ANTI-CD48 ANTIBODIES AND USES THEREOF

FIELD OF THE INVENTION

[0001] The present invention relates to antibodies, and antigen-binding fragments thereof, which are specific for human CD48.

BACKGROUND

[0002] CD48 is a GPI-anchored protein that exists in both membrane-bound and soluble forms. CD48 is a high-affinity ligand for 2B4 and a low-affinity ligand for CD2. CD48 has also been referred to in the scientific literature as "TCT.1" (Mami-Chouaib *et al.*, *J. Exp. Med.* *172*:1071-1082 (1990)), "B-LAST 1" (Thorley-Lawson *et al.*, *Cell 30*:415-425 (1982)), and SLAMF2 (Detre *et al.*, *Semin. Immunopathol. 32*:157-171 (2010)). The interaction between CD48 and 2B4 was shown to cause NK cell activation. CD48 has also been shown to stimulate T-cell activation *in vitro*, and CD48-knockout mice display an impaired T-cell proliferation response.

[0003] Certain lines of evidence suggest a role for CD48 in asthma progression and inflammatory bowel disease (IBD). For example, CD48 was shown to be upregulated in experimental asthma (*e.g.*, ovalbumin- and *Aspergillus*-induced allergic eosinophilic airway inflammation models). *See*, *e.g.*, Munitz *et al.*, *Am. J. Respir. Crit. Care Med. 175*:911-918 (2007). Moreover, CD48 knock-out mice failed to develop inflammatory colitis in the adoptive transfer model of IBD. Furthermore, CD48 antagonism was shown to produce beneficial effects in previously established colitis.

[0004] The use of CD48 antagonists for therapeutic purposes are mentioned in, *e.g.*, US 2007/0212353 and WO 97/35614. Nonetheless, there remains a need in the art for novel CD48 modulating agents, including anti-CD48 antibodies, that can be used to treat CD48-mediated diseases and conditions.

BRIEF SUMMARY

[0005] The present disclosure provides antibodies that bind human CD48. The antibodies of the disclosure are useful, *inter alia*, for inhibiting CD48-mediated signaling and for treating diseases and disorders caused by or related to CD48 activity and/or signaling.

[0005a] In one aspect, there is provided an isolated antibody, or antigen-binding fragment thereof, that specifically binds human CD48 (SEQ ID NO:384) and blocks the interaction between human CD48 and a CD48 receptor, wherein the antibody or antigen binding fragment thereof comprises complementarity determining regions (CDRs) of a heavy chain variable region (HCVR) amino acid sequence having an amino acid sequence of SEQ ID NO:368 and the CDRs of a light chain variable region (LCVR) having an amino acid sequence of SEQ ID NO:370.

[0006] The antibodies of the present disclosure block the interaction between CD48 and a CD48 receptor (*e.g.*, 2B4 and/or CD2), and/or inhibit the activation of primary human peripheral blood mononuclear cells (PBMCs). According to certain embodiments, the antibodies of the disclosure bind an epitope within the first immunoglobulin domain of human CD48.

[0007] The antibodies of the disclosure can be full-length (for example, an IgG1 or IgG4 antibody) or may comprise only an antigen-binding portion (for example, a Fab, $F(ab')_2$ or scFv fragment), and may be modified to affect functionality, e.g., to eliminate residual effector

functions (Reddy et al., 2000, J. Immunol. 164:1925-1933).

[0008] The present disclosure provides an antibody or antigen-binding fragment of an antibody comprising a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NO: 2, 18, 34, 50, 66, 82, 98, 114, 130, 162, 178, 192, 208, 212, 228, 232, 248, 252, 268, 272, 288, 292, 308, 312, 328, 332, 348, 352, and 368, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0009] The present disclosure also provides an antibody or antigen-binding fragment of an antibody comprising a light chain variable region (LCVR) having an amino acid sequence selected from the group consisting of SEQ ID NO: 10, 26, 42, 58, 74, 90, 106, 122, 138, 146, 154, 170, 180, 182, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 372, and 380, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0010] The present disclosure also provides an antibody or antigen-binding fragment thereof comprising a HCVR and LCVR (HCVR/LCVR) sequence pair selected from the group consisting of SEQ ID NO: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 162/170, 178/180, 162/182, 178/190, 192/200, 208/210, 212/220, 228/230, 232/240, 248/250, 252/260, 268/270, 272/280, 288/290, 292/300, 308/310, 312/320, 328/330, 332/340, 348/350, 352/360, 368/370, 352/372, and 368/380.

[0011] The present disclosure also provides an antibody or antigen-binding fragment of an antibody comprising a heavy chain CDR3 (HCDR3) domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 8, 24, 40, 56, 72, 88, 104, 120, 136, 168, 198, 218, 238, 258, 278, 298, 318, 338, and 358, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a light chain CDR3 (LCDR3) domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 16, 32, 48, 64, 80, 96, 112, 128, 144, 152, 160, 176, 188, 206, 226, 246, 266, 286, 306, 326, 346, 366, and 378, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0012] In certain embodiments, the antibody or antigen-binding portion of an antibody comprises a HCDR3/LCDR3 amino acid sequence pair selected from the group consisting of SEQ ID NO: 8/16, 24/32, 40/48, 56/64, 72/80, 88/96, 104/112, 120/128, 136/144, 168/176, 168/188, 198/206, 218/226, 238/246, 258/266, 278/286, 298/306, 318/326, 338/346, 358/366, and 358/378.

[0013] The present disclosure also provides an antibody or fragment thereof further comprising a heavy chain CDR1 (HCDR1) domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 4, 20, 36, 52, 68, 84, 100, 116, 132, 164, 194, 214, 234, 254, 274, 294, 314, 334, and 354, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a heavy chain CDR2 (HCDR2) domain

having an amino acid sequence selected from the group consisting of SEQ ID NO: 6, 22, 38, 54, 70, 86, 102, 118, 134, 166, 196, 216, 236, 256, 276, 296, 316, 336, and 356, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a light chain CDR1 (LCDR1) domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 28, 44, 60, 76, 92, 108, 124, 140, 148, 156, 172, 184, 202, 222, 242, 262, 282, 302, 322, 342, 362, and 374, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a light chain CDR2 (LCDR2) domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 14, 30, 46, 62, 78, 94, 110, 126, 142, 150, 158, 174, 186, 204, 224, 244, 264, 284, 304, 324, 344, 364, and 376, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0014] Certain non-limiting, exemplary antibodies and antigen-binding fragments of the disclosure comprise HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 domains, respectively, having the amino acid sequences selected from the group consisting of: SEQ ID NOs: 4-6-8-12-14-16 (*e.g.* H2M1707N); 20-22-24-28-30-32 (*e.g.* H2M1709N); 36-38-40-44-46-48 (*e.g.* H2M1710N); 52-54-56-60-62-64 (*e.g.* H2M1712N); 68-70-72-76-78-80 (*e.g.* H2M1713N); 84-86-88-92-94-96 (*e.g.* H2M1763N); 100-102-104-108-110-112 (*e.g.* H2M1764N); 116-118-120-124-126-128 (*e.g.* H2M1811N); 132-134-136-140-142-144 (*e.g.* H2M1766N); 164-166-168-172-174-176 (*e.g.* H4H1769N-a); 164-166-168-184-186-188 (*e.g.* H4H1769N-b); 194-196-198-202-204-206 (*e.g.* H4H1770N); 214-216-218-222-224-226 (*e.g.* H4H1771N); 234-236-238-242-244-246 (*e.g.* H4H1772N); 254-256-258-262-264-266 (*e.g.* H4H1774N); 274-276-278-282-284-286 (*e.g.* H4H1775N); 294-296-298-302-304-306 (*e.g.* H4H1778N); 314-316-318-322-324-326 (*e.g.* H4H1779N); 334-336-338-342-344-346 (*e.g.* H4H1781N); 354-356-358-362-364-366 (*e.g.* H4H1789Na and Pa); and 354-356-358-374-376-378 (*e.g.* H4H1789Nb and Pb).

[0015] The present disclosure also includes antibodies, or antigen-binding fragments thereof, which comprise HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 domains, respectively, having the following amino acid sequences: HCDR1 = GFTFSNYG (SEQ ID NO:254); HCDR2 = IWYDDSXK, wherein X is S or N (SEQ ID NO:381); HCDR3 = ARDRWTYSHXFEY, wherein X is Y or F (SEQ ID NO:382); LCDR1 = QXISSW, wherein X is D or G (SEQ ID NO:383); LCDR2 = AAS (SEQ ID NO:264); and LCDR3 = QQANSFPRT (SEQ ID NO:266). Non-limiting exemplary antibodies of the disclosure which have these sequence characteristics include H4H1774N, H4H1775N, H4H1778N, H4H1779N, and H4H1781N.

[0016] In a related embodiment, the disclosure includes an antibody or antigen-binding fragment of an antibody which specifically binds CD48, wherein the antibody or fragment comprises the heavy and light chain CDR domains contained within heavy and light chain sequences selected from the group consisting of SEQ ID NO: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 162/170, 178/180, 162/182, 178/190, 192/200, 208/210, 212/220, 228/230, 232/240, 248/250, 252/260, 268/270, 272/280, 288/290, 292/300, 308/310,

312/320, 328/330, 332/340, 348/350, 352/360, 368/370, 352/372, and 368/380. Methods and techniques for identifying CDRs within HCVR and LCVR amino acid sequences are well known in the art and can be used to identify CDRs within the specified HCVR and/or LCVR amino acid sequences disclosed herein. Exemplary conventions that can be used to identify the boundaries of CDRs include, *e.g.*, the Kabat definition, the Chothia definition, and the AbM definition. In general terms, the Kabat definition is based on sequence variability, the Chothia definition is based on the location of the structural loop regions, and the AbM definition is a compromise between the Kabat and Chothia approaches. *See, e.g.*, Kabat, "Sequences of Proteins of Immunological Interest," National Institutes of Health, Bethesda, Md. (1991); Al-Lazikani *et al.*, *J. Mol. Biol. 273*:927-948 (1997); and Martin *et al.*, *Proc. Natl. Acad. Sci. USA 86*:9268-9272 (1989). Public databases are also available for identifying CDR sequences within an antibody.

[0017] In another embodiment, the disclosure provides nucleic acid molecules encoding anti-CD48 antibodies or fragments thereof. Recombinant expression vectors carrying the nucleic acids of the disclosure, and host cells into which such vectors have been introduced, are also encompassed by the disclosure, as are methods of producing the antibodies by culturing the host cells under conditions permitting production of the antibodies, and recovering the antibodies produced.

[0018] In one embodiment, the disclosure provides an antibody or fragment thereof comprising a HCVR encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, 17, 33, 49, 65, 81, 97, 113, 129, 161, 177, 191, 207, 211, 227, 231, 247, 251, 267, 271, 287, 291, 307, 311, 327, 331, 347, 351, and 367, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof.

[0019] The present disclosure also provides an antibody or fragment thereof comprising a LCVR encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 9, 25, 41, 57, 73, 89, 105, 121, 137, 145, 153, 169, 179, 181, 189, 199, 209, 219, 229, 239, 249, 259, 269, 279, 289, 299, 309, 319, 329, 339, 349, 359, 369, 371, and 379, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof.

[0020] The present disclosure also provides an antibody or antigen-binding fragment of an antibody comprising a HCDR3 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 7, 23, 39, 55, 71, 87, 103, 119, 135, 167, 197, 217, 237, 257, 277, 297, 317, 337, and 357, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof; and a LCDR3 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 15, 31, 47, 63, 79, 95, 111, 127, 143, 151, 159, 175, 187, 205, 225, 245, 265, 285, 305, 325, 345, 365, and 377, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof.

[0021] The present disclosure also provides an antibody or fragment thereof which further comprises a HCDR1 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 3, 19, 35, 51, 67, 83, 99, 115, 131, 163, 193, 213, 233, 253, 273, 293, 313, 333, and 353 or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof; a HCDR2 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 5, 21, 37, 53, 69, 85, 101, 117, 133, 165, 195, 215, 235, 255, 275, 295, 315, 335, and 355, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof; a LCDR1 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 11, 27, 43, 59, 75, 91, 107, 123, 139, 147, 155, 171, 183, 201, 221, 241, 261, 281, 301, 321, 341, 361, and 373, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof; and a LCDR2 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 13, 29, 45, 61, 77, 93, 109, 125, 141, 149, 157, 173, 185, 203, 223, 243, 263, 283, 303, 323, 343, 363, and 375, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof.

[0022] According to certain embodiments, the antibody or fragment thereof comprises the heavy and light chain CDR sequences encoded by the nucleic acid sequences of SEQ ID NOs: SEQ ID NOs: 1 and 9 (*e.g.* H2M1707N), 17 and 25 (*e.g.* H2M1709N), 33 and 41 (*e.g.* H2M1710N), 49 and 57 (*e.g.* H2M1712N), 65 and 73 (*e.g.* H2M1713N), 81 and 89 (*e.g.* H2M1763N), 97 and 105 (*e.g.* H2M1764N), 113 and 121 (*e.g.* H2M1811N), 129 and 137 (*e.g.* H2M1766N), 161 and 169 (*e.g.* H4H1769N-a), 177 and 179 (*e.g.*, H4H1769P-a), 161 and 181 (*e.g.* H4H1769N-b), 177 and 189 (*e.g.*, H4H1769P-b), 191 and 199 (*e.g.* H4H1770N), 207 and 209 (*e.g.*, H4H1770P), 211 and 219 (*e.g.* H4H1771N), 227 and 229 (*e.g.*, H4H1771P), 231 and 239 (*e.g.* H4H1772N), 247 and 249 (*e.g.*, H4H1772P), 251 and 259 (*e.g.* H4H1774N), 267 and 269 (*e.g.*, H4H1774P), 271 and 279 (*e.g.* H4H1775N), 287 and 289 (*e.g.*, H4H1775P), 291 and 299 (*e.g.* H4H1778N), 307 and 309 (*e.g.*, H4H1778P), 311 and 319 (*e.g.* H4H1779N), 327 and 329 (*e.g.*, H4H1779P), 331 and 339 (*e.g.* H4H1781N), 347 and 349 (*e.g.*, H4H1781P), 351 and 359 (*e.g.* H4H1789Na), 367 and 369 (*e.g.*, H4H1789Pa), 351 and 371 (*e.g.* H4H1789Nb), or 367 and 379 (*e.g.*, H4H1789Pb).

[0023] The present disclosure includes anti-CD48 antibodies having a modified glycosylation pattern. In some applications, modification to remove undesirable glycosylation sites may be useful, or an antibody lacking a fucose moiety present on the oligosaccharide chain, for example, to increase antibody dependent cellular cytotoxicity (ADCC) function (see Shield et al. (2002) JBC 277:26733). In other applications, modification of galactosylation can be made in order to modify complement dependent cytotoxicity (CDC).

[0023a] In one aspect, there is provided a pharmaceutical composition comprising the antibody or antigen-binding fragment as defined herein and a pharmaceutically acceptable carrier or diluent.

[0024] In another embodiment, the disclosure provides a pharmaceutical composition comprising a recombinant human antibody or fragment thereof which specifically binds CD48 and a

pharmaceutically acceptable carrier. In a related embodiment, the disclosure features a composition which is a combination of a CD48 inhibitor and a second therapeutic agent. In one embodiment, the CD48 inhibitor is an antibody or fragment thereof. In one embodiment, the second therapeutic agent is any agent that is advantageously combined with a CD48 inhibitor. Exemplary agents that may be advantageously combined with a CD48 inhibitor include, without limitation, other agents that inhibit CD48 activity (including other antibodies or antigen-binding fragments thereof, peptide inhibitors, small molecule antagonists, etc) and/or agents which interfere with CD48 upstream or downstream signaling.

[0025] In yet another embodiment, the disclosure provides methods for inhibiting CD48 activity using an anti-CD48 antibody or antigen-binding portion of an antibody of the disclosure, wherein the therapeutic methods comprise administering a therapeutically effective amount of a pharmaceutical composition comprising an antibody or antigen-binding fragment of an antibody of the disclosure. The disorder treated is any disease or condition which is improved, ameliorated, inhibited or prevented by removal, inhibition or reduction of CD48 activity. The anti-CD48 antibody or antibody fragment of the disclosure may function to block the interaction between CD48 and a CD48 receptor (*e.g.*, 2B4 and/or CD2), or otherwise inhibit the signaling activity of CD48.

[0025a] In one aspect, there is provided the pharmaceutical composition as defined herein, for use in treating a patient afflicted with, diagnosed with, or at risk of being afflicted with a disease or disorder that is treatable by blocking the interaction between CD48 and a CD48 receptor.

[0026] The present disclosure also includes the use of an anti-CD48 antibody or antigen binding portion of an antibody of the disclosure in the manufacture of a medicament for the treatment of a disease or disorder related to or caused by CD48 activity in a patient.

[0026a] In one aspect, there is provided the use of the isolated antibody or antigen-binding fragment as defined herein, or the pharmaceutical composition as defined herein, in the manufacture of a medicament for use in treating a patient afflicted with, diagnosed with, or at risk of being afflicted with a disease or disorder that is treatable by blocking the interaction between CD48 and a CD48 receptor.

[0027] Other embodiments will become apparent from a review of the ensuing detailed description.

BRIEF DESCRIPTION OF THE FIGURES

[0028] **Fig. 1.** Linear depiction of human CD48 polypeptide (SEQ ID NO:384). The portion represented by amino acids 1-26 is the signal sequence; The portion represented by amino acids 29-127 is Ig Domain 1; The portion represented by amino acids 132-212 is Ig Domain 2. The regions of SEQ ID NO:384 corresponding to amino acids 60-68 (YTFDQKIVE) and 107-125 (YIMRVLKKTGNEQEWKIKL) represent the epitope determined for the exemplary antibody designated H4H1789Pa (see Example 4).

[0029] **Fig. 2.** Graphical representations of the effects of anti-CD48 antibody treatment in a mouse model of graft-versus-host disease. (see Example 9). Panel A shows the weight loss observed in treated and control animals; Panel B shows the percent survival of treated and control animals. For both Panels (●) indicates no treatment; (▲) indicates hIgG4 isotype control treatment; and (■) indicates treatment with the exemplary anti-CD48 antibody H4H1789Pa.

DETAILED DESCRIPTION

[0030] Before the present invention is described, it is to be understood that this invention is

not limited to particular methods and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0031] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. As used herein, the term "about," when used in reference to a particular recited numerical value, means that the value may vary from the recited value by no more than 1%. For example, as used herein, the expression "about 100" includes 99 and 101 and all values in between (*e.g.*, 99.1, 99.2, 99.3, 99.4, etc.).

[0032] Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described.

Definitions

[0033] The expressions "CD48" and "CD48 fragment," as used herein refer to the human CD48 protein or fragment unless specified as being from a non-human species (*e.g.*, "mouse CD48," "mouse CD48 fragment," "monkey CD48," "monkey CD48 fragment," etc.). Human CD48 has the amino acid sequence of SEQ ID NO:384. Amino acid sequences of CD48 molecules from non-human species (*e.g.*, mouse, monkey, rabbit, dog, pig, etc.) are available from public sources such as GenBank (*e.g.*, GenBank accession numbers BAE96326.1 (mouse); DAA31966.1 (bovine); EDL94663.1 (rat); etc).

[0034] The term "CD48 receptor," as used herein, means a protein with which the human CD48 protein interacts to transmit a biological signal *in vivo*. The term "CD48 receptor" includes human 2B4 and human CD2. The term "human 2B4," as used herein, means a protein comprising the amino acid sequence of SEQ ID NO:390, or a portion thereof which is capable of interacting with CD48. The term "human CD2," as used herein, means a protein comprising the amino acid sequence of SEQ ID NO:392, or a portion thereof which is capable of interacting with CD48.

[0035] The term "antibody", as used herein, is intended to refer to immunoglobulin molecules comprising four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, as well as multimers thereof (*e.g.*, IgM). Each heavy chain comprises a heavy chain variable region (abbreviated herein as HCVR or V_H) and a heavy chain constant region. The heavy chain constant region comprises three domains, C_H1, C_H2 and C_H3. Each light chain comprises a light chain variable region (abbreviated herein as LCVR or V_L) and a light chain constant region. The light chain constant region comprises one domain (C_L1). The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and

four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. In different embodiments of the invention, the FRs of the anti-CD48 antibody (or antigen-binding portion thereof) may be identical to the human germline sequences, or may be naturally or artificially modified. An amino acid consensus sequence may be defined based on a side-by-side analysis of two or more CDRs.

[0036] The term "antibody," as used herein, also includes antigen-binding fragments of full antibody molecules. The terms "antigen-binding portion" of an antibody, "antigen-binding fragment" of an antibody, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. Antigen-binding fragments of an antibody may be derived, *e.g.*, from full antibody molecules using any suitable standard techniques such as proteolytic digestion or recombinant genetic engineering techniques involving the manipulation and expression of DNA encoding antibody variable and optionally constant domains. Such DNA is known and/or is readily available from, *e.g.*, commercial sources, DNA libraries (including, *e.g.*, phage-antibody libraries), or can be synthesized. The DNA may be sequenced and manipulated chemically or by using molecular biology techniques, for example, to arrange one or more variable and/or constant domains into a suitable configuration, or to introduce codons, create cysteine residues, modify, add or delete amino acids, etc.

[0037] Non-limiting examples of antigen-binding fragments include: (i) Fab fragments; (ii) F(ab')2 fragments; (iii) Fd fragments; (iv) Fv fragments; (v) single-chain Fv (scFv) molecules; (vi) dAb fragments; and (vii) minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (*e.g.*, an isolated complementarity determining region (CDR) such as a CDR3 peptide), or a constrained FR3-CDR3-FR4 peptide. Other engineered molecules, such as domain-specific antibodies, single domain antibodies, domain-deleted antibodies, chimeric antibodies, CDR-grafted antibodies, diabodies, triabodies, tetrabodies, minibodies, nanobodies (*e.g.* monovalent nanobodies, bivalent nanobodies, etc.), small modular immunopharmaceuticals (SMIPs), and shark variable IgNAR domains, are also encompassed within the expression "antigen-binding fragment," as used herein.

[0038] An antigen-binding fragment of an antibody will typically comprise at least one variable domain. The variable domain may be of any size or amino acid composition and will generally comprise at least one CDR which is adjacent to or in frame with one or more framework sequences. In antigen-binding fragments having a V_H domain associated with a V_L domain, the V_H and V_L domains may be situated relative to one another in any suitable arrangement. For example, the variable region may be dimeric and contain V_H-V_H, V_H-V_L or V_L-V_L dimers. Alternatively, the antigen-binding fragment of an antibody may contain a monomeric V_H or V_L domain.

[0039] In certain embodiments, an antigen-binding fragment of an antibody may contain at least one variable domain covalently linked to at least one constant domain. Non-limiting,

exemplary configurations of variable and constant domains that may be found within an antigen-binding fragment of an antibody of the present invention include: (i) V_H-C_H1; (ii) V_H-C_H2; (iii) V_H-C_H3; (iv) V_H-C_H1-C_H2; (v) V_H-C_H1-C_H2-C_H3; (vi) V_H-C_H2-C_H3; (vii) V_H-C_L; (viii) V_L-C_H1; (ix) V_L-C_H2; (x) V_L-C_H3; (xi) V_L-C_H1-C_H2; (xii) V_L-C_H1-C_H2-C_H3; (xiii) V_L-C_H2-C_H3; and (xiv) V_L-C_L. In any configuration of variable and constant domains, including any of the exemplary configurations listed above, the variable and constant domains may be either directly linked to one another or may be linked by a full or partial hinge or linker region. A hinge region may consist of at least 2 (*e.g.*, 5, 10, 15, 20, 40, 60 or more) amino acids which result in a flexible or semi-flexible linkage between adjacent variable and/or constant domains in a single polypeptide molecule. Moreover, an antigen-binding fragment of an antibody of the present invention may comprise a homo-dimer or hetero-dimer (or other multimer) of any of the variable and constant domain configurations listed above in non-covalent association with one another and/or with one or more monomeric V_H or V_L domain (*e.g.*, by disulfide bond(s)).

[0040] As with full antibody molecules, antigen-binding fragments may be monospecific or multispecific (*e.g.*, bispecific). A multispecific antigen-binding fragment of an antibody will typically comprise at least two different variable domains, wherein each variable domain is capable of specifically binding to a separate antigen or to a different epitope on the same antigen. Any multispecific antibody format, including the exemplary bispecific antibody formats disclosed herein, may be adapted for use in the context of an antigen-binding fragment of an antibody of the present invention using routine techniques available in the art.

[0041] The antibodies of the present invention may function through complement-dependent cytotoxicity (CDC) or antibody-dependent cell-mediated cytotoxicity (ADCC). "Complement-dependent cytotoxicity" (CDC) refers to lysis of antigen-expressing cells by an antibody of the invention in the presence of complement. "Antibody-dependent cell-mediated cytotoxicity" (ADCC) refers to a cell-mediated reaction in which nonspecific cytotoxic cells that express Fc receptors (FcRs) (*e.g.*, Natural Killer (NK) cells, neutrophils, and macrophages) recognize bound antibody on a target cell and thereby lead to lysis of the target cell. CDC and ADCC can be measured using assays that are well known and available in the art. (*See, e.g.*, U.S. 5,500,362 and 5,821,337, and Clynes *et al.* (1998) Proc. Natl. Acad. Sci. (USA) 95:652-656). The constant region of an antibody is important in the ability of an antibody to fix complement and mediate cell-dependent cytotoxicity. Thus, the isotype of an antibody may be selected on the basis of whether it is desirable for the antibody to mediate cytotoxicity.

[0042] The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs and in particular CDR3. However, the term "human antibody", as used herein, is not intended to include

antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

[0043] The term "recombinant human antibody", as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell (described further below), antibodies isolated from a recombinant, combinatorial human antibody library (described further below), antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes (see e.g., Taylor et al. (1992) Nucl. Acids Res. 20:6287-6295) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies are subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the V_H and V_L regions of the recombinant antibodies are sequences that, while derived from and related to human germline V_H and V_L sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

[0044] Human antibodies can exist in two forms that are associated with hinge heterogeneity. In one form, an immunoglobulin molecule comprises a stable four chain construct of approximately 150-160 kDa in which the dimers are held together by an interchain heavy chain disulfide bond. In a second form, the dimers are not linked via inter-chain disulfide bonds and a molecule of about 75-80 kDa is formed composed of a covalently coupled light and heavy chain (half-antibody). These forms have been extremely difficult to separate, even after affinity purification.

[0045] The frequency of appearance of the second form in various intact IgG isotypes is due to, but not limited to, structural differences associated with the hinge region isotype of the antibody. A single amino acid substitution in the hinge region of the human IgG4 hinge can significantly reduce the appearance of the second form (Angal et al. (1993) Molecular Immunology 30:105) to levels typically observed using a human IgG1 hinge. The instant invention encompasses antibodies having one or more mutations in the hinge, C_H2 or C_H3 region which may be desirable, for example, in production, to improve the yield of the desired antibody form.

[0046] An "isolated antibody," as used herein, means an antibody that has been identified and separated and/or recovered from at least one component of its natural environment. For example, an antibody that has been separated or removed from at least one component of an organism, or from a tissue or cell in which the antibody naturally exists or is naturally produced, is an "isolated antibody" for purposes of the present invention. An isolated antibody also includes an antibody *in situ* within a recombinant cell. Isolated antibodies are antibodies that

have been subjected to at least one purification or isolation step. According to certain embodiments, an isolated antibody may be substantially free of other cellular material and/or chemicals.

[0047] The term "specifically binds," or the like, means that an antibody or antigen-binding fragment thereof forms a complex with an antigen that is relatively stable under physiologic conditions. Methods for determining whether an antibody specifically binds to an antigen are well known in the art and include, for example, equilibrium dialysis, surface plasmon resonance, and the like. For example, an antibody that "specifically binds" human CD48, as used in the context of the present invention, includes antibodies that bind human CD48 or portion thereof with a K_D of less than about 1000 nM, less than about 500 nM, less than about 300 nM, less than about 200 nM, less than about 100 nM, less than about 90 nM, less than about 80 nM, less than about 70 nM, less than about 60 nM, less than about 50 nM, less than about 40 nM, less than about 30 nM, less than about 20 nM, less than about 10 nM, less than about 5 nM, less than about 4 nM, less than about 3 nM, less than about 2 nM, less than about 1 nM or less than about 0.5 nM, as measured in a surface plasmon resonance assay. (See, *e.g.*, Example 3, herein). An isolated antibody that specifically binds human CD48 may, however, have cross-reactivity to other antigens, such as CD48 molecules from other (non-human) species.

[0048] A "neutralizing" or "blocking" antibody, as used herein, is intended to refer to an antibody whose binding to CD48: (i) interferes with the interaction between CD48 or a CD48 fragment and a CD48 receptor (*e.g.*, 2B4 and/or CD2), and/or (ii) results in inhibition of at least one biological function of CD48. The inhibition caused by a CD48 neutralizing or blocking antibody need not be complete so long as it is detectable using an appropriate assay. Exemplary assays for detecting CD48 inhibition are described herein.

[0049] The anti-CD48 antibodies disclosed herein may comprise one or more amino acid substitutions, insertions and/or deletions in the framework and/or CDR regions of the heavy and light chain variable domains as compared to the corresponding germline sequences from which the antibodies were derived. Such mutations can be readily ascertained by comparing the amino acid sequences disclosed herein to germline sequences available from, for example, public antibody sequence databases. The present invention includes antibodies, and antigen-binding fragments thereof, which are derived from any of the amino acid sequences disclosed herein, wherein one or more amino acids within one or more framework and/or CDR regions are mutated to the corresponding residue(s) of the germline sequence from which the antibody was derived, or to the corresponding residue(s) of another human germline sequence, or to a conservative amino acid substitution of the corresponding germline residue(s) (such sequence changes are referred to herein collectively as "germline mutations"). A person of ordinary skill in the art, starting with the heavy and light chain variable region sequences disclosed herein, can easily produce numerous antibodies and antigen-binding fragments which comprise one or more individual germline mutations or combinations thereof. In certain embodiments, all of the

framework and/or CDR residues within the V_H and/or V_L domains are mutated back to the residues found in the original germline sequence from which the antibody was derived. In other embodiments, only certain residues are mutated back to the original germline sequence, *e.g.*, only the mutated residues found within the first 8 amino acids of FR1 or within the last 8 amino acids of FR4, or only the mutated residues found within CDR1, CDR2 or CDR3. In other embodiments, one or more of the framework and/or CDR residue(s) are mutated to the corresponding residue(s) of a different germline sequence (*i.e.*, a germline sequence that is different from the germline sequence from which the antibody was originally derived). Furthermore, the antibodies of the present invention may contain any combination of two or more germline mutations within the framework and/or CDR regions, *e.g.*, wherein certain individual residues are mutated to the corresponding residue of a particular germline sequence while certain other residues that differ from the original germline sequence are maintained or are mutated to the corresponding residue of a different germline sequence. Once obtained, antibodies and antigen-binding fragments that contain one or more germline mutations can be easily tested for one or more desired property such as, improved binding specificity, increased binding affinity, improved or enhanced antagonistic or agonistic biological properties (as the case may be), reduced immunogenicity, etc. Antibodies and antigen-binding fragments obtained in this general manner are encompassed within the present invention.

[0050] The present invention also includes anti-CD48 antibodies comprising variants of any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein having one or more conservative substitutions. For example, the present invention includes anti-CD48 antibodies having HCVR, LCVR, and/or CDR amino acid sequences with, *e.g.*, 10 or fewer, 8 or fewer, 6 or fewer, 4 or fewer, etc. conservative amino acid substitutions relative to any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein.

[0051] The term "surface plasmon resonance", as used herein, refers to an optical phenomenon that allows for the analysis of real-time interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIAcore™ system (Biacore Life Sciences division of GE Healthcare, Piscataway, NJ).

[0052] The term "K_D", as used herein, is intended to refer to the equilibrium dissociation constant of a particular antibody-antigen interaction.

[0053] The term "epitope" refers to an antigenic determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope. A single antigen may have more than one epitope. Thus, different antibodies may bind to different areas on an antigen and may have different biological effects. Epitopes may be either conformational or linear. A conformational epitope is produced by spatially juxtaposed amino acids from different segments of the linear polypeptide chain. A linear epitope is one produced by adjacent amino acid residues in a polypeptide chain. In certain circumstance, an epitope may include moieties of saccharides, phosphoryl groups, or sulfonyl groups on the antigen.

[0054] The term "substantial identity" or "substantially identical," when referring to a nucleic acid or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 95%, and more preferably at least about 96%, 97%, 98% or 99% of the nucleotide bases, as measured by any well-known algorithm of sequence identity, such as FASTA, BLAST or Gap, as discussed below. A nucleic acid molecule having substantial identity to a reference nucleic acid molecule may, in certain instances, encode a polypeptide having the same or substantially similar amino acid sequence as the polypeptide encoded by the reference nucleic acid molecule.

[0055] As applied to polypeptides, the term "substantial similarity" or "substantially similar" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 95% sequence identity, even more preferably at least 98% or 99% sequence identity. Preferably, residue positions which are not identical differ by conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well-known to those of skill in the art. *See, e.g.*, Pearson (1994) Methods Mol. Biol. 24: 307-331. Examples of groups of amino acids that have side chains with similar chemical properties include (1) aliphatic side chains: glycine, alanine, valine, leucine and isoleucine; (2) aliphatic-hydroxyl side chains: serine and threonine; (3) amide-containing side chains: asparagine and glutamine; (4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; (5) basic side chains: lysine, arginine, and histidine; (6) acidic side chains: aspartate and glutamate, and (7) sulfur-containing side chains are cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine. Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet *et al.* (1992) Science 256: 1443-1445. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[0056] Sequence similarity for polypeptides, which is also referred to as sequence identity, is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG software contains programs such as Gap and Bestfit which can be used with default parameters

to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. *See, e.g.*, GCG Version 6.1. Polypeptide sequences also can be compared using FASTA using default or recommended parameters, a program in GCG Version 6.1. FASTA (e.g., FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson (2000) supra). Another preferred algorithm when comparing a sequence of the invention to a database containing a large number of sequences from different organisms is the computer program BLAST, especially BLASTP or TBLASTN, using default parameters. *See, e.g.*, Altschul *et al.* (1990) J. Mol. Biol. 215:403-410 and Altschul *et al.* (1997) Nucleic Acids Res. 25:3389-402.

Biological Characteristics of the Antibodies

[0057] The antibodies of the present invention block the interaction between human CD48 and at least one CD48 receptor. As used herein, the expression "blocks the interaction between human CD48 and at least one CD48 receptor" means that, in an assay in which the physical interaction between CD48 and a CD48 receptor (*e.g.*, human 2B4 and/or human CD2) can be detected and/or quantified, the addition of an antibody of the invention reduces the interaction between CD48 and the receptor by at least 50%. A non-limiting, exemplary assay that can be used to determine if an antibody blocks the interaction between human CD48 and a CD48 receptor (*e.g.*, human 2B4) is illustrated in Example 5, herein. In this Example, antibodies are mixed with CD48 protein, and then the antibody/CD48 mixture is applied to a surface coated with 2B4 protein. After washing away unbound molecules, the amount of CD48 bound to the 2B4-coated surface is measured. By using varying amounts of antibody in this assay format, the amount of antibody required to block 50% of CD48 binding to 2B4 can be calculated and expressed as an IC_{50} value. The present invention includes anti-CD48 antibodies that exhibit an IC_{50} of less than about 400 pM when tested in a CD48/CD48 receptor binding assay as described above, or a substantially similar assay. For example, the invention includes anti-CD48 antibodies that exhibit an IC_{50} of less than about 400, 300, 290, 280, 270, 260, 250, 240, 230, 220, 210, 200, 190, 180, 170, 160, 150, 140, 130, 120, 110, 100, 90, 80, 70, 60, 50, 40, 30, 20, 10, 18, 16, 14, 12, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 pM when tested in a CD48/CD48 receptor binding assay as described above, or a substantially similar assay.

[0058] The antibodies of the present invention are also able to inhibit activation of primary human peripheral blood mononuclear cells (PBMCs). As used herein, the expression "inhibits activation of primary human peripheral blood mononuclear cells" means that, in an assay in which the activation of PBMCs can be detected and/or quantified (*e.g.*, by measuring release of IFN-gamma or other cytokine), the addition of an antibody of the invention reduces the amount of IFN-gamma (or other cytokine) that is released by at least 50%. Non-limiting, exemplary assays that can be used to determine if an antibody inhibits activation of PBMCs are illustrated

in Examples 6 and 7, herein. In one example, anti-CD48 antibody is added to isolated human PBMCs prior to activation of the PBMCs with agonist anti-CD3 and anti-CD28 antibodies. After a period of incubation, the amount of IFN-gamma released is measured. By using varying amounts of antibody in this assay format, the amount of antibody required to inhibit 50% of maximal IFN-gamma release can be calculated and expressed as an IC_{50} value. The present invention includes anti-CD48 antibodies that exhibit an IC_{50} of less than about 500 pM when tested in a PBMC activation assay as described above, or a substantially similar assay. For example, the invention includes anti-CD48 antibodies that exhibit an IC_{50} of less than about 500, 400, 300, 290, 280, 270, 260, 250, 240, 230, 220, 210, 200, 190, 180, 170, 160, 150, 140, 130, 120, 110, 100, 90, 80, 70, 60, 50, 40, 30, 20, 10, 18, 16, 14, 12, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 pM when tested in a PBMC activation assay as described above, or a substantially similar assay.

Epitope Mapping and Related Technologies

[0059] The human CD48 protein contains two immunoglobulin-like domains, referred to herein as "Ig domain 1" and "Ig domain 2". Ig domain 1 is the sequence of amino acids represented by amino acids 29 through 127 of SEQ ID NO:384, and Ig domain 2 is the sequence of amino acids represented by amino acids 132 through 212 of SEQ ID NO:384 (see Figure 1).

[0060] The present invention includes anti-CD48 antibodies which bind specifically to an epitope within Ig domain 1 of human CD48. The epitope may consist of a single contiguous sequence of 3 or more (*e.g.*, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more) amino acids located within Ig domain 1 of CD48. Alternatively, the epitope may consist of a plurality of non-contiguous amino acids (or amino acid sequences) located within Ig domain 1 of CD48. According to certain embodiments of the present invention, anti-CD48 antibodies are provided which interact with one or more amino acids located within amino acids 60 to 125 of SEQ ID NO:384. For example, the present invention includes anti-CD48 antibodies which interact with one or more amino acids located within amino acids 60 to 68 and/or 107 to 125 of SEQ ID NO:384.

[0061] Various techniques known to persons of ordinary skill in the art can be used to determine whether an antibody "interacts with one or more amino acids" within a polypeptide or protein. Exemplary techniques include, *e.g.*, routine cross-blocking assay such as that described Antibodies, Harlow and Lane (Cold Spring Harbor Press, Cold Spring Harb., NY), alanine scanning mutational analysis, peptide blots analysis (Reineke, 2004, Methods Mol Biol 248:443-463), and peptide cleavage analysis. In addition, methods such as epitope excision, epitope extraction and chemical modification of antigens can be employed (Tomer, 2000, Protein Science 9:487-496). Another method that can be used to identify the amino acids within a polypeptide with which an antibody interacts is hydrogen/deuterium exchange detected by mass spectrometry. (See, *e.g.*, Example 4 herein). In general terms, the hydrogen/deuterium exchange method involves deuterium-labeling the protein of interest, followed by binding the antibody to the deuterium-labeled protein. Next, the protein/antibody complex is transferred to

water to allow hydrogen-deuterium exchange to occur at all residues except for the residues protected by the antibody (which remain deuterium-labeled). After dissociation of the antibody, the target protein is subjected to protease cleavage and mass spectrometry analysis, thereby revealing the deuterium-labeled residues which correspond to the specific amino acids with which the antibody interacts. *See, e.g.*, Ehring (1999) *Analytical Biochemistry* 267(2):252-259; Engen and Smith (2001) *Anal. Chem. 73*:256A-265A.

[0062] The present invention further includes anti-CD48 antibodies that bind to the same epitope as any of the specific exemplary antibodies described herein (*e.g.*, H4H1789Pa, H4H1763N, H2M1707N, H2M1709N, H4H1770N, H4H1771N, etc.). Likewise, the present invention also includes anti-CD48 antibodies that compete for binding to CD48 or a CD48 fragment with any of the specific exemplary antibodies described herein (*e.g.*, H4H1789Pa, H4H1763N, H2M1707N, H2M1709N, H4H1770N, H4H1771N, etc.).

[0063] One can easily determine whether an antibody binds to the same epitope as, or competes for binding with, a reference anti-CD48 antibody by using routine methods known in the art. For example, to determine if a test antibody binds to the same epitope as a reference anti-CD48 antibody of the invention, the reference antibody is allowed to bind to a CD48 protein or peptide under saturating conditions. Next, the ability of a test antibody to bind to the CD48 molecule is assessed. If the test antibody is able to bind to CD48 following saturation binding with the reference anti-CD48 antibody, it can be concluded that the test antibody binds to a different epitope than the reference anti-CD48 antibody. On the other hand, if the test antibody is not able to bind to the CD48 molecule following saturation binding with the reference anti-CD48 antibody, then the test antibody may bind to the same epitope as the epitope bound by the reference anti-CD48 antibody of the invention. Additional routine experimentation (*e.g.*, peptide mutation and binding analyses) can then be carried out to confirm whether the observed lack of binding of the test antibody is in fact due to binding to the same epitope as the reference antibody or if steric blocking (or another phenomenon) is responsible for the lack of observed binding. Experiments of this sort can be performed using ELISA, RIA, Biacore, flow cytometry or any other quantitative or qualitative antibody-binding assay available in the art. In accordance with certain embodiments of the present invention, two antibodies bind to the same (or overlapping) epitope if, *e.g.*, a 1-, 5-, 10-, 20- or 100-fold excess of one antibody inhibits binding of the other by at least 50% but preferably 75%, 90% or even 99% as measured in a competitive binding assay (see, e.g., Junghans et al., Cancer Res. 1990:50:1495-1502). Alternatively, two antibodies are deemed to bind to the same epitope if essentially all amino acid mutations in the antigen that reduce or eliminate binding of one antibody reduce or eliminate binding of the other. Two antibodies are deemed to have "overlapping epitopes" if only a subset of the amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

[0064] To determine if an antibody competes for binding with a reference anti-CD48 antibody,

the above-described binding methodology is performed in two orientations: In a first orientation, the reference antibody is allowed to bind to a CD48 molecule under saturating conditions followed by assessment of binding of the test antibody to the CD48 molecule. In a second orientation, the test antibody is allowed to bind to a CD48 molecule under saturating conditions followed by assessment of binding of the reference antibody to the CD48 molecule. If, in both orientations, only the first (saturating) antibody is capable of binding to the CD48 molecule, then it is concluded that the test antibody and the reference antibody compete for binding to CD48. As will be appreciated by a person of ordinary skill in the art, an antibody that competes for binding with a reference antibody may not necessarily bind to the same epitope as the reference antibody, but may sterically block binding of the reference antibody by binding an overlapping or adjacent epitope.

Preparation of Human Antibodies

[0065] Methods for generating monoclonal antibodies, including fully human monoclonal antibodies are known in the art. Any such known methods can be used in the context of the present invention to make human antibodies that specifically bind to human CD48.

[0066] Using VELOCIMMUNE™ technology or any other known method for generating monoclonal antibodies, high affinity chimeric antibodies to CD48 are initially isolated having a human variable region and a mouse constant region. As in the experimental section below, the antibodies are characterized and selected for desirable characteristics, including affinity, selectivity, epitope, etc. The mouse constant regions are replaced with a desired human constant region to generate the fully human antibody of the invention, for example wild-type or modified IgG1 or IgG4. While the constant region selected may vary according to specific use, high affinity antigen-binding and target specificity characteristics reside in the variable region.

Bioequivalents

[0067] The anti-CD48 antibodies and antibody fragments of the present invention encompass proteins having amino acid sequences that vary from those of the described antibodies but that retain the ability to bind human CD48. Such variant antibodies and antibody fragments comprise one or more additions, deletions, or substitutions of amino acids when compared to parent sequence, but exhibit biological activity that is essentially equivalent to that of the described antibodies. Likewise, the anti-CD48 antibody-encoding DNA sequences of the present invention encompass sequences that comprise one or more additions, deletions, or substitutions of nucleotides when compared to the disclosed sequence, but that encode an anti-CD48 antibody or antibody fragment that is essentially bioequivalent to an anti-CD48 antibody or antibody fragment of the invention. Examples of such variant amino acid and DNA sequences are discussed above.

[0068] Two antigen-binding proteins, or antibodies, are considered bioequivalent if, for example, they are pharmaceutical equivalents or pharmaceutical alternatives whose rate and

extent of absorption do not show a significant difference when administered at the same molar dose under similar experimental conditions, either single does or multiple dose. Some antibodies will be considered equivalents or pharmaceutical alternatives if they are equivalent in the extent of their absorption but not in their rate of absorption and yet may be considered bioequivalent because such differences in the rate of absorption are intentional and are reflected in the labeling, are not essential to the attainment of effective body drug concentrations on, e.g., chronic use, and are considered medically insignificant for the particular drug product studied.

[0069] In one embodiment, two antigen-binding proteins are bioequivalent if there are no clinically meaningful differences in their safety, purity, and potency.

[0070] In one embodiment, two antigen-binding proteins are bioequivalent if a patient can be switched one or more times between the reference product and the biological product without an expected increase in the risk of adverse effects, including a clinically significant change in immunogenicity, or diminished effectiveness, as compared to continued therapy without such switching.

[0071] In one embodiment, two antigen-binding proteins are bioequivalent if they both act by a common mechanism or mechanisms of action for the condition or conditions of use, to the extent that such mechanisms are known.

[0072] Bioequivalence may be demonstrated by in vivo and in vitro methods. Bioequivalence measures include, e.g., (a) an in vivo test in humans or other mammals, in which the concentration of the antibody or its metabolites is measured in blood, plasma, serum, or other biological fluid as a function of time; (b) an in vitro test that has been correlated with and is reasonably predictive of human in vivo bioavailability data; (c) an in vivo test in humans or other mammals in which the appropriate acute pharmacological effect of the antibody (or its target) is measured as a function of time; and (d) in a well-controlled clinical trial that establishes safety, efficacy, or bioavailability or bioequivalence of an antibody.

[0073] Bioequivalent variants of anti-CD48 antibodies of the invention may be constructed by, for example, making various substitutions of residues or sequences or deleting terminal or internal residues or sequences not needed for biological activity. For example, cysteine residues not essential for biological activity can be deleted or replaced with other amino acids to prevent formation of unnecessary or incorrect intramolecular disulfide bridges upon renaturation. In other contexts, bioequivalent antibodies may include anti-CD48 antibody variants comprising amino acid changes which modify the glycosylation characteristics of the antibodies, *e.g.*, mutations which eliminate or remove glycosylation.

Species Selectivity and Species Cross-Reactivity

[0074] According to certain embodiments of the invention, the anti-CD48 antibodies bind to human CD48 but not to CD48 from other species. The present invention also includes anti-CD48 antibodies that bind to human CD48 and to CD48 from one or more non-human species.

For example, the anti-CD48 antibodies of the invention may bind to human CD48 and may bind or not bind, as the case may be, to one or more of mouse, rat, guinea pig, hamster, gerbil, pig, cat, dog, rabbit, goat, sheep, cow, horse, camel, cynomologous, marmoset, rhesus or chimpanzee CD48.

Immunoconjugates

[0075] The invention encompasses anti-CD48 monoclonal antibodies conjugated to a therapeutic moiety ("immunoconjugate"), such as a cytotoxin, a chemotherapeutic drug, an immunosuppressant or a radioisotope. Cytotoxic agents include any agent that is detrimental to cells. Examples of suitable cytotoxic agents and chemotherapeutic agents for forming immunoconjugates are known in the art, (see for example, WO 05/103081).

Multispecific Antibodies

[0076] The antibodies of the present invention may be monospecific, bi-specific, or multispecific. Multispecific antibodies may be specific for different epitopes of one target polypeptide or may contain antigen-binding domains specific for more than one target polypeptide. See, e.g., Tutt et al., 1991, J. Immunol. 147:60-69; Kufer *et al.*, 2004, Trends Biotechnol. 22:238-244. The anti-CD48 antibodies of the present invention can be linked to or co-expressed with another functional molecule, e.g., another peptide or protein. For example, an antibody or fragment thereof can be functionally linked (e.g., by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody or antibody fragment to produce a bi-specific or a multispecific antibody with a second binding specificity. For example, the present invention includes bi-specific antibodies wherein one arm of an immunoglobulin is specific for human CD48 or a fragment thereof, and the other arm of the immunoglobulin is specific for a second therapeutic target or is conjugated to a therapeutic moiety such as a trypsin inhibitor.

[0077] An exemplary bi-specific antibody format that can be used in the context of the present invention involves the use of a first immunoglobulin (Ig) C_H3 domain and a second Ig C_H3 domain, wherein the first and second Ig C_H3 domains differ from one another by at least one amino acid, and wherein at least one amino acid difference reduces binding of the bispecific antibody to Protein A as compared to a bi-specific antibody lacking the amino acid difference. In one embodiment, the first Ig C_H3 domain binds Protein A and the second Ig C_H3 domain contains a mutation that reduces or abolishes Protein A binding such as an H95R modification (by IMGT exon numbering; H435R by EU numbering). The second C_H3 may further comprise a Y96F modification (by IMGT; Y436F by EU). Further modifications that may be found within the second C_H3 include: D16E, L18M, N44S, K52N, V57M, and V82I (by IMGT; D356E, L358M, N384S, K392N, V397M, and V422I by EU) in the case of IgG1 antibodies; N44S, K52N, and V82I (IMGT; N384S, K392N, and V422I by EU) in the case of IgG2 antibodies; and Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (by IMGT; Q355R, N384S, K392N, V397M, R409K,

E419Q, and V422I by EU) in the case of IgG4 antibodies. Variations on the bi-specific antibody format described above are contemplated within the scope of the present invention.

Therapeutic Formulation and Administration

[0078] The invention provides pharmaceutical compositions comprising the anti-CD48 antibodies or antigen-binding fragments thereof of the present invention. The pharmaceutical compositions of the invention are formulated with suitable carriers, excipients, and other agents that provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. See also Powell et al. "Compendium of excipients for parenteral formulations" PDA (1998) J Pharm Sci Technol 52:238-311.

[0079] The dose of antibody administered to a patient may vary depending upon the age and the size of the patient, target disease, conditions, route of administration, and the like. The preferred dose is typically calculated according to body weight or body surface area. When an antibody of the present invention is used for treating a condition or disease associated with CD48 activity in an adult patient, it may be advantageous to intravenously administer the antibody of the present invention normally at a single dose of about 0.01 to about 20 mg/kg body weight, more preferably about 0.02 to about 7, about 0.03 to about 5, or about 0.05 to about 3 mg/kg body weight. Depending on the severity of the condition, the frequency and the duration of the treatment can be adjusted. Effective dosages and schedules for administering CD48 antibodies may be determined empirically; for example, patient progress can be monitored by periodic assessment, and the dose adjusted accordingly. Moreover, interspecies scaling of dosages can be performed using well-known methods in the art (*e.g.*, Mordenti *et al.*, 1991, *Pharmaceut. Res. 8*:1351).

[0080] Various delivery systems are known and can be used to administer the pharmaceutical composition of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the mutant viruses, receptor mediated endocytosis (see, e.g., Wu et al., 1987, J. Biol. Chem. 262:4429-4432). Methods of introduction include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The composition may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local.

[0081] A pharmaceutical composition of the present invention can be delivered subcutaneously or intravenously with a standard needle and syringe. In addition, with respect to subcutaneous delivery, a pen delivery device readily has applications in delivering a pharmaceutical composition of the present invention. Such a pen delivery device can be reusable or disposable. A reusable pen delivery device generally utilizes a replaceable cartridge that contains a pharmaceutical composition. Once all of the pharmaceutical composition within the cartridge has been administered and the cartridge is empty, the empty cartridge can readily be discarded and replaced with a new cartridge that contains the pharmaceutical composition. The pen delivery device can then be reused. In a disposable pen delivery device, there is no replaceable cartridge. Rather, the disposable pen delivery device comes prefilled with the pharmaceutical composition held in a reservoir within the device. Once the reservoir is emptied of the pharmaceutical composition, the entire device is discarded.

[0082] Numerous reusable pen and autoinjector delivery devices have applications in the subcutaneous delivery of a pharmaceutical composition of the present invention. Examples include, but are not limited to AUTOPEN™ (Owen Mumford, Inc., Woodstock, UK), DISETRONIC™ pen (Disetronic Medical Systems, Bergdorf, Switzerland), HUMALOG MIX 75/25™ pen, HUMALOG™ pen, HUMALIN 70/30™ pen (Eli Lilly and Co., Indianapolis, IN), NOVOPEN™ I, II and III (Novo Nordisk, Copenhagen, Denmark), NOVOPEN JUNIOR™ (Novo Nordisk, Copenhagen, Denmark), BD™ pen (Becton Dickinson, Franklin Lakes, NJ), OPTIPEN™, OPTIPEN PRO™, OPTIPEN STARLET™, and OPTICLIK™ (sanofi-aventis, Frankfurt, Germany), to name only a few. Examples of disposable pen delivery devices having applications in subcutaneous delivery of a pharmaceutical composition of the present invention include, but are not limited to the SOLOSTAR™ pen (sanofi-aventis), the FLEXPEN™ (Novo Nordisk), and the KWIKPEN™ (Eli Lilly), the SURECLICK™ Autoinjector (Amgen, Thousand Oaks, CA), the PENLET™ (Haselmeier, Stuttgart, Germany), the EPIPEN (Dey, L.P.), and the HUMIRA™ Pen (Abbott Labs, Abbott Park IL), to name only a few.

[0083] In certain situations, the pharmaceutical composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, supra; Sefton, 1987, CRC Crit. Ref. Biomed. Eng. 14:201). In another embodiment, polymeric materials can be used; see, Medical Applications of Controlled Release, Langer and Wise (eds.), 1974, CRC Pres., Boca Raton, Florida. In yet another embodiment, a controlled release system can be placed in proximity of the composition's target, thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, 1984, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138). Other controlled release systems are discussed in the review by Langer, 1990, Science 249:1527-1533.

[0084] The injectable preparations may include dosage forms for intravenous, subcutaneous, intracutaneous and intramuscular injections, drip infusions, etc. These injectable preparations may be prepared by methods publicly known. For example, the injectable preparations may be

prepared, *e.g.*, by dissolving, suspending or emulsifying the antibody or its salt described above in a sterile aqueous medium or an oily medium conventionally used for injections. As the aqueous medium for injections, there are, for example, physiological saline, an isotonic solution containing glucose and other auxiliary agents, etc., which may be used in combination with an appropriate solubilizing agent such as an alcohol (e.g., ethanol), a polyalcohol (e.g., propylene glycol, polyethylene glycol), a nonionic surfactant [e.g., polysorbate 80, HCO-50 (polyoxyethylene (50 mol) adduct of hydrogenated castor oil)], etc. As the oily medium, there are employed, e.g., sesame oil, soybean oil, etc., which may be used in combination with a solubilizing agent such as benzyl benzoate, benzyl alcohol, etc. The injection thus prepared is preferably filled in an appropriate ampoule.

[0085] Advantageously, the pharmaceutical compositions for oral or parenteral use described above are prepared into dosage forms in a unit dose suited to fit a dose of the active ingredients. Such dosage forms in a unit dose include, for example, tablets, pills, capsules, injections (ampoules), suppositories, etc. The amount of the aforesaid antibody contained is generally about 5 to about 500 mg per dosage form in a unit dose; especially in the form of injection, it is preferred that the aforesaid antibody is contained in about 5 to about 100 mg and in about 10 to about 250 mg for the other dosage forms.

Therapeutic Uses of the Antibodies

[0086] The antibodies of the invention are useful, *inter alia*, for the treatment, prevention and/or amelioration of any disease or disorder associated with or mediated by CD48 activity or treatable by blocking the interaction between CD48 and a CD48 receptor. Exemplary diseases and disorders that can be treated with the anti-CD48 antibodies of the present invention include, *e.g.*, asthma, allergy, atopic dermatitis, conjunctivitis, inflammatory bowel disease (*e.g.*, ulcerative colitis), celiac disease, and cancer (*e.g.*, blood cell cancer, brain cancer, breast cancer, colon cancer, head and neck cancer, liver cancer, lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer, stomach cancer, etc.). The anti-CD48 antibodies of the present invention can also be administered to patients to treat psoriasis. Other diseases and disorders that can be treated with the anti-CD48 antibodies of the present invention include, *e.g.*, systemic lupus erythematosis (SLE), rheumatoid arthritis, graft-versus-host disease (GvHD) (*e.g.*, chronic GvHD or acute GvHD), and transplant rejection.

Combination Therapies

[0087] The present invention includes therapeutic administration regimens which comprise administering an anti-CD48 antibody of the present invention in combination with at least one additional therapeutically active component. Non-limiting examples of such additional therapeutically active components include other CD48 antagonists (*e.g.*, a second anti-CD48 antibody or small molecule inhibitor of CD48), cytokine inhibitors (*e.g.*, an interleukin-1 (IL-1) inhibitor (such as rilonacept or anakinra, a small molecule IL-1 antagonist, or an anti-IL-1

antibody); IL-18 inhibitor (such as a small molecule IL-18 antagonist or an anti-IL-18 antibody); IL-4 inhibitor (such as a small molecule IL-4 antagonist, an anti-IL-4 antibody or an anti-IL-4 receptor antibody); IL-6 inhibitor (such as a small molecule IL-6 antagonist, an anti-IL-6 antibody or an anti-IL-6 receptor antibody); aspirin; NSAIDs; steroids (*e.g.*, prednisone, methotrexate, etc.); low dose cyclosporine A; tumor necrosis factor (TNF) or TNF receptor inhibitors (*e.g.*, a small molecule TNF or TNFR antagonist or an anti-TNF or TNFR antibody); uric acid synthesis inhibitors (*e.g.*, allopurinol); uric acid excretion promoters (*e.g.*, probenecid, sulfinpyrazone, benzbromarone, etc.); other inflammatory inhibitors (*e.g.*, inhibitors of caspase-1, p38, IKK1/2, CTLA-4Ig, etc.); and/or corticosteroids. The additional therapeutically active component(s) may be administered prior to, concurrent with, or after the administration of an anti-CD48 antibody of the present invention (for purposes of the present disclosure, such administration regimens are considered the administration of an anti-CD48 antibody "in combination with" a therapeutically active component of the invention).

Diagnostic Uses of the Antibodies

[0088] The anti-CD48 antibodies of the present invention may also be used to detect and/or measure CD48 in a sample, *e.g.*, for diagnostic purposes. For example, an anti-CD48 antibody, or fragment thereof, may be used to diagnose a condition or disease characterized by aberrant expression (*e.g.*, over-expression, under-expression, lack of expression, etc.) of CD48. Exemplary diagnostic assays for CD48 may comprise, *e.g.*, contacting a sample, obtained from a patient, with an anti-CD48 antibody of the invention, wherein the anti-CD48 antibody is labeled with a detectable label or reporter molecule. Alternatively, an unlabeled anti-CD48 antibody can be used in diagnostic applications in combination with a secondary antibody which is itself detectably labeled. The detectable label or reporter molecule can be a radioisotope, such as ^{3}H, ^{14}C, ^{32}P, ^{35}S, or ^{125}I; a fluorescent or chemiluminescent moiety such as fluorescein isothiocyanate, or rhodamine; or an enzyme such as alkaline phosphatase, beta-galactosidase, horseradish peroxidase, or luciferase. Specific exemplary assays that can be used to detect or measure CD48 in a sample include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence-activated cell sorting (FACS).

[0089] Samples that can be used in CD48 diagnostic assays according to the present invention include any tissue or fluid sample obtainable from a patient which contains detectable quantities of CD48 protein, or fragments thereof, under normal or pathological conditions. Generally, levels of CD48 in a particular sample obtained from a healthy patient (*e.g.*, a patient not afflicted with a disease or condition associated with abnormal CD48 levels or activity) will be measured to initially establish a baseline, or standard, level of CD48. This baseline level of CD48 can then be compared against the levels of CD48 measured in samples obtained from individuals suspected of having a CD48 related disease or condition.

EXAMPLES

[0090] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1. Generation of Human Antibodies to Human CD48

[0091] An immunogen comprising the ecto-domain of human CD48 (amino acids 27-220 of SEQ ID NO:384) was administered directly, with an adjuvant to stimulate the immune response, to a VELOCIMMUNE® mouse comprising DNA encoding human Immunoglobulin heavy and kappa light chain variable regions. The antibody immune response was monitored by a CD48-specific immunoassay. When a desired immune response was achieved splenocytes were harvested and fused with mouse myeloma cells to preserve their viability and form hybridoma cell lines. The hybridoma cell lines were screened and selected to identify cell lines that produce CD48-specific antibodies. Using this technique several anti-CD48 chimeric antibodies (*i.e.*, antibodies possessing human variable domains and mouse constant domains) were obtained; exemplary antibodies generated in this manner were designated as follows: H2M1707N, H2M1709N, H2M1710N, H2M1712N, H2M1713N, H2M1763N, H2M1811N, H3M1766N, H2M1798N, and H2M1711N.

[0092] Anti-CD48 antibodies were also isolated directly from antigen-positive B cells without fusion to myeloma cells, as described in U.S. 2007/0280945A1. Using this method, several fully human anti-CD48 antibodies (*i.e.*, antibodies possessing human variable domains and human constant domains) were obtained; exemplary antibodies generated in this manner were designated as follows: H4H1769Na, H4H1769Pa, H4H1769Nb, H4H1769Pb, H4H1770N, H4H1770P, H4H1771N, H4H1771P, H4H1772N, H4H1772P, H4H1774N, H4H1774P, H4H1775N, H4H1775P, H4H1778N, H4H1778P, H4H1779N, H4H1779P, H4H1781N, H4H1781P, H4H1789Na, H4H1789Pa, H4H1789Nb, H4H1789Pb.

[0093] The biological properties of the exemplary anti-CD48 antibodies generated in accordance with the methods of this Example are described in detail in the Examples set forth below.

Example 2. Heavy and Light Chain Variable Region Amino Acid Sequences

[0094] Table 1 sets forth the heavy and light chain variable region amino acid sequence pairs of selected anti-CD48 antibodies and their corresponding antibody identifiers. The N, P and G designations refer to antibodies having heavy and light chains with identical CDR sequences

but with sequence variations in regions that fall outside of the CDR sequences (i.e., in the framework regions). Thus, N, P and G variants of a particular antibody have identical CDR sequences within their heavy and light chain variable regions but differ from one another within their framework regions. The H2M, H3M, H4H, etc. prefixes on the antibody designations used herein indicate the particular Fc region of the antibody. For example, an "H2M" antibody has a mouse IgG2 Fc, whereas an "H4H" antibody has a human IgG4 Fc. As will be appreciated by a person of ordinary skill in the art, an H2M or H3M antibody can be converted to an H4H antibody, and vice versa, but the variable domains (including the CDRs) will remain the same.

Table 1

	SEQ ID NOs:							
Antibody Designation	HCVR	HCDR1	HCDR2	HCDR3	LCVR	LCDR1	LCDR2	LCDR3
H2M1707N	2	4	6	8	10	12	14	16
H2M1709N	18	20	22	24	26	28	30	32
H2M1710N	34	36	38	40	42	44	46	48
H2M1712N	50	52	54	56	58	60	62	64
H2M1713N	66	68	70	72	74	76	78	80
H2M1763N	82	84	86	88	90	92	94	96
H2M1764N	98	100	102	104	106	108	110	112
H2M1811N	114	116	118	120	122	124	126	128
H3M1766N	130	132	134	136	138	140	142	144
H4H1769Na	162	164	166	168	170	172	174	176
H4H1769Pa	178	164	166	168	180	172	174	176
H4H1769Nb	162	164	166	168	182	184	186	188
H4H1769Pb	178	164	166	168	190	184	186	188
H4H1770N	192	194	196	198	200	202	204	206
H4H1770P	208	194	196	198	210	202	204	206
H4H1771N	212	214	216	218	220	222	224	226
H4H1771P	228	214	216	218	230	222	224	226
H4H1772N	232	234	236	238	240	242	244	246
H4H1772P	248	234	236	238	250	242	244	246
H4H1774N	252	254	256	258	260	262	264	266
H4H1774P	268	254	256	258	270	262	264	266
H4H1775N	272	274	276	278	280	282	284	286
H4H1775P	288	274	276	278	290	282	284	286
H4H1778N	292	294	296	298	300	302	304	306
H4H1778P	308	294	296	298	310	302	304	306
H4H1779N	312	314	316	318	320	322	324	326
H4H1779P	328	314	316	318	330	322	324	326
H4H1781N	332	334	336	338	340	342	344	346

H4H1781P	348	334	336	338	350	342	344	346
H4H1789Na	352	354	356	358	360	362	364	366
H4H1789Pa	368	354	356	358	370	362	364	366
H4H1789Nb	352	354	356	358	372	374	376	378
H4H1789Pb	368	354	356	358	380	374	376	378

Example 3. Antibody Binding to Soluble CD48 Determined by Surface Plasmon Resonance

[0095] Binding affinities and kinetic constants of human monoclonal anti-hCD48 antibodies binding to human and monkey soluble recombinant CD48 were determined by surface plasmon resonance at both 25°C and 37°C and for both monomeric and dimeric configurations of CD48. Measurements were conducted on a T100-2 BIACORE™ instrument (Biacore Life Sciences division of GE Healthcare, Piscataway, NJ). Antibodies (either with mouse Fc [designated "H2M" or "H3M"] or human IgG4 Fc [designated "H4H"]) were captured through an anti-Fc chip surface, and CD48 was flowed over the surface either in a monomeric (soluble CD48 expressed with a myc-myc-hexa-histidine C-terminal tag [mmH]) or dimeric (soluble CD48 expressed with a C-terminal Fc fusion [mFc]) format. The amino acid sequence identifiers of the reagents used in this Example are set forth in Table 2.

Table 2

Construct	SEQ ID NO:
Monomeric human CD48-mmH	385
Monomeric monkey CD48-mmH	386
Dimeric human CD48-mFc	387
Dimeric monkey CD48-mFc	388

[0096] The soluble CD48 was applied to the flow cell in separate injections at different concentrations, and kinetic association (k_a) and dissociation (k_d) rate constants were determined by fits to the kinetic binding data using Biacore software. Binding dissociation equilibrium constants and dissociation half-times were calculated from the kinetic rate constants as: $K_D = k_d / k_a$; $t_{1/2} = (\ln 2/k_d)$ (units: ka = $M^{-1}*s^{-1}$; kd = s^{-1}; K_D = M; T½ = min) (Tables 3-6).

Table 3 (25°C)

	monomeric human CD48				dimeric human CD48			
Antibody	ka	kd	K_D	T½	ka	kd	K_D	T½
H2M1707N	1.31E+05	6.42E-04	4.92E-09	18	7.14E+05	6.59E-05	9.23E-11	175
H4H1707N	1.68E+05	6.01E-04	3.57E-09	19	3.85E+05	2.83E-05	7.36E-11	408
H2M1709N	2.92E+05	5.80E-	1.98E-	2	9.28E+05	2.88E-	3.11E-	40

		03	08			04	10	
H2M1710N	3.73E+04	1.46E-03	3.91E-08	8	1.12E+05	9.38E-05	8.41E-10	123
H2M1711N	6.92E+05	1.13E-03	1.64E-09	10	1.64E+06	1.02E-04	6.23E-11	113
H2M1712N	5.24E+05	6.19E-03	1.18E-08	2	1.84E+06	5.30E-04	2.88E-10	22
H2M1713N	1.61E+05	2.17E-04	1.35E-09	53	8.26E+05	1.11E-05	1.34E-11	1043
H4H1713N	1.94E+05	1.74E-04	8.93E-10	67	8.94E+05	1.90E-05	2.12E-11	609
H2M1763N	5.48E+05	6.48E-03	1.18E-08	2	1.95E+06	4.99E-04	2.56E-10	23
H4H1763N	7.69E+05	6.31E-03	8.20E-09	2	2.05E+06	5.48E-05	2.68E-11	211
H2M1764N	1.21E+05	2.85E-03	2.35E-08	4	2.59E+05	2.66E-04	1.03E-09	43
H4H1764N	1.05E+05	2.48E-03	2.36E-08	5	2.68E+05	6.05E-05	2.26E-10	191
H3M1766N	6.34E+04	1.77E-04	2.79E-09	65	1.29E+05	6.57E-05	5.10E-10	176
H4H1769P	1.93E+05	7.79E-05	4.03E-10	148	9.69E+05	1.08E-05	1.11E-11	1071
H4H1770P	5.89E+05	1.53E-04	2.59E-10	76	1.71E+06	5.54E-06	3.25E-12	2084
H4H1771P	8.05E+05	2.09E-04	2.60E-10	55	2.07E+06	1.52E-05	7.36E-12	759
H4H1772P	1.27E+05	1.76E-04	1.38E-09	66	3.76E+05	1.29E-05	3.44E-11	894
H4H1774P	1.04E+05	4.72E-05	4.54E-10	245	3.11E+05	4.65E-06	1.49E-11	2486
H4H1775P	1.45E+05	4.92E-05	3.40E-10	235	7.34E+05	1.31E-05	1.79E-11	881
H4H1778P	9.86E+04	4.69E-05	4.76E-10	246	2.94E+05	8.95E-06	3.04E-11	1291
H4H1779P	1.58E+05	3.90E-05	2.46E-10	296	7.74E+05	1.17E-05	1.51E-11	990
H4H1781P	1.70E+05	3.30E-05	1.94E-10	351	8.54E+05	1.23E-05	1.44E-11	940
H4H1789Pa	1.43E+05	4.58E-04	3.20E-09	25	3.82E+05	3.66E-05	9.58E-11	315
H2M1798N	9.14E+05	2.47E-03	2.70E-09	5	2.05E+06	2.71E-04	1.32E-10	43
H2M1811N	5.00E+04	8.49E-04	1.70E-08	14	1.44E+05	2.30E-04	1.60E-09	50

Table 4 (37°C)

	monomeric human CD48				dimeric human CD48			
Antibody	ka	kd	K_D	T½	ka	kd	K_D	T½
H2M1707N	2.14E+05	3.14E-03	1.47E-08	4	9.20E+05	2.27E-04	2.47E-10	51
H4H1707N	2.84E+05	2.73E-03	9.63E-09	4	9.81E+05	6.42E-05	6.54E-11	180
H2M1709N	1.14E+06	4.00E-02	3.51E-08	0	1.52E+06	3.77E-04	2.48E-10	31
H2M1710N	3.40E+04	5.20E-03	1.53E-07	2	4.81E+05	4.26E-04	8.85E-10	27
H2M1711N	9.26E+05	3.02E-03	3.26E-09	4	2.08E+06	2.39E-04	1.15E-10	48
H2M1712N	8.46E+05	1.40E-02	1.66E-08	1	2.72E+06	6.85E-04	2.52E-10	17
H2M1713N	2.35E+05	6.44E-04	2.74E-09	18	1.25E+06	8.27E-05	6.63E-11	140
H4H1713N	2.86E+05	6.36E-04	2.23E-09	18	1.26E+06	4.53E-05	3.60E-11	255
H2M1763N	1.49E+06	2.01E-02	1.35E-08	1	3.02E+06	8.83E-04	2.93E-10	13
H4H1763N	1.33E+06	1.69E-02	1.27E-08	1	2.35E+06	6.28E-05	2.67E-11	184
H2M1764N	1.69E+05	1.60E-02	9.47E-08	1	7.02E+05	4.12E-04	5.86E-10	28
H4H1764N	1.05E+05	1.14E-02	1.09E-07	1	3.01E+05	9.94E-05	3.30E-10	116
H3M1766N	1.61E+05	6.20E-04	3.84E-09	19	9.49E+05	3.24E-04	3.42E-10	36
H4H1769P	2.25E+05	3.14E-04	1.40E-09	37	1.21E+06	3.63E-05	3.01E-11	319
H4H1770P	8.04E+05	5.33E-04	6.62E-10	22	2.23E+06	3.08E-05	1.38E-11	375
H4H1771P	1.12E+06	5.47E-04	4.88E-10	21	2.89E+06	4.81E-05	1.66E-11	240
H4H1772P	1.72E+05	1.02E-03	5.94E-09	11	9.20E+05	5.98E-05	6.50E-11	193
H4H1774P	1.30E+05	1.07E-04	8.24E-10	108	3.63E+05	1.60E-05	4.39E-11	724
H4H1775P	1.77E+05	9.48E-05	5.36E-10	122	5.22E+05	2.27E-05	4.34E-11	509
H4H1778P	1.35E+05	1.13E-04	8.37E-10	102	4.73E+05	3.53E-05	7.46E-11	327
H4H1779P	2.07E+05	1.02E-04	4.95E-10	113	7.72E+05	4.59E-05	5.94E-11	252
H4H1781P	2.26E+05	1.01E-04	4.48E-10	114	8.12E+05	4.89E-05	6.03E-11	236
H4H1789Pa	1.99E+05	1.79E-03	8.97E-09	6	2.21E+05	3.57E-05	1.61E-10	324
H2M1798N	1.41E+06	8.35E-03	5.94E-09	1	2.91E+06	2.17E-04	7.47E-11	53
H2M1811N	1.30E+05	2.95E-03	2.27E-08	4	2.65E+05	2.56E-04	9.66E-10	45

Table 5 (25°C)

	monomeric monkey CD48				dimeric monkey CD48			
Antibody	ka	kd	K_D	T½	ka	kd	K_D	T½
H4H1707N	2.89E+05	3.62E-04	1.25E-09	32	8.05E+05	1.19E-03	1.48E-09	10
H4H1713N	3.15E+05	2.65E-04	8.43E-10	44	8.61E+05	2.42E-04	2.81E-10	48
H4H1763N	5.27E+05	1.24E-03	2.34E-09	9	1.33E+06	6.88E-05	5.19E-11	168
H4H1764N	8.77E+04	2.06E-04	2.34E-09	56	2.51E+05	1.32E-04	5.25E-10	88
H4H1769P	5.88E+05	3.93E-04	6.69E-10	29	4.82E+05	2.56E-05	5.31E-11	451
H4H1770P	7.20E+05	2.15E-04	2.98E-10	54	8.58E+05	1.13E-04	1.31E-10	103
H4H1771P	1.05E+06	4.77E-04	4.53E-10	24	9.39E+05	5.51E-05	5.87E-11	210
H4H1772P	4.61E+05	5.08E-04	1.10E-09	23	5.19E+05	5.02E-05	9.68E-11	230
H4H1774P	2.63E+05	7.63E-05	2.89E-10	151	3.95E+05	2.07E-05	5.25E-11	558
H4H1775P	2.91E+05	4.07E-05	1.40E-10	284	4.88E+05	6.60E-06	1.35E-11	1750
H4H1778P	2.76E+05	8.98E-05	3.25E-10	129	3.84E+05	1.77E-05	4.60E-11	654
H4H1779P	3.01E+05	1.13E-04	3.75E-10	102	5.22E+05	3.17E-05	6.06E-11	364
H4H1781P	2.98E+05	7.80E-05	2.62E-10	148	5.99E+05	2.13E-05	3.56E-11	542
H4H1789Pa	4.16E+05	1.38E-03	3.31E-09	8	6.51E+05	2.76E-04	4.23E-10	42

Table 6 (37°C)

	monomeric monkey CD48				dimeric monkey CD48			
Antibody	ka	kd	K_D	T½	ka	kd	K_D	T½
H4H1707N	3.27E+05	1.22E-03	3.72E-09	9	1.64E+06	5.24E-03	3.20E-09	2
H4H1713N	3.20E+05	3.82E-04	1.19E-09	30	1.72E+06	5.16E-04	3.00E-10	22
H4H1763N	5.34E+05	1.33E-03	2.49E-09	9	2.42E+06	1.14E-04	4.70E-11	101
H4H1764N	2.13E+05	6.20E-04	2.91E-09	19	1.92E+05	1.42E-03	7.40E-09	8
H4H1769P	2.80E+05	1.10E-03	3.92E-09	11	6.97E+05	9.80E-05	1.41E-10	118
H4H1770P	3.96E+05	1.74E-04	4.40E-10	66	1.53E+06	1.39E-04	9.11E-11	83

H4H1771P	6.57E+05	8.77E-04	1.34E-09	13	1.64E+06	1.02E-04	6.21E-11	113
H4H1772P	3.20E+05	1.45E-03	4.53E-09	8	7.72E+05	7.31E-05	9.47E-11	158
H4H1774P	1.97E+05	2.67E-04	1.36E-09	43	5.08E+05	5.40E-05	1.07E-10	214
H4H1775P	2.60E+05	1.29E-04	4.98E-10	90	6.69E+05	3.97E-05	5.93E-11	291
H4H1778P	1.89E+05	2.53E-04	1.34E-09	46	5.25E+05	3.60E-05	6.86E-11	321
H4H1779P	2.77E+05	2.89E-04	1.04E-09	40	7.21E+05	5.98E-05	8.29E-11	193
H4H1781P	3.50E+05	3.07E-04	8.75E-10	38	8.75E+05	6.61E-05	7.55E-11	175
H4H1789Pa	4.37E+05	2.06E-03	4.71E-09	6	9.90E+05	7.68E-04	7.76E-10	15

[0097] As shown in Tables 3-6, the exemplary antibodies tested in this Example exhibited high affinity binding to both human and monkey CD48 soluble protein. A significant increase in binding affinity was observed when bivalent CD48 was used as the solution-phase analyte in comparison to monovalent CD48.

Example 4. Epitope Mapping of H4H1789Pa Binding to CD48 by H/D Exchange

[0098] Experiments were conducted to determine the amino acid residues of CD48 with which H4H1789Pa interacts. For this purpose H/D exchange epitope mapping was carried out. A general description of the H/D exchange method is set forth in *e.g.*, Ehring (1999) *Analytical Biochemistry* 267(2):252-259; and Engen and Smith (2001) *Anal. Chem. 73*:256A-265A.

[0099] To map the epitope of CD48 for binding H4H1789Pa via H/D exchange, a recombinant human CD48-myc-myc-his fusion construct was used ("hCD48-mmH"; SEQ ID NO:391). hCD48-mmH was first deglycosylated with PNGase F (New England BioLabs) under native conditions. H4H1789Pa was then covalently attached to anti-human IgG-agarose beads (Sigma) by crosslinker BS^3 (Thermo Scientific).

[0100] In the 'on-solution/off-beads' (on-exchange in solution followed by off-exchange on beads) experiment, the ligand (deglycosyated hCD48-mmH) was deuterated for 5 min or 10 min in PBS buffer prepared with D_2O, and then bound to H4H1789Pa beads during a 2 min incubation. The CD48-bound beads were washed with PBS aqueous buffer (prepared with H_2O) and incubated for half of the on-exchange time. After the off-exchange, the bound CD48 was eluted from beads with an ice-cold low pH TFA solution. The eluted CD48 was then digested with immobilized pepsin (Thermo Scientific) for 5 min. The resulting peptides were isolated using ZipTip® chromatographic pipette tips and immediately analyzed by UltrafleXtreme matrix assisted laser desorption ionization time of flight (MALDI-TOF)-TOF mass spectrometry (MS).

[0101] In the 'on-beads/off-beads' (on-exchange on beads followed by off-exchange on beads) experiment, CD48 was first bound to H4H1789Pa beads and then incubated for 5 min or

10 min in D_2O for on-exchange. The following steps (off-exchange, pepsin digestion, and MS analysis) were carried out as described for the 'on-solution/off-beads' procedure. The centroid values of all the detected peptides were calculated and compared between these two sets of experiments.

[0102] The results are summarized in Table 7a which provides a quantitative comparison of the centroid mass-to-charge ratios for all the detected peptides identified by liquid chromatography-matrix assisted laser desorption ionization (LC-MALDI) MS following the H/D exchange and peptic digest procedure. While the majority of the observed peptic peptides gave similar centroid mass-to-charge ratios for both the on-solution/off-beads and on-beads/off-beads experiments, three different peptic peptides corresponding to residues 60–76 of SEQ ID NO:384 and seven peptides corresponding to residues 107–125 of SEQ ID NO:384 gave consistently higher centroid values, (*i.e.*, higher deuterium retention) in the 'on-solution/off-beads' experiment. For purposes of the present Example, a positive difference (Δ) of at least 0.20 indicates amino acids protected by antibody binding. Such residues are indicated by bold text and an asterisk (*) in Table 7a.

Table 7a: H4H1789Pa Binding to hCD48-mmH

	5 min on- / 2.5 min off-exchange			10 min on- / 5 min off-exchange		
Residues (of SEQ ID NO:384)	On-solution / Off Beads	On-Beads / Off-Beads	Δ	On-solution / Off Beads	On-Beads / Off-Beads	Δ
27 – 33	804.97	804.95	0.03	804.93	804.98	-0.05
32 – 50	2001.88	2001.78	0.11	2001.68	2001.77	-0.09
45 – 59	1856.18	1856.19	-0.01	1856.37	1856.32	0.04
53 – 59	986.77	986.75	0.02	986.26	986.15	0.12
60 – 68*	1143.72	1143.33	**0.39**	1143.78	1143.41	**0.37**
60 – 76*	2195.08	2194.74	**0.34**	2195.13	2194.82	**0.31**
63 – 76*	1783.45	1783.24	**0.21**	1783.51	1783.26	**0.25**
69 – 76	1070.35	1070.29	0.06	1070.30	1070.29	0.01
77 – 94	2036.78	2036.65	0.13	2036.73	2036.87	-0.13
95 – 106	1413.74	1413.70	0.03	1413.64	1413.65	-0.01
95 – 107	1576.79	1576.86	-0.07	1576.83	1576.84	-0.02
107 – 120*	1711.01	1710.73	**0.28**	1710.97	1710.73	**0.24**
107 – 125*	2379.65	2379.19	**0.47**	2379.64	2379.33	**0.31**
108 – 120*	1547.48	1547.04	**0.44**	1547.38	1547.12	**0.26**
108 – 125*	2216.50	2216.02	**0.48**	2216.54	2216.15	**0.39**
110 – 120*	1303.49	1303.23	**0.26**	1303.46	1303.24	**0.22**
110 – 121*	1489.25	1488.79	**0.46**	1489.22	1488.89	**0.34**
110 – 125*	1972.12	1971.70	**0.42**	1972.10	1971.77	**0.33**
161 – 175	1902.09	1902.05	0.04	1902.07	1902.05	0.02

161 – 178	2257.67	2257.70	-0.02	2257.70	2257.92	-0.22
161 – 181	2557.23	2557.13	0.10	2557.09	2557.35	-0.27
163 – 175	1688.45	1688.40	0.05	1688.31	1688.29	0.01
163 – 181	2343.05	2343.03	0.02	2342.94	2343.22	-0.28
166 – 181	1893.07	1892.97	0.10	1892.89	1893.21	-0.33
168 – 181	1672.12	1672.13	-0.02	1672.14	1672.21	-0.07
225 – 236	1318.53	1318.53	-0.01	1318.51	1318.63	-0.12
225 – 248	2828.26	2828.14	0.12	2828.09	2828.30	-0.21
228 – 248	2498.76	2498.69	0.07	2498.71	2498.83	-0.12
237 – 248	1528.63	1528.56	0.07	1528.49	1528.58	-0.08

[0103] The H/D exchange results summarized in Table 7a demonstrate that the two regions (corresponding to amino acids 60–76 and 107–125 of SEQ ID NO:384) are protected from proton off-exchange by H4H1789Pa binding to these specific epitopes on CD48 after on-exchange. Since the peptides corresponding to residues 69–76 did not show significant mass difference, the N-terminal epitope region can be reduced to residues 60–68 of SEQ ID NO:384. Therefore, the two segments (corresponding to amino acids 60–76 and 107–125 of SEQ ID NO:384) on CD48 are defined by the H/D exchange method as a discontinuous epitope for antibody H4H1789Pa binding to the soluble human CD48 protein. These areas of antibody interaction are located within Ig Domain 1 of CD48 and are depicted diagrammatically in Fig 1.

[0104] To confirm the validity and accuracy of the aforementioned epitope mapping assay, additional experiments were conducted using a control antibody having a previously defined epitope. In particular, H/D exchange experiments were performed using the CD48-mmH construct described above (SEQ ID NO:391) and the anti-Myc antibody 9E10. The Myc epitope corresponds to amino acids 195–216 of SEQ ID NO:391.

[0105] All the experimental procedures for these confirmatory experiments were the same as for the H4H1789Pa experiments described above except that the anti-Myc antibody 9E10 was used as the test antibody. The results of the confirmatory anti-Myc experiments are summarized in Table 7b. (Note that the residue numbering shown in the first column of Table 7b corresponds to the actual amino acid numbering of the hCD48-mmH construct [SEQ ID NO:391], whereas the residue numbering shown in the first column of Table 7a has been adjusted to correspond to the amino acid number of the full length human CD48 polypeptide [SEQ ID NO:384]). As before, a positive difference (Δ) of at least 0.20 indicates amino acids protected by antibody binding. Such residues are indicated by bold text and an asterisk (*) in Table 7b.

Table 7b: 9E10 Binding to hCD48-mmH

	5 min on- / 2.5 min off-exchange			10 min on- / 5 min off-exchange		
Residues (of SEQ ID NO:391)	On-solution / Off Beads	On-Beads / Off-Beads	Δ	On-solution / Off Beads	On-Beads / Off-Beads	Δ
1-7	804.93	804.91	0.02	804.99	804.95	0.04
19-33	1856.16	1856.24	-0.09	***	***	
34-50	2194.74	2194.68	0.06	2194.75	2194.64	0.10
37-50	1783.23	1783.21	0.02	1783.27	1783.19	0.08
43-50	1070.04	1070.07	-0.03	1070.09	1070.08	0.01
51-68	2036.51	2036.52	0.00	2036.53	2036.50	0.02
69-80	1413.96	1413.89	0.07	1413.78	1413.80	-0.02
69-81	1576.94	1577.07	-0.13	1577.05	1577.09	-0.04
81-94	1710.59	1710.62	-0.02	1710.59	1710.57	0.03
81-99	2379.16	2379.23	-0.07	2379.26	2379.24	0.02
82-94	1546.91	1546.84	0.07	1546.90	1546.84	0.06
82-99	2215.89	2215.89	0.00	2215.88	2215.87	0.00
84-94	1303.32	1303.34	-0.02	1303.29	1303.20	0.09
84-99	1972.13	1972.14	-0.02	1972.21	1972.11	0.10
135-155	2556.75	2556.64	0.12	2556.71	2556.66	0.05
137-145	1186.27	1186.33	-0.06	1186.27	1186.29	-0.02
137-155	2342.71	2342.93	-0.22	2342.99	2342.94	0.05
140-155	1891.85	1891.90	-0.05	1891.90	1891.92	-0.02
199-210	1318.80	1318.57	0.22	1318.59	1318.61	-0.02
199-222*	2830.27	2828.66	**1.61**	2829.99	2828.63	**1.36**
202-222*	2500.60	2499.30	**1.30**	2500.31	2499.28	**1.03**
211-220*	1255.00	1254.38	**0.62**	1254.86	1254.38	**0.48**
211-222*	1529.30	1528.69	**0.60**	1529.21	1528.76	**0.45**

[0106] As expected, the epitope recognized by 9E10 is identified as being in the region defined by amino acids 199--222 of SEQ ID NO:391. The results summarized in Table 7b thus confirm that the H/D methodology used in this Example can be used to reliably and accurately identify the amino acid residues on a polypeptide antigen with which an antibody interacts.

Example 5. Antibody Blocking of CD48 – 2B4 Interaction Measured by ELISA

[0107] The ability of human anti-CD48 monoclonal antibodies to block human CD48 and monkey CD48 binding to the human cognate ligand, human 2B4, was measured using a competition sandwich ELISA (Table 8). Constant amounts of biotinylated dimeric human CD48 protein expressed as a fusion with mouse IgG2a Fc ("bio-hCD48-mFc" [SEQ ID NO:387]) or monomeric monkey CD48 expressed with a tag consisting of 2 myc and one 6-His epitopes

("mfCD48-mmH" [SEQ ID NO:386]) were separately titrated with varying amounts of antibodies. These antibody-protein complexes were incubated (1hr, 25°C) before being transferred to microtiter plates coated with a dimeric construct expressing the human 2B4 receptor fused to a either human IgG1 (h2B4-hFc [R&D Systems, Inc., Minneapolis, MN, Cat. No. 1039-2B]) or mouse IgG2a Fc (h2B4-mFc [SEQ ID NO:389). After a 1 hr at 25°C the wells were washed and bound human CD48 was detected with streptavidin conjugated with horse-radish peroxidase (HRP), and monkey CD48 was detected with an HRP conjugated anti-myc polyclonal antibody. The ELISA was developed with a TMB solution to produce a colorimetric reaction and quenched with sulfuric acid before reading absorbance at 450nm on a Victor X5 plate reader. Sigmoidal dose response curves were fit to the data using Prism™ software. The calculated IC_{50} value, defined as the concentration of antibody required to block 50% of hCD48 or mfCD48 binding to 2B4, was used as an indicator of blocking potency. (Tables 8 and 9, NB = no binding observed)

Table 8

Antibody	h2B4-hFc + bio-hCD48-mFc IC_{50} (molar)	h2B4-hFc + mfCD48-mmH IC_{50} (molar)
H2M1707N	2.2E-11	2.3E-11
H2M1709N	4.5E-11	1.2E-10
H2M1710N	5.8E-10	3.7E-10
H2M1711N	5.9E-12	2.9E-11
H2M1712N	1.4E-11	1.7E-11
H2M1713N	2.6E-11	2.4E-11
H2M1763N	8.8E-12	1.3E-11
H2M1764N	6.4E-11	2.4E-10
H3M1766N	NB	NB
H2M1798N	5.0E-12	NB
H2M1811N	4.9E-10	2.2E-10

Table 9

Antibody	h2B4-mFc + bio-hCD48-mFc IC_{50} (molar)	h2B4-mFc + mfCD48-mmH IC_{50} (molar)
H2M1707N	8.6E-12	4.9E-12
H4H1707N	2.2E-11	4.5E-11
H2M1710N	4.9E-10	2.6E-10
H2M1711N	4.1E-12	7.7E-13
H2M1713N	2.2E-11	2.5E-12
H4H1713N	1.8E-11	3.4E-12
H2M1763N	5.5E-12	2.3E-12
H4H1763N	7.4E-12	7.2E-12
H2M1764N	2.9E-11	6.2E-11

H4H1764N	7.6E-11	1.6E-10
H4H1769P	1.5E-11	1.8E-11
H4H1770P	2.0E-11	2.2E-11
H4H1771P	9.9E-12	2.2E-12
H4H1772P	1.4E-11	4.4E-12
H4H1774P	2.5E-11	1.6E-11
H4H1775P	4.4E-11	4.3E-11
H4H1778P	5.6E-11	8.4E-12
H4H1779P	1.6E-11	5.7E-12
H4H1781P	1.8E-11	8.1E-12
H4H1789Pa	5.3E-11	3.8E-11

[0108] As shown in Tables 8 and 9, all tested antibodies effectively blocked the interaction between CD48 and 2B4.

Example 6. Antibody Blocking of IFN-gamma Release in Primary Human PBMCs

[0109] The ability of human anti-CD48 monoclonal antibodies of the present invention to block activation of primary human peripheral blood mononuclear cells (PBMC) was measured using an IFNγ release assay. Human PBMC were isolated from white blood cell concentrate of several donors (Leukopak, New York Blood Center, New York, NY) by centrifugation on Ficoll-Hypaque gradient. Cells were re-suspended in RPMI-1640 medium supplemented with 10% fetal bovine serum at final concentration of 1million/ml and plated in 96-well microtiter plates (200µl per well). Cells were incubated with varying amounts of CD48-specific antibodies (1hr, 37°C) prior to addition of agonist anti-CD3 (B&D Pharmingen, San Jose, California, Cat# 555336) and anti-CD28 (B&D Pharmingen, San Jose, California, Cat# 555725) antibodies (both at final concentration of 40ng/ml) pre-mixed with protein G (at final concentration of 0.8mg/ml, Sigma-Aldrich, St. Louis, MO, Cat# P4689). After 48h at 37°C and 5%CO_2, supernatants were collected and IFNγ concentration was determined using ELISA kit (B&D Biosciences, San Jose, California, Cat# 555142) per manufacturer's protocol. Sigmoidal dose response curves were fit to the data using Prism™ software. The calculated IC_{50} values, defined as the concentration of antibody required to block 50% of maximal IFNγ release, was used as an indicator of blocking potency. (Tables 10-12; NB = no binding observed; ND = not determined; NC = data not convergent).

Table 10

	Inhibition of IFN-gamma Release IC_{50} (Molar)				
Antibody	**Donor 1**	**Donor 2**	**Donor 3**	**Donor 4**	**Donor 5**
H2M1707N	4.9E-11	3.0E-11	1.1E-10	1.7E-10	1.5E-11
H2M1709N	4.8E-11	5.7E-11	1.2E-10	1.1E-10	3.2E-11

H2M1763N	4.9E-11	3.4E-11	1.3E-10	7.5E-11	1.9E-11
H2M1798N	4.3E-11	3.4E-11	9.5E-11	5.7E-10	6.0E-11
H2M1764N	2.7E-11	1.1E-10	1.9E-10	9.5E-11	1.9E-11
H2M1711N	NB	NB	NB	NB	NB
H2M1710N	7.8E-10	1.9E-10	1.1E-06	1.5E-09	2.7E-10
H2M1712N	2.0E-11	2.8E-11	7.2E-11	4.8E-11	1.1E-11
H3M1766N	NB	NB	NB	NB	NB
H2M1811N	9.1E-11	4.6E-10	8.3E-11	4.8E-10	9.4E-11
H2M1713N	2.1E-11	7.6E-11	NB	1.1E-10	1.1E-11

Table 11

	Inhibition of IFN-gamma Release IC_{50} (Molar)	
Antibody	**Donor 6**	**Donor 7**
H2M1707N	9.3E-12	1.9E-11
H2M1763N	2.6E-11	1.8E-11
H2M1764N	1.0E-10	not tested
H2M1710N	5.0E-11	1.4E-10
H2M1713N	2.2E-11	1.0E-10
H4H1769Pa	NB	NC
H4H1770P	NB	NB
H4H1771P	NB	NB
H4H1772P	2.0E-12	2.2E-11
H4H1774P	2.5E-11	9.5E-12
H4H1775P	NC	5.1E-12
H4H1778P	9.8E-12	NC
H4H1779P	1.7E-12	1.5E-11
H4H1781P	3.6E-11	1.5E-11
H4H1789Pa	1.2E-11	1.8E-11

Table 12

	Inhibition of IFN-gamma Release IC_{50} (Molar)			
Antibody	**Donor 8**	**Donor 9**	**Donor 10**	**Donor 11**
H4H1707N	NC	5.10E-11	NC	NC
H4H1763N	8.60E-12	9.30E-12	1.00E-12	3.40E-12
H4H1764N	5.30E-12	3.10E-11	5.10E-12	2.30E-11
H4H1769Pa	NB	5.70E-12	NB	1.90E-11
H4H1771P	NC	NB	NB	1.50E-11
H4H1772P	NB	NC	1.40E-12	8.60E-11
H4H1775P	3.10E-12	2.30E-12	2.00E-11	NC
H4H1789Pa	3.70E-11	1.60E-11	1.30E-11	1.30E-11

[0110] As shown in Tables 10, 11, and 12, majority of the tested antibodies effectively blocked the IFNγ release in primary human PBMC.

Example 7. Antibody Blocking of IFN-gamma Release in Primary Human Mixed Lymphocyte Reaction (MLR) Assays

[0111] The ability of human anti-CD48 monoclonal antibodies to block activation of primary human peripheral blood mononuclear cells (PBMC) was measured using a mixed lymphocyte reaction assay (Tables 13 and 14). Human PBMC were isolated from white blood cell concentrate (Leukopak, New York Blood Center, New York, NY) or whole blood samples (obtained through an in-house blood collection program) by centrifugation on Ficoll-Hypaque gradient. Cells were re-suspended in RPMI-1640 medium supplemented with 10% fetal bovine serum to a final concentration of 1 million/ml. Freshly isolated cells were mixed with previously irradiated cells from a different donor at 10:1 ratio and plated in 96-well microtiter plates (200µl per well). Immediately after plating cells, antibodies were added to final concentrations ranging from 0.01 nM to 10 nM. Cells were incubated for 7 days at 37°C and 5%CO_2. Supernatants were collected and IFNγ concentration was determined using ELISA kit (B&D Biosciences, San Jose, California, Cat# 555142) per the manufacturer's protocol. Sigmoidal dose response curves were fit to the data using Prism™ software. The IFNγ blockade level was defined as the difference between the maximum and minimum values of the curves fitted through the dose-response data, normalized by the maximum value ("% blockade" in Tables 13 and 14; "nb" = no binding observed; NC = data not convergent). The calculated IC_{50} value, defined as the concentration of antibody required to block 50% of maximal IFNγ release, was used as an indicator of blocking potency (Table 14).

Table 13

Antibody	% Blockade for nine individual donors (D1-D9)								
	D1	D2	D3	D4	D5	D6	D7	D8	D9
H4H1769Pa	nb	60	39	nb	43	60	51	40	nb
H4H1771P	78	79	88	nb	47	50	36	nb	32
H4H1772P	nb	65	62	39	62	63	58	25	59
H4H1775P	42	77	87	37	70	72	69	22	60
H4H1789Pa	65	66	88	36	89	82	75	65	66
H4H1707N	66	87	78	20	62	77	62	42	60
H4H1763N	59	89	68	52	63	75	48	30	53
H4H1764N	69	46	80	nb	75	59	48	nb	nb
Orencia®	nb	52	nb	nb	68	80	53	58	75
Amevive®	54	53	76	66	78	40	79	nb	nb
belatacept	81	77	74	90	82	83	80	64	66

Table 14

Donor	H4H1763N		H4H1789Pa	
	% Blockade	IC_{50} (M)	% Blockade	IC_{50} (M)
D1	59	~$1.0x10^{-11}$	65	~$1.4x10^{-12}$
D2	89	NC	66	~$9.3x10^{-12}$
D3	68	~$6.4x10^{-12}$	88	NC
D4	52	$2.1x10^{-10}$	36	$2.3x10^{-10}$
D5	63	~$2.7x10^{-11}$	89	~$9.0x10^{-11}$
D6	75	$2.3x10^{-11}$	82	$2.8x10^{-12}$
D7	48	NC	75	NC
D8	30	~$3.7x10^{-9}$	65	$2.9x10^{-11}$
D9	53	~$4.1x10^{-9}$	66	$1.8x10^{-10}$
D10	79	~$8.4x10^{-11}$	77	~$5.0x10^{-11}$
D11	72	$7.3x10^{-11}$	80	~$9.2x10^{-11}$
D12	79	~$5.6x10^{-11}$	55	~$3.0x10^{-10}$
D13	72	$9.2x10^{-11}$	64	~$9.6x10^{-11}$
D14	90	$6.3x10^{-11}$	83	$2.0x10^{-10}$
D15	82	~$5.9x10^{-14}$	95	~$2.7x10^{-12}$
D16	90	$7.6x10^{-12}$	85	~$1.2x10^{-11}$

[0112] As shown in Tables 13 and 14, all tested antibodies effectively blocked the IFNγ release in primary human mixed lymphocyte reaction (PBMC MLR). The ability of the candidate anti-CD48 antibodies to effectively block IFNγ release in primary human PBMCs, as demonstrated in Examples 6 and 7, suggests that these antibodies are useful in blocking inappropriate autoimmune reactions in diseases and conditions such as systemic lupus erythematosus, rheumatoid arthritis, graft-versus-host disease, inflammatory bowel disease, allergy, celiac disease, psoriasis, asthma, etc.

Example 8. Antibody Blocking of Human Cytokine Release in $Rag2^{-/-}\gamma_c^{-/-}$ Mice Reconstituted with Human Hematopoietic Stem Cells

[0113] The ability of human anti-CD48 monoclonal antibodies to block activation of human immune cells *in vivo* was measured using a chimeric animal model consisting of immunodeficient $Rag2^{-/-}\gamma_c^{-/-}$ mice reconstituted with human hematopoietic stem cells. Animals were reconstituted with human stem cells shortly after birth and reconstitution was confirmed by flow cytometry. Fully reconstituted animals (12-16 weeks old) were intraperitoneally injected with 5mg/kg of CD48-specific antibodies or appropriate isotype control (in-house produced human IgG4(S108P) antibody) 24h prior to treatment with an agonist mouse anti-human CD3 antibody (OKT3, 1µg/ animal, IP injection, BioLegend, San Diego, California, Cat#317315) or the isotype control (in-house produced mouse IgG2a antibody). Blood samples were obtained 24h after stimulation and serum levels of nine human cytokines were measured using MSD multiplex kit (Human Proinflammatory 9-Plex Ultra-Sensitive Kit, MesoScale Discovery,

Gaithersburg, Maryland, Cat#K15007C-2) per manufacturer's protocol. Both antibodies tested inhibited production of human cytokines (Tables 15 and 16).

Table 15

Treatment Group	Serum cytokine level (pg/ml)				
	IFNγ	IL-6	IL-10	TNFα	IL-2
IgG2a + hIgG4mut	206.5±30.1	145.9±18.5	394.0±37.8	261.0±25.6	173.2±21.4
IgG2a + H4H1763N	166.3±9.8	153.9±13.7	343.8±15.0	301.2±16.8	194.7±11.5
IgG2a + H4H1789Pa	185.6±14.5	169.1±13.1	380.1±11.5	326.3±27.3	184.9±15.5
OKT3	8383.1± 425.8	1144.1± 120.6	21128.3± 1276.0	1457.4±69.4	2900.6± 209.7
OKT3 + hIgG4mut	5967.7± 1659.4	1124.4± 190.1	3853.8± 742.3	2668.4± 555.6	521.6±85.9
OKT3 + H4H1763N	493.9±55.4	180.2±12.4	536.2±34.0	497.1±56.6	295.1±35.3
OKT3 + H4H1789Pa	617.0±116.9	249.0±38.8	460.9±30.2	339.4±20.7	244.4±15.3

Table 16

Treatment Group	Serum cytokine level (pg/ml)			
	IL-8	IL-12 p70	IL-1β	GM-CSF
IgG2a + hIgG4mut	2186.8±885.4	202.7±7.3	490.7±37.7	177.7±14.8
IgG2a + H4H1763N	5808.0±926.0	217.9±10.4	437.4±26.7	175.6±10.3
IgG2a + H4H1789Pa	2740±651	213.4±11.4	408.8±15.9	172.3±7.7
OKT3	5241.8± 1237.0	225.6±13.0	445.0±8.8	1107.7±90.9
OKT3 + hIgG4mut	19899.0±3138.1	279.0±18.2	603.9±41.4	676.3±59.2
OKT3 + H4H1763N	3381.0±1169.0	182.1±10.5	410.4±22.4	243.3±10.4
OKT3 + H4H1789Pa	1585.7± 269.2	206.3±4.2	395.0±16.4	559.3±76.8

[0114] As shown in Tables 15 and 16, both tested antibodies effectively blocked cytokine release induced by polyclonal stimulation of human immune cells in a chimeric *in vivo* system ($Rag2^{-/-}\gamma_c^{-/-}$ mice reconstituted with human hematopoietic stem cells).

Example 9. Antibody Inhibition of Weight Loss and Prolonged Survival in a Xenogenic Model of Graft-versus-Host Disease (GvHD)

[0115] The ability of a human anti-CD48 monoclonal antibody, H4H1789Pa, to block activation of human immune cells *in vivo* was determined using a xenogeneic animal model of Graft-versus-Host Disease (GvHD) established in the immunodeficient NOD/scid/$^{-/-}\gamma_c^{-/-}$ (NSG) mice engrafted with human peripheral blood mononuclear cells (PBMC). Eight week-old mice were injected intravenously with freshly isolated PBMC from healthy normal volunteers (Day0). The engraftment success rate was evaluated as percentage of cells positive for human CD45 cell surface marker (detected by flow cytometry) in the peripheral blood three days after the PBMC injection. A single intraperitoneal dose of H4H1789Pa (10mg/kg) administered 24h prior to induction with human PBMC fully inhibited engraftment of human cells (<10% of the cells in lymphocyte gate compared to ~99% in the blood from mice treated with isotype control).

[0116] In 100% of untreated animals, successful engraftment of human cells was observed leading to development of GvHD symptoms (weight loss, hunched position, ruffled fur, weakness, etc.) 7-8 days after the PBMC injection. Mice treated with a single intraperitoneal dose of H4H1789Pa (10mg/kg) on Day3, showed delayed weight loss (statistically significant on Day10) and a trend towards prolonged survival (Figure 2). Further, human cytokine levels were measured in the sera obtained from terminal bleeds (Day 13 or earlier) using MSD multiplex kit (Human Proinflammatory 9-Plex Ultra-Sensitive Kit, MesoScale Discovery, Gaithersburg, MD, Cat. No. K15007C-2) per manufacturer's protocol. Treatment with H4H1789Pa resulted in reduced levels of human IL-8 (55.2±21.3 pg/ml compared to 142.7±22.2pg/ml in the isotype control group), IL-10 (85.4±2.6 pg/ml vs 171.8±44.2 pg/ml), IL-6 (20.5±12.3 pg/ml vs 30.0±8.9 pg/ml), and TNFα (35.3±4.5 pg/ml vs 51.6±1.1 pg/ml). No reduction in levels of human GM-CSF (554.9±131.8 pg/ml vs 348.9±20.7 pg/ml) and IFNγ (4.8±0.9 ng/ml vs 5.0±0.5 ng/ml) were observed. Levels of human IL-1β, IL-2 and IL-12p70 were below detection.

[0117] As shown in Figure 2, anti-CD48 antibody, H4H1789Pa, delayed weight loss ($p<0.05$, Dunnett's multiple comparison test) and prolonged survival in a xenogeneic model of GvHD (NSG mice engrafted with human PBMC). These results confirm the utility of anti-CD48 therapy in the treatment of GvHD.

Example 10. Clinical Trial of an Anti-CD48 Antibody for the Treatment of Celiac Disease

[0118] Celiac disease is a chronic HLA-DQ2 or DQ8 restricted CD4+ T cell mediated enteropathy resulting from the secretion of interferon-γ in response to specific dietary deamidated gluten peptides from wheat, barley and rye which have a high binding affinity to these specific HLA class II molecules.

[0119] A 6-week, double-blind, placebo-controlled, proof-of-mechanism study is conducted to evaluate the efficacy, safety, and tolerability of treatment with an anti-CD48 antibody in biopsy-proven quiescent celiac disease challenged with oral gluten. Twelve patients receive (3:1 randomization) a single intravenous infusion of 12 mg/kg of an anti-CD48 antibody of the

invention (*e.g.*, H4H1789Pa) or placebo.

[0120] Patients included in this study will have been on a gluten free diet for at least 2 years (daily gluten intake ≤ 5 mg), lack gluten intolerance for 8 weeks, have a positive HLA-DQ2 genotype, have negative anti-transglutaminase antibodies (anti-TGA) and negative PBMC interferon-γ ELISpot response to gliadin incubation.

[0121] Two weeks after placebo controlled treatment all patients receive a gluten dose of 16 g per day (equivalent to approximately four slices of bread per day) for 3 days consumed as a flour slurry mixed with orange juice or soy milk.

[0122] Celiac disease symptoms are recorded by means of a daily symptom diary beginning two weeks prior, during, and up to two weeks after gluten challenge, recording the presence and severity (on a three-point scale) of nausea, bloating, abdominal pain, lethargy, vomiting, diarrhea or other symptoms.

[0123] Small bowel biopsies will be taken from the second part of the duodenum for mRNA, histology, immunohistochemistry, determination of villous height/crypt depth (Vh/Cd) and counting of intraepithelial lymphocytes (IEL), two weeks post-randomization and 2 weeks post oral gluten challenge. Overall architecture of the small intestinal mucosa will be evaluated by the modified Marsh classification. Normal mucosa (Marsh 0), low grade inflammation with intraepithelial lymphocytosis (Marsh I), low grade inflammation with intraepithelial lymphocytosis and crypthyperplasia (Marsh II), and gold standard to establish a celiac disease diagnosis with intraepithelial lymphocytosis and crypthyperplasia and villous atrophy (Marsh III).

[0124] Prior to oral gluten challenge and one and 2 weeks after challenge, peripheral blood will be sampled from patients (all HLA-DQ2 or DQ8) and purified T lymphocytes will be incubated with the deamidated and control gliadin peptides (α-gliadin$_{57-73}$ native and the Q65E variant). T cell responses in these subjects will be quantitated by measuring the differential IFN-γ ELISpot response to the deamidated versus native α-gliadin$_{57-73}$ peptide and normalized to the IFN-γ ELISpot response to the pan-HLA-DR influenza peptide hemaglutinin peptide HA$_{307-319}$ which is not expected to be altered following gluten challenge.

[0125] Safety and tolerability will be assessed by physical examination, clinical laboratory testing, and adverse event reporting.

[0126] Primary endpoint, at 2 weeks post-gluten challenge, would be improvement in the median Vh/Cd index. Secondary endpoints would include improvement in overall Marsh intestinal biopsy histologic score, reduction of daily celiac symptom score, and reduction of PBMC interferon-γ ELISpot response to α-gliadin$_{57-73}$ peptide challenge. Exploratory endpoints would include change in tissue and WB mRNA, peripheral T cell phenotypes, tissue median IEL count, and serum cytokines measured by ELISA.

Example 11. Clinical Trial of an Anti-CD48 Antibody for the Treatment of Systemic Lupus Erythematosus (SLE)

[0127] SLE is an autoimmune disease characterized by multiple immune system

abnormalities including production of autoantibodies against endogenous nucleic acids of apoptosis that can lead to inflammation and tissue damage. Both humoral and cellular immune systems are activated. Autoantibody containing immune complexes and chronic viral infections stimulate Type I interferons which regulate both innate and adaptive immune systems including T cells, B cells, dendritic cells and NK cells creating a self-perpetuating cycle of autoimmunity.

[0128] A 12-week, double-blind, placebo-controlled, proof-of-mechanism study is conducted to evaluate the efficacy, safety, and tolerability of treatment with anti-CD48 antibody in stable mild-to-moderate to severe SLE. Twenty patients receive (4:1 randomization) a single intravenous infusion of 12 mg/kg of an anti-CD48 antibody of the invention (*e.g.*, H4H1789Pa) or placebo. Patients are evaluated on a regular basis with Safety of Estrogens and Lupus Erythematosus National Assessment (SELENA)-Systemic Lupus Erythematosus Disease Activity Index (SLEDAI), SLE Flare Index and the Physician's Global Disease Assessment (PGA). Biologic marker assessments include: (i) CD20+ B cells and CD138+ plasmacytoid cells, $CD40L^+$ T cells, anti-dsDNA, ANA, Immunoglobulins (IgG, IgM, IgE, and IgA) and complement components C1q, C3,C4, and C5a; (ii) levels of cytokines including interferon-α and interferon-γ, IL-10, IL-6, IL-15, IL-21 and BLyS by ELISA; (iii) total numbers of circulating T, B, NK and T-regulatory cells with surface marker analysis by FACS; and/or (iv) measurement of changes in leukocyte numbers in whole blood (*e.g.* normalization of leukopenia) and involved skin mRNA gene regulation. Laboratory measurements may also include serial PK and anti-drug antibodies.

[0129] Key study inclusion/exclusion criteria include: (i) adults age 18 to 70; (ii) stable SLE disease activity for at least two months before screening; and (iii) maintenance with no medication or with a stable treatment regimen of low-dose prednisone, antimalarials, NSAIDs, azathioprine or myclopnolate mofetil.

[0130] Patients selected for this study will have measurable anti-dsDNA, anti-Smith, anti-RNP, or anti-Sjogren's antibodies. Patients are excluded with active lupus nephritis requiring hemodialysis, cyclophosphamide or high dose prednisone. Patients are excluded with a history of serious infection within four weeks.

[0131] Efficacy is assessed by the reduction in anti-dsDNA antibodies and increase in complement C3 and C4 between baseline and week 4 of anti-CD48 treatment. Exploratory endpoints of improvement include SELENA-SLEDAI, PGA, and SLE Flare Index. Safety and tolerability is assessed by physical examination, clinical laboratory testing, and adverse event reporting.

Example 12. Clinical Trial of an Anti-CD48 Antibody for the Treatment of Psoriasis

[0132] The pathophysiology of psoriasis is related to aberrant immune mediated Th1 and Th17 lymphocyte inflammatory responses which lead to the secretion of cytokines, chemokines and growth factors that can activate and proliferate keratinocytes. These processes lead to the substantial leukocytic infiltration and epidermal thickening which characterize the hallmark

pathology of psoriatic plaques.

[0133] A 12-week, double-blind, placebo-controlled, proof-of-concept study is conducted to evaluate the efficacy, safety, and tolerability of treatment with an anti-CD48 antibody of the invention (*e.g.*, H4H1789Pa) in moderate to severe psoriasis. Twenty patients receive (4:1 randomization) a single intravenous infusion of 12 mg/kg of anti-CD48 antibody or placebo. Patients are evaluated on a regular basis with Psoriasis Area and Severity Index (PASI) scores, Physician Global Assessment (PGA) scores, DLQ, photography, clinical observations, and laboratory measurements including PK and anti-drug antibodies.

[0134] Efficacy is assessed by the change in PASI between baseline and week 8 of anti-CD48 treatment. The primary endpoint is 75% improvement in PASI score from baseline to week 8. Secondary endpoints include 50% improvement of PASI, PGA, and DLQ between baseline and week 8. Safety and tolerability are assessed by physical examination, clinical laboratory testing, and adverse event reporting.

[0135] Key study inclusion/exclusion criteria include: (i) adults age 18 to 70; (ii) chronic plaque psoriasis of at least six month's duration; (iii) BSA involvement of ≥ 10%; (iv) PASI of ≥ 12; (v) candidate for systemic or phototherapy; (vi) no current use of systemic therapies or use within the specified washout periods; and/or (vii) no previous discontinuation of a biologic therapy for psoriasis due to lack of efficacy.

Example 13. Clinical Trial of an Anti-CD48 Antibody for the Treatment of Ulcerative Colitis

[0136] The pathophysiology of ulcerative colitis is characterized by a hyper-reactive CD4+ T cell adaptive immune response in the gut wall directed against the intestinal commensal florae. Activated Th1 and Th17 lymphocytes release pro-inflammatory chemokines and cytokines which cause neutrophils and monocytes to be recruited to the intestine. Costimulation blockade by anti-CD48 has the potential to prevent microbially activated APCs from interacting with naïve T helper cells, which potentiates and sustains the chronic mucosal inflammatory response.

[0137] A 12-week, double-blind, placebo-controlled, proof-of-concept study is conducted to evaluate the efficacy, safety, and tolerability of 4 weeks of treatment with an anti-CD48 antibody in patients with moderate-to-severe symptoms of ulcerative colitis. Seventy patients will receive (1:1 randomization) an anti-CD48 antibody of the invention (*e.g.*, H4H1789Pa) or placebo as a single intravenous infusion at baseline. Patients included in this study are 18-70 years of age, and will have been diagnosed with ulcerative colitis with a Disease Activity Index score (12-point scale) of 6-10, inclusive, at screening. Patients will have been taking an amino-salicylate medication for at least 1 month prior to screening, with a stable dose for at least 2 weeks prior to randomization. If the patient is taking any other medications for ulcerative colitis prior to the study, the doses of such medications will be stable for at least 1 month prior to randomization. With regard to patients taking azathioprine or 6-mercaptopurine, only patients that have been taking these medication(s) for at least 3 months prior to screening are included in the study.

[0138] The primary efficacy analysis will evaluate the mean change in Ulcerative Colitis Disease Activity Index Score from baseline to Week 4 as a function of treatment group assignment. A sample size of 35 patients per treatment group (29 per group with evaluable data at week 4) has at least 80% power at the 5% level of significance to detect a difference between the placebo group and active treatment group, assuming a mean difference between treatment groups of at least 3 points, with a common SD of 4 or less.

[0139] A secondary efficacy analysis will evaluate the proportion of patients who have a reduction in Disease Activity Index score of 3 points or more from baseline to week 4 (responder). Assuming that the placebo response rate is no more than 60% and the rate of response in the active group is at least 40% more than in the placebo group (*e.g.*, 30% placebo group responders vs. $\geq$70% active group responders), the sample size of 35 subjects per group (29 per group with evaluable data at week 4) has at least 80% power at the 5% level of significance to detect a difference between the placebo group and the active treatment group.

[0140] The secondary efficacy endpoints (change in stool frequency, rectal bleeding, fecal continence, endoscopic appearance, Investigator's Global Assessment, and Patient's Global Assessment) are also compared as a function of treatment group from baseline to week 4. In addition, intestinal tissue biopsies are harvested for quantitation of leukocytic infiltration (T cells, activated macrophages) and confirmed by cellular phenotyping using immunohistochemical surface marker analysis.

[0141] The present invention is not to be limited in scope by the specific embodiments describe herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Claims

1. An isolated antibody, or antigen-binding fragment thereof, that specifically binds human CD48 (SEQ ID NO:384) and blocks the interaction between human CD48 and a CD48 receptor, wherein the antibody or antigen binding fragment thereof comprises complementarity determining regions (CDRs) of a heavy chain variable region (HCVR) amino acid sequence having an amino acid sequence of SEQ ID NO:368 and the CDRs of a light chain variable region (LCVR) having an amino acid sequence of SEQ ID NO:370.

2. The antibody or antigen-binding fragment of claim 1, wherein the CD48 receptor is human CD2 (SEQ ID NO:392).

3. The antibody or antigen-binding fragment of claim 1, wherein the CD48 receptor is human 2B4 (SEQ ID NO:390).

4. The antibody or antigen-binding fragment of any one of claims 1 to 3, wherein the antibody or antigen-binding fragment inhibits activation of primary human peripheral blood mononuclear cells (PBMCs) *in vitro*.

5. The antibody or antigen-binding fragment of any one of claims 1 to 4, wherein the antibody or antigen-binding fragment binds an epitope within Ig domain 1 of human CD48 (amino acids 29 to 127 of SEQ ID NO:384).

6. The antibody or antigen-binding fragment of claim 5, wherein the antibody or antigen-binding fragment interacts with one or more amino acids located within amino acids 60 to 125 of SEQ ID NO:384.

7. The antibody or antigen-binding fragment of claim 6, wherein the antibody or antigen-binding fragment interacts with one or more amino acids located within amino acids 60 to 68 of SEQ ID NO:384 and/or amino acids 107 to 125 of SEQ ID NO:384.

8. The antibody or antigen-binding fragment of claim 7, wherein the antibody or antigen-binding fragment interacts with one or more amino acids located within amino acids 60 to 68 of SEQ ID NO:384 and with one or more amino acids located within amino acids 107 to 125 of SEQ ID NO:384.

9. The antibody or antigen-binding fragment of claim 1, wherein the antibody or antigen-binding fragment comprises heavy and light chain CDRs HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, respectively, having the amino acid sequences of SEQ ID NOs:354, 356, 358, 362, 364 and 366.

10. The antibody or antigen-binding fragment of claim 1, wherein the antibody or antigen-binding fragment comprises an HCVR having the amino acid sequence of SEQ ID NO:368, and an LCVR having the amino acid sequence of SEQ ID NO:370.

11. A pharmaceutical composition comprising the antibody or antigen-binding fragment of any one of claims 1 to 10 and a pharmaceutically acceptable carrier or diluent.

12. The pharmaceutical composition of claim 11, for use in treating a patient afflicted with, diagnosed with, or at risk of being afflicted with a disease or disorder that is treatable by blocking the interaction between CD48 and a CD48 receptor.

13. The pharmaceutical composition of claim 12, wherein the disease or disorder that is treatable by blocking the interaction between CD48 and a CD48 receptor is a disease or disorder selected from the group consisting of celiac disease, systemic lupus erythematosus, ulcerative colitis, and psoriasis.

14. Use of the isolated antibody or antigen-binding fragment of any one of claims 1 to 10, or the pharmaceutical composition of claim 11 , in the manufacture of a medicament for use in treating a patient afflicted with, diagnosed with, or at risk of being afflicted with a disease or disorder that is treatable by blocking the interaction between CD48 and a CD48 receptor.

15. The use as defined in claim 14, wherein the disease or disorder that is treatable by blocking the interaction between CD48 and a CD48 receptor is a disease or disorder selected from the group consisting of celiac disease, systemic lupus erythematosus, ulcerative colitis, and psoriasis.