instance, an antibody of the invention may be co-administered with at least one additional therapeutic agent.
- [0217] Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and

separate administration, in which case, administration of the antibody of the invention can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent or agents. In one embodiment, administration of the anti-MASP-1 antibody and administration of an additional therapeutic agent occur within about one month, or within about one, two or three weeks, or within about one, two, three, four, five, or six days, of each other.

[0218] An antibody of the invention (and any additional therapeutic agent) can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Dosing can be by any suitable route, e.g. by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

[0219] Antibodies of the invention would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The antibody need not be, but is optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of antibody present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

[0220] For the prevention or treatment of disease, the appropriate dosage of an antibody of the invention (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the type of antibody, the severity and course of the disease, whether the antibody is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The antibody is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 micro g/kg to 15 mg/kg (e.g. 0.1mg/kg-10mg/kg) of antibody can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate adminis-

trations, or by continuous infusion. One typical daily dosage might range from about 1 micro g/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of the antibody would be in the range from about 0.05 mg/kg to about 10 mg/kg. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the patient. Such doses may be administered intermittently, e.g. every week or every three weeks (e.g. such that the patient receives from about two to about twenty, or e.g. about six doses of the antibody). An initial higher loading dose, followed by one or more lower doses may be administered. The progress of this therapy is easily monitored by conventional techniques and assays.

[0221] It is understood that any of the above formulations or therapeutic methods may be carried out using an immunoconjugate of the invention in place of or in addition to an anti-MASP-1 antibody.

[0222] H. Articles of Manufacture

In another aspect of the invention, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label on or a package insert associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active ingredient in the composition is an antibody of the invention. The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises an antibody of the invention; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including

other buffers, diluents, filters, needles, and syringes.

- [0223] It is understood that any of the above articles of manufacture may include an immunoconjugate of the invention in place of or in addition to an anti-MASP-1 antibody.

Examples

- [0224] III. EXAMPLES

The following are examples of methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

- [0225] Example 1

Expression and purification of recombinant human MASP-1 (CCP1-CCP2-SP), human MASP-1 (CCP1-CCP2-SP S646A), and human MASP-3 (CCP1-CCP2-SP S664A)

Human MASP-1 (CCP1-CCP2-SP) (SEQ ID NO: 15) fused with a Flag-tag on its N-terminus was expressed transiently using FreeStyle293-F cell line (Thermo Fisher). Conditioned media expressing human MASP-1 (CCP1-CCP2-SP) was applied to a column packed with anti-Flag M2 affinity resin (Sigma) and eluted with Flag peptide (Sigma). Fractions containing human MASP-1 (CCP1-CCP2-SP) were collected and subsequently subjected to a Superdex 200 gel filtration column (GE healthcare) equilibrated with 1x PBS. Fractions containing human MASP-1 (CCP1-CCP2-SP) were then pooled and stored at -80 degrees C.

- [0226] In order to prevent self-digestion, Ser at position 646 of human MASP-1 was substituted with Ala, and Ser at position 664 of MASP-3 was substituted with Ala. The human MASP-1 CCP1-CCP2-SP domain carrying the Ser646Ala mutation was designated as human MASP-1 (CCP1-CCP2-SP S646A) (SEQ ID NO: 16) and the human MASP-3 CCP1-CCP2-SP domain carrying the Ser664Ala mutation was designated as human MASP-3 (CCP1-CCP2-SP S664A) (SEQ ID NO: 17)). Position 646 of MASP-1 corresponds to the amino acid position 376 of SEQ ID NO: 15 and 16, and position 664 of human MASP-3 corresponds to the amino acid position 394 of SEQ ID NO: 17. Expression and purification of N-terminally Flag-tagged human MASP-1 (CCP1-CCP2-SP S646A) and N-terminally Flag-tagged human MASP-3 (CCP1-CCP2-SP S664A) were performed exactly the same way as N-terminally Flag-tagged human MASP-1 (CCP1-CCP2-SP).

- [0227] Example 2

Expression and purification of recombinant antibodies

Unless indicated otherwise, recombinant antibodies were expressed transiently using FreeStyle293-F cell line (Thermo Fisher). Purification from the conditioned media expressing the antibodies was done with a conventional method using protein A. Gel

filtration was further conducted if necessary.

[0228] Example 3

Generation of anti-MASP-1 antibodies

3.1. Antibody screening

Anti-MASP1 antibodies were prepared, selected and assayed as follows:

Twelve to sixteen week old NZW rabbits were immunized intradermally with human MASP-1 (CCP1-CCP2-SP) or human MASP-1 (CCP1-CCP2-SP S646A) (50-100 micro g/dose/rabbit). This dose was repeated 3 times over 1 month. One week after the final immunization, the spleen and blood were collected from the immunized rabbits. Antigen-specific B-cells were stained with labelled antigen, sorted with FCM cell sorter (FACS aria III, BD), and plated in 96-well plates at a density of one cell/well together with 25,000 cells/well of EL4 cells (European Collection of Cell Cultures) and an activated rabbit T-cell conditioned medium diluted 20 times, and were cultured for 7-12 days. EL4 cells were treated with mitomycin C (Sigma, Cat No. M4287) for 2 hours and washed 3 times in advance. The activated rabbit T-cell conditioned medium was prepared by culturing rabbit thymocytes in RPMI-1640 containing Phytohemagglutinin-M (Roche, Cat No. 1 1082132-001), phorbol 12-myristate 13-acetate (Sigma, Cat No. P1585) and 2% FBS. After cultivation, B-cell culture supernatants were collected for further analysis and pellets were cryopreserved.

[0229] ELISA assay was used to test specificity of antibodies in B-cell culture supernatant. Streptavidin (GeneScript, Cat No. Z02043) was coated onto a 384-well MAXISorp (Nunc, Cat No. 164688) at 50nM in PBS for 1 hour at room temperature. Plates were then blocked with Blocking One (Nacalai Tesque, Cat No. 03953-95) diluted 5 times. Human MASP-1 (CCP1-CCP2-SP S646A) was labelled with NHS-PEG4-Biotin (PIERCE, Cat No. 21329) and was added to the blocked ELISA plates, incubated for 1 hour and washed with PBS containing 0.05% Tween20. B-cell culture supernatants were added to the ELISA plates, incubated for 1 hour and washed. Binding was detected by goat anti-rabbit IgG-Horseradish peroxidase (BETHYL, Cat No. A120-111P) followed by the addition of ABTS (KPL, Cat No. 50-66-06).

[0230] A total of 15,524 B-cell lines were screened for binding to human MASP-1 (CCP1-CCP2-SP S646A) and 94 lines were selected and designated as MTA0095-0188. Their RNA was purified from cryopreserved cell pellets by using ZR-96 Quick-RNA kits (ZYMO RESEARCH, Cat No. R1053). The DNAs of their antibody heavy chain variable regions were amplified by reverse transcription PCR and recombined with the DNA encoding F760G4 (SEQ ID NO: 18), which is a modified human IgG4 heavy chain constant region with Fc gamma receptors (Fc gamma Rs) silent. The DNAs of their antibody light chain variable regions were amplified by reverse transcription PCR and recombined with the DNA encoding

k0MTC (SEQ ID NO: 19), which is a modified human kappa light chain constant region. Cloned antibodies were expressed in FreeStyle™ 293-F Cells (Invitrogen) and purified from culture supernatant to evaluate functional activity. Inhibiting activities of the chimeric monoclonal antibodies against human MASP-1 were evaluated by testing inhibition of hC4c (human C4c) deposition assay as described later (Example 4).

[0231] 3.2 Cross-reactivity against human MASP-1 and human MASP-3

ELISA assay was used to check the cross-reactivity of the chimeric monoclonal antibodies generated in 3.1 against human MASP-1 and human MASP-3. Goat anti-human IgG polyclonal antibody (Thermo Scientific, H10500) was diluted to 1 micro g/ml with PBS (-) and added to 384-well MAXISorp plates (Nunc, Cat No. 164688). The plates were incubated for 1 hour at room temperature, and blocked with Blocking One (Nacalai Tesque, Cat No. 03953-95) diluted 5 times. The plates were incubated for 1 hour, washed, and anti-MASP-1 antibody was added and incubated for 1 hour. Human MASP-1 (CCP1-CCP2-SP S646A) or human MASP-3 (CCP1-CCP2-SP S664A) was labelled with NHS-PEG4-Biotin (PIERCE, Cat No. 21329). After incubation, the plates were washed and 8nM of labeled antigen was added and incubated for 1 hour. After incubation, the plates were washed and Streptavidin-HRP conjugate (Thermo Scientific 21130) were added. After 1 hour incubation, binding of biotinylated antigen was detected by the addition of ABTS (KPL, Cat No. 50-66-06). Anti-MASP-1 monoclonal antibodies listed in Table 5 were selected as MASP-1 specific antibodies and used for further analysis. The sequences of the variable regions and the HVRs of the selected antibodies are shown in Tables 6 and 7, respectively.

[0232] [Table 5]

Specific binding of selected monoclonal antibodies against human MASP-1 (CCP1-CCP2-SP S646A)

Antibody	Human MASP-1 (CCP1-CCP2-SP S646A)	Human MASP-3 (CCP1-CCP2-SP S664A)
MAS0102dd	1.9795	-0.027
MAS0114bb	1.7905	-0.068
MAS0116aa	1.7195	0.055
MAS0141aa	1.5095	-0.147
MAS0154dd	1.9515	0.078
MAS0177hh	0.8985	0.388

The values show absorbance at 405nm after background subtraction.

[0233]

[Table 6]

Sequences of variable regions of selected monoclonal antibodies

Antibody	Variable Region	SEQ ID NO
MAS0102dd	Heavy chain variable region	20
	Light chain variable region	21
MAS0114bb	Heavy chain variable region	22
	Light chain variable region	23
MAS0116aa	Heavy chain variable region	24
	Light chain variable region	25
MAS0141aa	Heavy chain variable region	26
	Light chain variable region	27
MAS0154dd	Heavy chain variable region	28
	Light chain variable region	29
MAS0177hh	Heavy chain variable region	30
	Light chain variable region	31

[0234]

[Table 7]

Sequences of HVRs of selected antibodies

Antibody	HVR	Sequence	SEQ ID NO
MAS0102dd	HVR H1	SYAMS	32
	HVR H2	IITNLDNTYYANWAKG	33
	HVR H3	ERIKRKYSYDDYGGWAFDP	34
	HVR L1	QAIENIYRSLA	35
	HVR L2	DTSDLAS	36
	HVR L3	QSYYYSSVTYNA	37
MAS0114bb	HVR H1	NYAIH	38
	HVR H2	YIYARSGVTWYASWVKG	39
	HVR H3	VLAGGGYANTGLSL	40
	HVR L1	QASQNIGYSSA	41
	HVR L2	GASTLAS	42
	HVR L3	QSNYISSGSVDGT	43
MAS0116aa	HVR H1	SCYMS	44
	HVR H2	TIYTGSGATYYASWAKG	45
	HVR H3	AIYPGSSSSNLCAFDL	46
	HVR L1	QASQSVYDNNWLA	47
	HVR L2	QASSLAS	48
	HVR L3	QGYIAGVLYS	49
MAS0141aa	HVR H1	NYYMT	50
	HVR H2	IINTDGGTTYASWAKG	51
	HVR H3	GVFTGGWTGMRTNL	52
	HVR L1	QASEDIYILLA	53
	HVR L2	AASNLES	54
	HVR L3	QCTYGSSSSNA	55
MAS0154dd	HVR H1	NYNMG	56
	HVR H2	IISSQGDIIYYATWAKG	57
	HVR H3	ARHVGSAADSYFHL	58
	HVR L1	QASEEIYRNLA	59
	HVR L2	GASNLAS	60
	HVR L3	QRYAWGKNSADGNT	61
MAS0177hh	HVR H1	SAYMN	62
	HVR H2	IYASGSTWYASWAKG	63
	HVR H3	DDYGTADL	64
	HVR L1	QASQSIGSSLA	65
	HVR L2	GASTLVS	66
	HVR L3	QNYYLSSSSADRRT	67

[0235] Example 44.1 Inhibition of lectin pathway by anti-MASP-1 monoclonal antibodies

The lectin pathway (LP) is activated upon binding of mannan-binding lectin (MBL) to mannan. The MBL is subsequently associated with MASPs. Without intending to be

bound by any theory, upon binding, MASP-1 will first autoactivate and then it activates MASP-2 and MASP-3. The activated MASP-2 cleaves C4 into C4a, C4b, and C4c, while activated MASP-1 and MASP-2 cleave C2 into C2a, and C2b. The assembly of the C4a and C2b fragments results in the formation of the C3 convertase C4bC2a. The C4bC2a cleaves C3 into C3a and C3b, followed by cleavage of C3b into C3c and C3d by Factor I. The anti-MASP-1 chimeric mAbs obtained in Example 3 were tested for inhibition of the lectin pathway of the complement system by C4c or C3c ELISA assay. Mannan (20 micro g/mL) was coated on 96-well plates at 4 degrees C overnight. The plates were blocked with TBS blocking buffer (Block Ace Powder, DS Pharma Biomedical) containing 0.5% bovine serum albumin (Roche Diagnostics) for 2 hours at 37 degrees C. Fifty-five microliters of normal human serum (4%) (Biopredic, SER019) was mixed with 55 micro L of diluted anti-MASP-1 mAb in a 96-well plate and incubated on a shaker for 1 hour at 25 degrees C. Serum samples containing anti-MASP-1 mab were transferred into the mannan-coated plates and incubated for 1 hour at 37 degrees C. After washing the plates, 100 micro L of rabbit anti-human C4c (Dako) was added to each well and reacted for 1 hour at R/T. Then the plates were washed and C4c deposition was detected by goat anti-rabbit IgG-Horseradish peroxidase (BETHYL) followed by the addition of ABTS (KPL). Figure 3 shows data indicating that anti-MASP-1 mAbs: MAS0102dd, MAS0114bb, MAS0116aa, MAS0154dd, and MAS0177hh, inhibited human C4c deposition. Similarly, these antibodies inhibited cynomolgus monkey C3c deposition (Figure 3B). All of the antibodies except MAS0116aa inhibited mouse C4c deposition as well (Figure 3C).

[0236] 4.2 IC 50 values of anti-MASP-1 mAbs

From the result of C4c ELISA assay using human serum described in 4.1, the IC50 value of each chimeric monoclonal antibody was calculated with GraphPad Prism (registered trademark). The calculated IC50 values are shown in Table 8.

[0237] [Table 8]

Antibody	IC50 (μg/mL)
MAS0102dd	0.29
MAS0114bb	0.14
MAS0116aa	0.47
MAS0141aa	0.45
MAS0154dd	0.11
MAS0177hh	3.29

[0238] 4.3 Stability of anti-MASP-1 mAb

The anti-MASP-1 chimeric mAb: MAS0141aa, was incubated in PBS at 40 degrees C for 2 weeks to evaluate the stability of the antibody. After the 2-week incubation, MAS0141aa was tested for inhibition of lectin pathway of the complement system by cynomolgus monkey C3c ELISA assay. As shown in Figure 4, MAS0141aa significantly inhibited cynomolgus monkey C3c deposition after the incubation. There was no difference in neutralizing activity of MAS0141aa between pre and post-incubation.

[0239] 4.4 Inhibition of enzymatic activity of the active form of MASP-1 by anti-MASP-1 mAbs

Anti-MASP-1 mAbs (MAS0102dd and MAS0116aa) were tested for inhibition of recombinant human MASP-1 (CCP1-CCP2-SP) (SEQ ID NO: 15) by a peptide cleavage assay. Boc-VPR-AMC was used as an artificial fluorogenic substrate of MASP-1. Twenty-five microliters of recombinant active-form human MASP-1 (CCP1-CCP2-SP) (0.4 micro g/mL) was mixed with 25 micro L of diluted mAb and 50 micro L of Boc-VPR-AMC (0.2mM, R&D system) as a substrate in a 96-well plate (MS-8596K, Sumitomo Bakelite). The plate was incubated for 30 min at 37 degrees C, and relative fluorescence units (RFU) were measured at an excitation wavelength of 380 nm and an emission wavelength of 460 nm. Figure 5 shows that both MAS0102dd and MAS0116aa are capable of neutralizing the active form of human MASP-1.

[0240] Example 5

In vivo study of anti-MASP1 mAb in C57BL mice

In vivo neutralization of the lectin pathway was assessed after the administration of anti-MASP-1 mAb in C57BL mice (In Vivos, Singapore).

[0241] MAS0154dd01-mF18 was generated from the chimeric mAb MAS0154dd obtained in Example 3. The heavy chain variable region of MAS0154dd01-mF18 was the same as MAS0154dd. The light chain variable region of MAS0154dd01-mF18 (SEQ ID NO: 68) was generated by substituting Cys at position 80 in the light chain variable region of MAS0154dd (SEQ ID NO: 29) with Ser to remove Cys residue in framework 3 of the light chain variable region. The heavy and light chain constant regions of MAS0154dd were changed to mF18 (SEQ ID NO: 69) and mk1 (SEQ ID NO: 70), respectively.

[0242] MAS0154-mF18 (3 mg/ml) was administered once at a dose of 10 ml/kg into the caudal vein. Blood was collected 1 day, 3 days, and 7 days after administration. The collected blood was immediately centrifuged at 14,000 rpm and 4 degrees C for 10 min to separate the plasma. The separated plasma was stored in a refrigerator at -80 degrees C before assay. To measure C4 activation, a 96-well plate was coated with mannan (20 micro g/mL) at 4 degrees C overnight followed by blocking with TBS blocking buffer

(Block Ace Powder, DS Pharma Biomedical) containing 0.5% bovine serum albumin (Roche Diagnostics) for 2 hours at 37 degrees C. Fifty microliters of mouse plasma samples (0.5%) were transferred into the mannan-coated plates and incubated for 1 hour at 37 degrees C. After washing the plates, C4c deposition was detected using a rabbit anti-C4c antibody (Dako) biotinylated in-house, followed by Streptavidin-HRP and development with ABTS (KPL). As shown in Figure 6, anti-MASP-1 mab MAS0154-mF18 inhibited the lectin pathway activity in mice.

[0243] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literature cited herein are expressly incorporated in their entirety by reference.

Claims

- [Claim 1] An anti-MASP-1 antibody which specifically binds to MASP-1.
- [Claim 2] An anti-MASP-1 antibody which binds to MASP-1 but does not bind to MASP-3.
- [Claim 3] An anti-MASP-1 antibody which binds to the serine protease domain of MASP-1.
- [Claim 4] The anti-MASP-1 antibody according to any one of claims 1 to 3, wherein the antibody inhibits MASP-1 activity.
- [Claim 5] The anti-MASP-1 antibody according to any one of claims 1 to 4, wherein the antibody does not inhibit MASP-3 activity.
- [Claim 6] The anti-MASP-1 antibody according to claim 5, wherein the MASP-1 activity is MASP-1 mediated complement lectin-pathway activation and the MASP-3 activity is MASP-3 mediated complement lectin-pathway activation.
- [Claim 7] The anti-MASP-1 antibody according to any one of claims 1 to 6, wherein the antibody competes for binding to MASP-1 with an antibody selected from the group consisting of:
- (a) an antibody comprising the HVR-H1 sequence of SEQ ID NO: 32, the HVR-H2 sequence of SEQ ID NO: 33, the HVR-H3 sequence of SEQ ID NO: 34, the HVR-L1 sequence of SEQ ID NO: 35, the HVR-L2 sequence of SEQ ID NO: 36, and the HVR-L3 sequence of SEQ ID NO: 37;
 - (b) an antibody comprising the HVR-H1 sequence of SEQ ID NO: 38, the HVR-H2 sequence of SEQ ID NO: 39, the HVR-H3 sequence of SEQ ID NO: 40, the HVR-L1 sequence of SEQ ID NO: 41, the HVR-L2 sequence of SEQ ID NO: 42, and the HVR-L3 sequence of SEQ ID NO: 43;
 - (c) an antibody comprising the HVR-H1 sequence of SEQ ID NO: 44, the HVR-H2 sequence of SEQ ID NO: 45, the HVR-H3 sequence of SEQ ID NO: 46, the HVR-L1 sequence of SEQ ID NO: 47, the HVR-L2 sequence of SEQ ID NO: 48, and the HVR-L3 sequence of SEQ ID NO: 49;
 - (d) an antibody comprising the HVR-H1 sequence of SEQ ID NO: 50, the HVR-H2 sequence of SEQ ID NO: 51, the HVR-H3 sequence of SEQ ID NO: 52, the HVR-L1 sequence of SEQ ID NO: 53, the HVR-L2 sequence of SEQ ID NO: 54, and the HVR-L3 sequence of SEQ ID NO: 55;

(e) an antibody comprising the HVR-H1 sequence of SEQ ID NO: 56, the HVR-H2 sequence of SEQ ID NO: 57, the HVR-H3 sequence of SEQ ID NO: 58, the HVR-L1 sequence of SEQ ID NO: 59, the HVR-L2 sequence of SEQ ID NO: 60, and the HVR-L3 sequence of SEQ ID NO: 61; and

(f) an antibody comprising the HVR-H1 sequence of SEQ ID NO: 62, the HVR-H2 sequence of SEQ ID NO: 63, the HVR-H3 sequence of SEQ ID NO: 64, the HVR-L1 sequence of SEQ ID NO: 65, the HVR-L2 sequence of SEQ ID NO: 66, and the HVR-L3 sequence of SEQ ID NO: 67.

[Claim 8]

The anti-MASP-1 antibody according to any one of claims 1 to 7, wherein the antibody binds to the same epitope to which a reference antibody binds, wherein the reference antibody is selected from the group consisting of:

(a) an antibody comprising the HVR-H1 sequence of SEQ ID NO: 32, the HVR-H2 sequence of SEQ ID NO: 33, the HVR-H3 sequence of SEQ ID NO: 34, the HVR-L1 sequence of SEQ ID NO: 35, the HVR-L2 sequence of SEQ ID NO: 36, and the HVR-L3 sequence of SEQ ID NO: 37;

(b) an antibody comprising the HVR-H1 sequence of SEQ ID NO: 38, the HVR-H2 sequence of SEQ ID NO: 39, the HVR-H3 sequence of SEQ ID NO: 40, the HVR-L1 sequence of SEQ ID NO: 41, the HVR-L2 sequence of SEQ ID NO: 42, and the HVR-L3 sequence of SEQ ID NO: 43;

(c) an antibody comprising the HVR-H1 sequence of SEQ ID NO: 44, the HVR-H2 sequence of SEQ ID NO: 45, the HVR-H3 sequence of SEQ ID NO: 46, the HVR-L1 sequence of SEQ ID NO: 47, the HVR-L2 sequence of SEQ ID NO: 48, and the HVR-L3 sequence of SEQ ID NO: 49;

(d) an antibody comprising the HVR-H1 sequence of SEQ ID NO: 50, the HVR-H2 sequence of SEQ ID NO: 51, the HVR-H3 sequence of SEQ ID NO: 52, the HVR-L1 sequence of SEQ ID NO: 53, the HVR-L2 sequence of SEQ ID NO: 54, and the HVR-L3 sequence of SEQ ID NO: 55;

(e) an antibody comprising the HVR-H1 sequence of SEQ ID NO: 56, the HVR-H2 sequence of SEQ ID NO: 57, the HVR-H3 sequence of SEQ ID NO: 58, the HVR-L1 sequence of SEQ ID NO: 59, the HVR-L2 sequence of SEQ ID NO: 60, and the HVR-L3 sequence of SEQ ID

NO: 61; and

(f) an antibody comprising the HVR-H1 sequence of SEQ ID NO: 62, the HVR-H2 sequence of SEQ ID NO: 63, the HVR-H3 sequence of SEQ ID NO: 64, the HVR-L1 sequence of SEQ ID NO: 65, the HVR-L2 sequence of SEQ ID NO: 66, and the HVR-L3 sequence of SEQ ID NO: 67

- [Claim 9] The anti-MASP-1 antibody according to any one of claims 1 to 8, wherein the antibody is capable of binding to, and inhibiting activity of, human MASP-1, cynomolgus monkey MASP-1, and/or mouse MASP-1.
- [Claim 10] The anti-MASP-1 antibody according to any one of claims 1 to 9, wherein the antibody inhibits C3 and/or C4 activation with an IC₅₀ of less than 0.35 micro g/mL.
- [Claim 11] The anti-MASP-1 antibody according to any one of claims 1 to 10, wherein the antibody is a human, humanized or chimeric antibody.
- [Claim 12] A nucleic acid comprising a nucleotide sequence encoding the antibody of any one of claims 1 to 11.
- [Claim 13] A pharmaceutical composition comprising the antibody according to any one of claims 1 to 11 and a pharmaceutically acceptable carrier.
- [Claim 14] A method of inhibiting C3 and/or C4 activation in serum, comprising contacting the antibody according to any one of claims 1 to 11 with mannan and a biological sample containing MBL (mannan-binding lectin), MASP-1, C2 and C4 under conditions permissive for binding of the antibody to MASP-1.
- [Claim 15] A method of detecting the presence of MASP-1 comprising:
(a) contacting a biological sample with the antibody according to any one of claims 1 to 11 under conditions permissive for binding of the antibody to MASP-1; and
(b) detecting whether a complex is formed between the antibody and MASP-1.

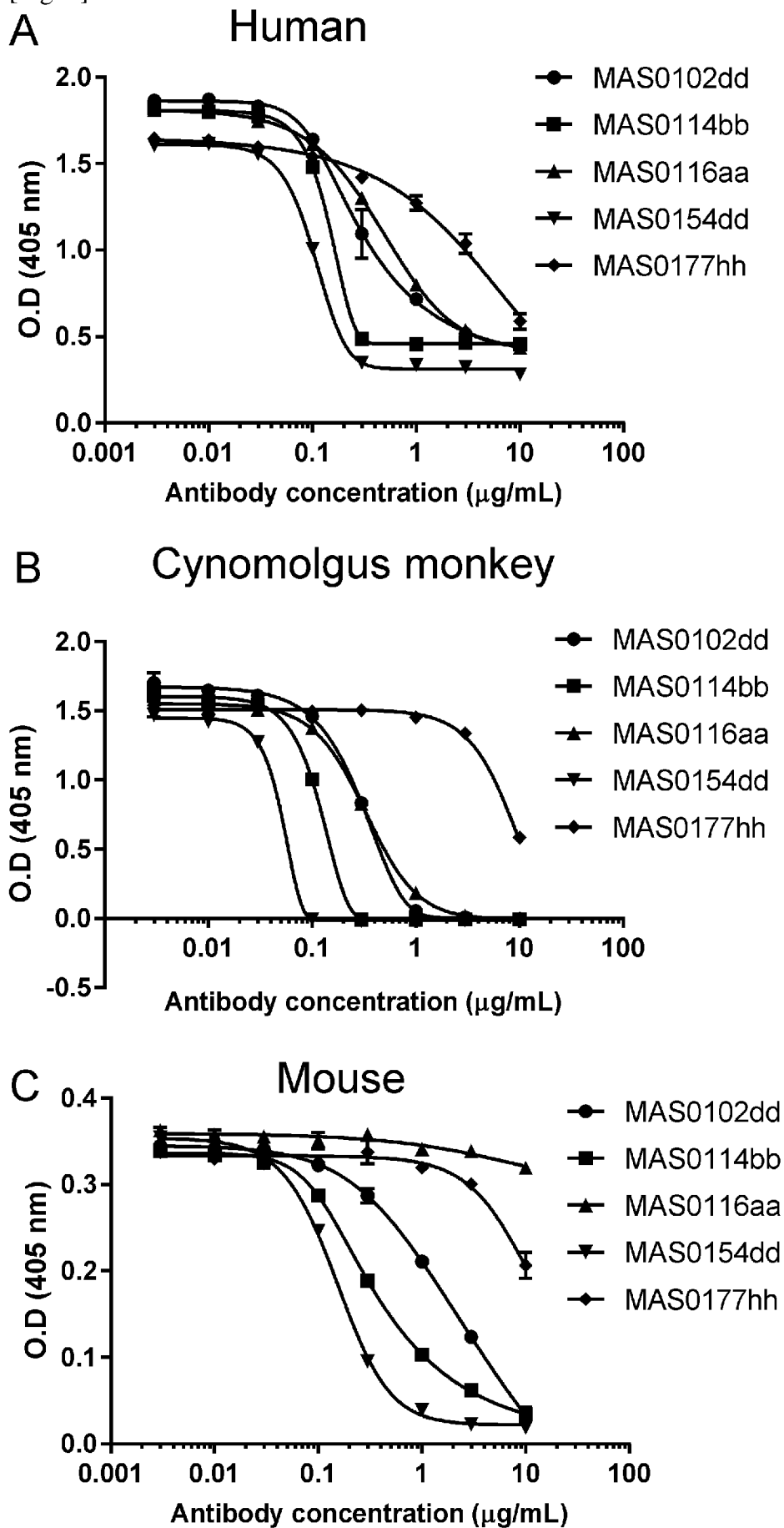
[Fig. 1]

SEQ ID NO: 7	1	IFNGRPAQKGTTTPWIAML-----SHL-NGQPFCCGGSLLGSSWIVTAAHCL	44
SEQ ID NO: 11	1	IIIGRNAEPGLFPWQALIVVEDTSRVPNDKWFGSGALLSASWILTAAHVL	50
SEQ ID NO: 7	45	HQSLDPEDPTLRDSDLL--SPSDFKIIILGKHWRLRSDENEQHLGVKHTTL	92
SEQ ID NO: 11	51	RSQ-----RRDTTVIPVSKHEVTVYLGHL-DVRDKSGAVNSSAARVVL	92
SEQ ID NO: 7	93	HPQYDPNTFENDVALVELLESFVLNFAVMPICLP----EGPQQEGAMVIV	138
SEQ ID NO: 11	93	HPDFNIONYNHDIALVOLQEPVPLGPHVMPVCLPRLEPEGPAPHMLGLVA	142
SEQ ID NO: 7	139	SGWG-----KQFLQRFPETLMEIEIPIVDHSTCQKAYAPLKKK-	176
SEQ ID NO: 11	143	GWGISNPNVTVDIISSGTRTSLDVLQYVKLPVVPHPAECKTSYESRSGNY	192
SEQ ID NO: 7	177	-VTRDMICAGEKEGGKDACAGDSGGPMVTLNRERGQWYLVGTVSWG--DD	223
SEQ ID NO: 11	193	SVTENMEFCAGYYEGGKDTCLGDSGGAFFIFDDLQSRWVVGGLVSWGGP	242
SEQ ID NO: 7	224	CGKKDRIYGVYSYIHHNKDWIQRVTG-----VRN	251
SEQ ID NO: 11	243	CGSKQVYGVYTKVSNYVDWVWEQMGCLPQSVVEPQVER	279

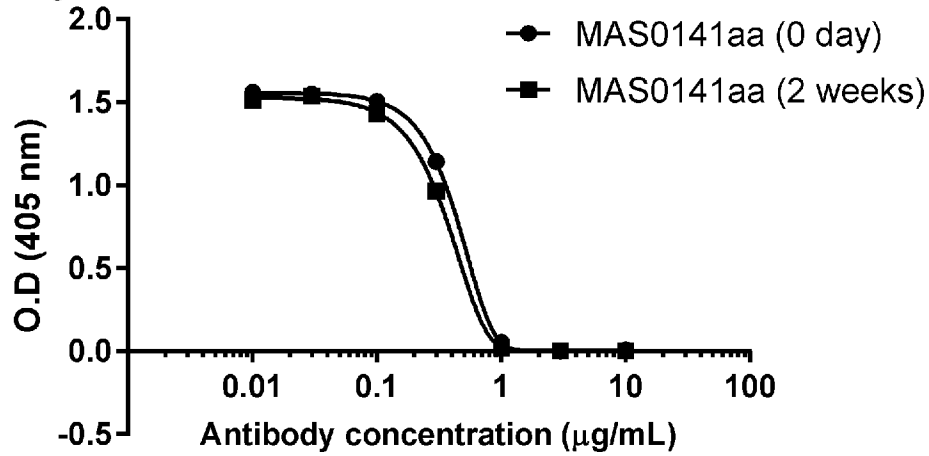
[Fig. 2]

SEQ ID NO: 7	1	IFNGRPAQKGTTTPWIAMLSHLNGQPFCCGGSLLGSSWIVTAAHCLHQSLDP	50
SEQ ID NO: 8	1	IFNGRPAQKGTTTPWIAMLSHLNGQPFCCGGSLLGSSWIVTAAHCLHQSLDP	50
SEQ ID NO: 9	1	IFNGRPAQKGTTTPWIAMLSHLNGQPFCCGGSLLGSSWIVTAAHCLHQSLDP	50
SEQ ID NO: 7	51	EDPTLRDSDLLSPSDFKIIILGKHWRLRSDENEQHLGVKHTTLHPQYDPNT	100
SEQ ID NO: 8	51	EDPTLRDSDLLSPSDFKIIILGKHWRLRSDENEQHLGVKHTTLHPQYDPST	100
SEQ ID NO: 9	51	EEPTLRDSDLLSPSDFKIIILGKHWRLRSDENEQHLGVKHTTLHPQYDPST	100
SEQ ID NO: 7	101	FENDVALVELLESFVLNFAVMPICLPEGPQQEGAMVIVSGWGKQFLQRF	150
SEQ ID NO: 8	101	FENDVALVELLESFVLNFAVMPICLPEGPQQEGAMVIVSGWGKQFLQRF	150
SEQ ID NO: 9	101	FENDLGLVELLESFVLNFAVMPICLPEGPQQEGAMVIVSGWGKQFLQRF	150
SEQ ID NO: 7	151	ETLMEIEIPIVDHSTCQKAYAPLKKKVTRDMICAGEKEGGKDACAGDSGG	200
SEQ ID NO: 8	151	ETLMEIEIPIVDHSTCQKAYAPLKKKVTRDMICAGEKEGGKDACAGDSGG	200
SEQ ID NO: 9	151	ENLMEIEIPIVNSDTCQKAYAPLKKKVTRDMICAGEKEGGKDACAGDSGG	200
SEQ ID NO: 7	201	PMVTLNRERGQWYLVGTVSWGDDCGKKDRIYGVYSYIHHNKDWIQRVTGVR	250
SEQ ID NO: 8	201	PMVTLNRERGQWYLVGTVSWGDDCGKKDRIYGVYSYIHHNKDWIQRVTGVR	250
SEQ ID NO: 9	201	PMVTKDAERDQWYLVGVVSWGDDCGKKDRIYGVYSYIHHNKDWIQRVTGVR	250
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SEQ ID NO: 8	251	N	251
SEQ ID NO: 9	251	N	251

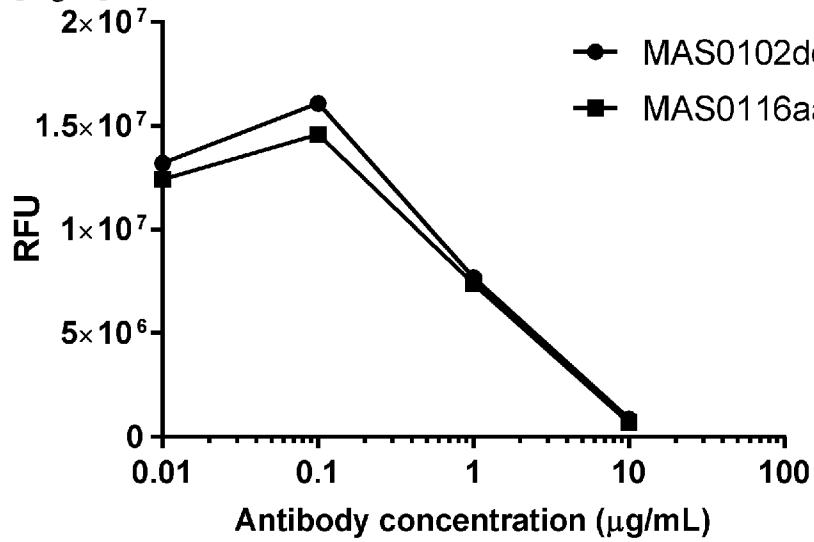
[Fig. 3]



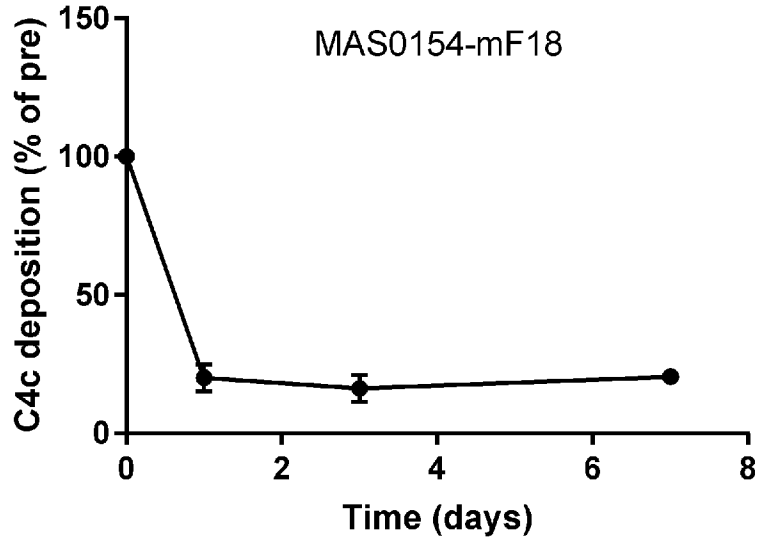
[Fig. 4]



[Fig. 5]



[Fig. 6]



INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP2018/014046

A. CLASSIFICATION OF SUBJECT MATTER		
Int.Cl. C07K16/40(2006.01)i, A61K39/00(2006.01)i, A61K39/395(2006.01)i, A61K45/00(2006.01)i, A61P7/00(2006.01)i		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols)		
Int.Cl. C07K16/40, A61K39/00, A61K39/395, A61K45/00, A61P7/00		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Published examined utility model applications of Japan 1922-1996 Published unexamined utility model applications of Japan 1971-2018 Registered utility model specifications of Japan 1996-2018 Published registered utility model applications of Japan 1994-2018		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
JSTPlus/JMEDPlus/JST/580 (JDreamIII), CAPplus/MEDLINE/EMBASE/BIOSIS (STN), WPIDS/WPIX (STN)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2013/192240 A2 (OMEROS CORPORATION) 2013.12.27, CLAIMS, p. 27-32 & JP 2015-521623 A & US 2013/0344073 A1 & EP 2861246 A2 & CA 2875567 A	1-15
X	WO 2013/180834 A2 (OMEROS CORPORATION) 2013.12.05, CLAIMS, p. 20-28 & JP 2015-514117 A & US 2013/0273053 A1 & EP 2833907 A2 & CA 2869326 A & AU 2013267909 A & KR 10-2015-0003785 A	1-15
☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.		
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Date of the actual completion of the international search		Date of mailing of the international search report
19.06.2018		26.06.2018
Name and mailing address of the ISA/JP		Authorized officer
Japan Patent Office		OTA, Yuzo
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