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 ~~OF THE INVENTION~~

[0005] The present disclosure~~invention~~ provides antibodies that bind human CD48. The antibodies of the disclosure~~invention~~ 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~~invention~~ 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~~invention~~ bind an epitope within the first immunoglobulin domain of human CD48.

[0007] The antibodies of the disclosure~~invention~~ 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~~invention~~ 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~~invention~~ 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~~invention~~ 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~~invention~~ 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~~invention~~ 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~~invention~~ 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~~invention~~ 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~~invention~~ which have these sequence characteristics include H4H1774N, H4H1775N, H4H1778N, H4H1779N, and H4H1781N.

[0016] In a related embodiment, the disclosure~~invention~~ 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~~aspect~~, the disclosure~~invention~~ provides nucleic acid molecules encoding anti-CD48 antibodies or fragments thereof. Recombinant expression vectors carrying the nucleic acids of the disclosure~~invention~~, and host cells into which such vectors have been introduced, are also encompassed by the disclosure~~invention~~, 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~~invention~~ 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~~invention~~ 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~~invention~~ 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 disclosureinvention 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 disclosureinvention 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 embodimentaspect, the disclosureinvention provides a pharmaceutical composition comprising a recombinant human antibody or fragment thereof which specifically binds CD48 and a

pharmaceutically acceptable carrier. In a related embodimentaspect, the disclosureinvention 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 embodimentaspect, the disclosureinvention provides methods for inhibiting CD48 activity using an anti-CD48 antibody or antigen-binding portion of an antibody of the disclosureinvention, 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 disclosureinvention. 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 disclosureinvention 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 disclosureinvention also includes the use of an anti-CD48 antibody or antigen binding portion of an antibody of the disclosureinvention 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