HUMAN ANTIBODIES TO CLOSTRIDIUM DIFFICILE TOXINS

FIELD OF THE INVENTION

[0001] The present invention is related to human antibodies and antigen-binding fragments of human antibodies that specifically bind to toxin A and/or toxin B of *Clostridium difficile*, compositions comprising these antibodies and therapeutic methods of using these antibodies.

STATEMENT OF RELATED ART

[0002] *Clostridium difficile* (*C. difficile*) is a gram positive, anaerobic, spore forming bacterium, which is a major cause of hospital-acquired gastrointestinal disease in humans, resulting in symptoms ranging from mild to severe diarrhea and colitis. It is believed that treatment with broad spectrum antibiotics, such as ampicillin, amoxicillin, cephalosporins, fluoroquinolones and clindamycin, may result in disruption of normal intestinal flora, which then allows for colonization of the gut with *C. difficile* (Kelly and Lamont, (1998), Ann. Rev. Med. 49:375-90). Treatment of *C. difficile* infections may involve stopping or modifying the use of broad spectrum antibiotics and requires commencing treatment with specific anticlostridial antibiotics, such as, for example, vancomycin, metronidazole, or fidaxomicin.

[0003] The diarrhea and inflammation observed in patients suffering from a *C. difficile* infection is believed to be due to the production of two toxins by the bacterium, enterotoxin (toxin A) and cytotoxin (toxin B). *C. difficile* toxins A and B are high molecular weight glucosyltransferases that inhibit members of the Rho family of GTPases. Toxin A has a molecular weight of 308 kDa and Toxin B has a molecular weight of 270 kDa. Both toxin A and toxin B deactivate small GTPases such as Rho, Rac and Cdc42 by glucosylation of a threonine residue. Inhibition of these GTPases causes the shutdown of signal transduction cascades leading to: depolymerization of the cytoskeleton, gene transcription of certain stress-activated protein kinases, a drop in synthesis of phosphatidylinositol bisphosphate, and possibly even the loss of cell polarity. Loss of cytoskeletal structure results in cell rounding, and this loss of structure may account for the host reactions to *C. difficile*. Toxin B is at least 1,000 times more cytotoxic than toxin A in cell rounding assays.

[0004] *C. difficile* toxins A and B are 63% homologous in amino acid content and have a similar three-dimensional structure (Davies, AH, (2011), Biochem. J., 436:517-526). The C-terminal third of each toxin is made up of sequences called clostridial repetitive oligopeptides (CROPs), which are highly antigenic. The remaining N-terminal two-thirds of toxins A and B are less similar to each other with respect to sequence homology; however, it is this portion of each protein that contains the glucosyltransferase activity.

[0005] Support for the role of toxin A and/or toxin B in the onset of diarrhea and inflammation following infection with *C. difficile* stems from observations in animal models. For example, oral dosing with the toxins mimics the disease (Kelly and Lamont, (1998), Ann. Rev. Med. 49:375-90). Mutant strains lacking toxin A and B have reduced or altered virulence (Lyras D, O'Connor

JR, Howarth PK *et al.*, *Nature* 458(7242), 1176–1179 (2009); Kuehne SA, Cartman ST, Heap JT, Kelly ML, Cockayne A, Minton NP, *Nature* 15, 467(7316), 711–713 (2010).). Furthermore, administration of polyclonal antibodies to the toxins has been shown to protect hamsters from the disease (Gianasca *et al.,* (1999), Infect. Immun. 66(2): 527-38). In the clinic, studies have shown that there is a correlation between the presence of anti-toxin A or anti-toxin B antibodies and protection against *C. difficile* associated diarrhea and disease recurrence (Warny, M. *et al.,* (1994), Inf. Immun. 62(2): 384-389; Kyne, L. *et al.* (2001), Lancet 357:189-193; Leav, B.A., (2010), Vaccine 28(4):965-969). Development of anti-toxin antibody is associated with asymptomatic carriers (Kyne, L. *et al.* (2000), NEJM 342(6), 390-397). Furthermore, a clinical trial using a combination of *C. difficile* anti-toxin A and anti-toxin B antibodies in conjunction with metronidazole or vancomycin resulted in a reduction in the rate of recurrent infection with *C. difficile* (Lowy, I. *et al.*, (2010), NEJM 362(3):197-205).

[0006] Monoclonal antibodies to *C. difficile* toxin A have been described by Wilkins, *et al.* in US patent 4,879,218. In addition, Rothman *et al*. described a murine monoclonal antibody that cross-reacts with *C. difficile* toxins A and B. Furthermore, Coughlin *et al.* described a monoclonal antibody specific for *C. difficile* toxin B, which did not cross-react with toxin A. Other antibodies to the *C. difficile* toxins have been described (See, for example, US7,151,159; US7,625,559; US8,236,311; US8,257,709; US publication Nos. 2009/0087478; US2010/0233182; US2010/0233181; US2012/0288508; US2012/012160; US2011/0020356; US2012/0121607; EP1766093B1; EP1024826B1; EP1568378A1; EP2305303A2; EP2305293A2; EP2405940A1; EP2261253A2; WO2006/121422; WO2011/130650; WO2010/094970; WO2009/108652; WO2011/063346 and WO2005/058353).

BRIEF SUMMARY OF THE INVENTION

[0007] The invention provides fully human monoclonal antibodies (mAbs) and antigen-binding fragments thereof that bind specifically to either toxin A or to toxin B produced by *Clostridium difficile (C. difficile)*, or which bind to both toxin A and toxin B of *C. difficile* (*ie.* human monoclonal antibodies that cross react with both toxin A and toxin B). Such antibodies may be useful to neutralize the toxicity associated with either toxin A or toxin B, or both, and as such, may act to lessen the severity of the primary *C. difficile*-associated condition or disease, or reduce the number, the duration, or the severity of disease recurrence, or ameliorate at least one symptom associated with the C. *difficile*-associated condition or disease. Such antibodies may be used alone or in conjunction with a second agent useful for treating a *C. difficile*-associated condition or disease. In certain embodiments, the antibodies specific for toxin A, toxin B, or both, may be given therapeutically in conjunction with a second agent to lessen the severity of the primary *C. difficile*-associated condition or disease, or to reduce the number, the duration, or the severity of disease recurrence, or ameliorate at least one symptom associated with the C. *difficile*-associated condition or disease. In certain embodiments, the antibodies

may be used prophylactically as stand-alone therapy to protect patients who are at risk for developing a *C. difficile*-associated condition or disease. For example, certain patient populations may be at risk for developing a *C. difficile* condition or disease, including elderly patients, or patients who have chronic and/or concomitant underlying medical conditions that may pre-dispose them to a *C. difficile* infection. Other at-risk patient populations include patients who are hospitalized for extended periods of time and who are taking broad spectrum antibiotics that may disrupt the normal intestinal flora and which may predispose them to infection with *C. difficile*. More recent data suggest that patients taking proton pump inhibitors (PPIs) are at risk for developing *C. difficile*-associated diarrhea (Yearsley, K. et al. (2006), Aliment. Pharmacol. Ther. 24(4):613-619; Lowe, DO, et al. Clin. Infect. Dis. (2006), 43(10):1272-1276). Other patient populations at risk for developing a *C. difficile* infection include patients who are undergoing any type of immunosuppressive therapy, such as, but not limited to an anti-cancer drug, general radiotherapy to treat certain cancers, or a drug or drug regimen to prevent tissue or organ graft rejection following a transplant. Patients who receive a hematopoietic stem cell transplant (HSCT) may be at particularly high risk for developing a *C. difficile* infection because of long hospitalizations, receipt of broad-spectrum antibiotics and chemotherapy-related disruption of enteric mucosal barriers (Thibault, A. *et al*., ((1991), Infect. Control Hosp. Epidemiol. 12:345-8; Anand, A. *et al.* (1993), Clin. Infect. Dis. 17:109-13). Patients who receive a solid organ transplant may also be at risk for developing a *C. difficile* infection. Included in the at-risk population are patients suffering from an autoimmune disease, or patients on dialysis. More recent studies demonstrated that patients who received either an autologous or allogeneic HSCT were not only at greater risk for developing a *C. difficile* infection, but these patients were also at higher risk of developing gastrointestinal graft versus host disease (GI-GVHD) (Alonso, C.D., *et. al*. (2012), Clin Inf. Dis, 54:1053-1063). While this study clearly demonstrated that *C. difficile* infections were a frequent early complication following HSCT, the exact relationship or interplay between *C. difficile* infections (CDI) and GVHD involving the GI tract needs to be explored in greater detail. Any of these patient populations may benefit from treatment with the antibodies of the invention, when given alone or in conjunction with a second agent, such as metronidazole, vancomycin or fidaxomicin.

[0008] The antibodies of the 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).

[0009] Accordingly, in a first aspect, the invention provides an isolated fully human monoclonal antibody or antigen-binding fragment thereof that binds to either toxin A, or to toxin B, or that binds to or cross reacts with both toxin A and toxin B of *Clostridium difficile,* wherein:

a) the isolated antibody or antigen-binding fragment thereof that specifically binds toxin A of *Clostridium difficile* comprises the three heavy chain complementarity determining regions

(HCDR1, HCDR2 and HCDR3) contained within a heavy chain variable region (HCVR) amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 98, 114, 130, 146 and 162; and the three light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3) contained within a light chain variable region (LCVR) amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 106, 122, 138, 154 and 170;

b) the isolated antibody or antigen-binding fragment thereof that specifically binds toxin B of *Clostridium difficile* comprises the HCDR1, HCDR2 and HCDR3 contained within a HCVR amino acid sequence selected from the group consisting of SEQ ID NOs: 178, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338 and 354; and the LCDR1, LCDR2 and LCDR3 contained within a LCVR amino acid sequence selected from the group consisting of SEQ ID NOs: 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346 and 362; and

c) the isolated antibody or antigen-binding fragment that binds to, or cross reacts with both toxin A and toxin B of *Clostridium difficile* comprises the HCDR1, HCDR2 and HCDR3 contained within a HCVR amino acid sequence selected from the group consisting of SEQ ID NOs: 18, 34, 50, 66 and 82; and the LCDR1, LCDR2 and LCDR3 contained within a LCVR amino acid sequence selected from the group consisting of SEQ ID NOs: 26, 42, 58, 74 and 90.

[0010] In one embodiment, the human monoclonal antibody that binds to/cross reacts with both toxin A and toxin B of *C. difficile* specifically binds to the carboxy terminal receptor binding domain (CBD) of both toxin A (CBD-A: SEQ ID NO: 375) and toxin B (CBD-B:SEQ ID NO: 376) of *C. difficile*.

[0011] In one embodiment, the isolated human antibody or antigen-binding fragment thereof which binds to/cross reacts with both toxin A and toxin B of *C. difficile* binds to toxin A and toxin B with a K_D equal to or less than 10^{-7} M.

[0012] In one embodiment, the isolated human antibody or antigen-binding fragment thereof which binds to/cross reacts with both toxin A and toxin B of *C. difficile* comprises the three heavy chain CDRs (HCDR1, HCDR2 and HCDR3) contained within any one of the heavy chain variable region (HCVR) sequences selected from the group consisting of SEQ ID NOs: 18, 34, 50, 66 and 82; and the three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within any one of the light chain variable region (LCVR) sequences selected from the group consisting of SEQ ID NOs: 26, 42, 58, 74 and 90. 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.*, (1997), *J. Mol. Biol. 273*:927-948;

and Martin *et al.*, (1989), *Proc. Natl. Acad. Sci. USA 86*:9268-9272. Public databases are also available for identifying CDR sequences within an antibody.

[0013] In one embodiment, the isolated human antibody or antigen-binding fragment thereof which binds to/cross reacts with both toxin A and toxin B of *C. difficile* comprises a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 18, 34, 50, 66 and 82.

[0014] In one embodiment, the isolated human antibody or antigen-binding fragment thereof which binds to/cross reacts with both toxin A and toxin B of *C. difficile* comprises a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 26, 42, 58, 74 and 90.

[0015] In one embodiment, the isolated human antibody or antigen-binding fragment thereof which binds to/cross reacts with both toxin A and toxin B of *C. difficile* comprises (a) a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 18, 34, 50, 66 and 82; and (b) a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 26, 42, 58, 74 and 90.

[0016] In one embodiment, the isolated human antibody or antigen-binding fragment thereof which binds to/cross reacts with both toxin A and toxin B of *C. difficile* comprises :

(a) a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 20, 36, 52, 68, and 84, ;

(b) a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 22, 38, 54, 70 and 86;

(c) a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 24, 40, 56, 72 and 88;

(d) a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 28, 44, 60, 76 and 92;

(f) a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 30, 46, 62, 78 and 94; and

(g) a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 32, 48, 64, 80 and 96.

[0017] In one embodiment, the human antibody or antigen binding fragment thereof that binds to/cross reacts with both toxin A and toxin B of *C. difficile* comprises the HCDR1, HCDR2 and HCDR3 amino acid sequences of SEQ ID NO: 20, 22 and 24, respectively and LCDR1, LCDR2 and LCDR3 amino acid sequences of SEQ ID NO: 28, 30 and 32, respectively.

[0018] In one embodiment, the human antibody or antigen binding fragment thereof that binds to/cross reacts with both toxin A and toxin B of *C. difficile* comprises the HCDR1, HCDR2 and HCDR3 amino acid sequences of SEQ ID NO: 36, 38 and 40, respectively and LCDR1, LCDR2 and LCDR3 amino acid sequences of SEQ ID NO: 44, 46 and 48, respectively.

[0019] In one embodiment, the isolated human antibody or antigen-binding fragment thereof which binds to/cross reacts with both toxin A and toxin B of *C. difficile* comprises a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 18/26, 34/42, 50/58, 66/74 and 82/90.

[0020] In one embodiment, the isolated human antibody or antigen-binding fragment thereof which binds to/cross reacts with both toxin A and toxin B of *C. difficile* comprises the HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 18/26 and 34/42.

[0021] In one embodiment, the isolated human antibody or antigen-binding fragment thereof that binds to/cross reacts with both toxin A and toxin B binds to:

an epitope within the carboxy terminal receptor binding domain of both toxin A and toxin B of *Clostridium difficile*, wherein the antibody comprises a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 18/26 and 34/42; or

an epitope outside of the carboxy terminal receptor binding domain of both toxin A and toxin B of *Clostridium difficile*, wherein the antibody comprises a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 50/58, 66/74 and 82/90.

[0022] In one embodiment, the invention provides a fully human monoclonal antibody or antigen-binding fragment thereof that binds to/cross reacts with both toxin A and toxin B of *C. difficile*, wherein the antibody or fragment thereof exhibits one or more of the following characteristics: (i) comprises a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 18, 34, 50, 66 and 82, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (ii) comprises a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 26, 42, 58, 74 and 90, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (iii) comprises a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 24, 40, 56, 72 and 88, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 32, 48, 64, 80 and 96, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (iv) comprises a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 20, 36, 52, 68 and 84, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 22, 38, 54, 70 and 86, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 28, 44, 60, 76 and 92, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR2 domain having an amino acid sequence selected

from the group consisting of SEQ ID NO: 30, 46, 62, 78 and 94, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (v) binds to toxin A and to toxin B with a K_D equal to or less than 10^{-9}M.

[0023] In one embodiment, the fully human monoclonal antibody or antigen binding fragment thereof that binds to/cross reacts with both toxin A and toxin B of *C. difficile* comprises a HCDR1 sequence comprising the formula $X^1 – X^2 – X^3 – X^4 – X^5 – X^6 – X^7 – X^8$ (SEQ ID NO: 381), wherein X^1 is Gly, X^2 is Phe, Val, or Ile, X^3 is Thr, Ala, or Ser, X^4 is Phe or Leu, X^5 is Ser, Arg, or Asn, X^6 is Gly, Thr, Asp, or Ser, X^7 is His, or Tyr, and X^8 is Gly, or Glu; a HCDR2 sequence comprising the formula $X^1 – X^2 – X^3 – X^4 – X^5 – X^6 – X^7 – X^8$ (SEQ ID NO: 382), wherein X^1 is Ile, X^2 is Leu, Ser, or Asp, X^3 is Tyr, Phe, or Ser, X^4 is Asp, or Ser, X^5 is Gly, X^6 is Ser, Gly, Asp, or Thr, X^7 is Ser, His, or Ile, and X^8 is Glu, Gln, or Ile; a HCDR3 sequence comprising the formula $X^1 – X^2 – X^3 – X^4 – X^5 – X^6 – X^7 – X^8 – X^9 – X^{10} – X^{11}$ - $X^{12} – X^{13} – X^{14} – X^{15} – X^{16} – X^{17}$ (SEQ ID NO: 383), wherein X^1 is Ala, or Val, X^2 is Lys, or Arg, X^3 is Gly, or Glu, X^4 is Ser, or Arg, X^5 is Ile, Asp, or Tyr, X^6 is Leu, Ser, or Asp, X^7 is Asn, Ser, Gln, or His, X^8 is Arg, Tyr, or Ser, X^9 is Pro, or Gly, X^{10} is Phe, or Tyr, X^{11} is Asp, Gly, or Tyr, X^{12} is Tyr, X^{13} is Phe, Leu, or absent, X^{14} is Gly, or absent, X^{15} is Met, or absent, X^{16} is Asp, or absent, X^{17} is Val, or absent; a LCDR1 sequence comprising the formula $X^1 – X^2 – X^3 – X^4 – X^5 – X^6 – X^7 – X^8 – X^9 – X^{10} – X^{11}$ - X^{12} (SEQ ID NO: 384), wherein X^1 is Gln, X^2 is Ser, or Glu, X^3 is Ile, Val, or Thr, X^4 is Leu, or Asp, X^5 is Phe, Lys, or Asn, and X^6 is Ser, or Trp, X^7 is Ser, or absent, X^8 is Asn, Asp, or absent, X^9 is Asn, or absent, X^{10} is Lys, or absent, X^{11} Ile, Asn, or absent, X^{12} is Tyr, or absent; a LCDR2 sequence comprising the formula $X^1 – X^2 – X^3$ (SEQ ID NO: 385), wherein X^1 is Trp, Lys, or Arg, X^2 is Ala or Thr, and X^3 is Ser; and a LCDR3 sequence comprising the formula $X^1 – X^2 – X^3 – X^4 – X^5 – X^6 – X^7 – X^8 – X^9$ (SEQ ID NO: 386), wherein X^1 is Gln or His, X^2 is Gln, or Glu, X^3 is Tyr, X^4 is Tyr, or Asn, X^5 is Thr, or Ser, X^6 is Leu, Ala, or Tyr, X^7 is Pro, Phe, or Ser, X^8 is Leu, Phe, or Arg and X^9 is Thr, or Ala.

[0024] In one embodiment, the invention provides an isolated human monoclonal antibody or antigen-binding fragment thereof that binds specifically to toxin A of *Clostridium difficile*, wherein the antibody comprises the three heavy chain CDRs (HCDR1, HCDR2 and HCDR3) contained within any one of the HCVR amino acid sequences selected from the group consisting of SEQ ID NOs: 2, 98, 114, 130, 146 and 162; and the three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within any one of the LCVR amino acid sequences selected from the group consisting of SEQ ID NOs: 10, 106, 122, 138, 154 and 170.

[0025] In one embodiment, the isolated human antibody or antigen-binding fragment thereof that binds specifically to toxin A of *Clostridium difficile*, comprises a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 98, 114, 130, 146 and 162.

[0026] In one embodiment, the isolated human antibody or antigen-binding fragment thereof that binds specifically to toxin A of *Clostridium difficile*, comprises a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 106, 122, 138, 154 and 170.

[0027] In one embodiment, the isolated human antibody or antigen-binding fragment thereof that binds specifically to toxin A of *Clostridium difficile*, comprises (a) a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 98, 114, 130, 146 and 162; and (b) a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 10, 106, 122, 138, 154 and 170.

[0028] In one embodiment, the isolated human antibody or antigen-binding fragment thereof that binds specifically to toxin A of *Clostridium difficile*, comprises:

(a) a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 100, 116, 132, 148 and 164;

(b) a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 6, 102, 118, 134, 150 and 166;

(c) a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 104, 120, 136, 152 and 168;

(d) a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 12, 108, 124, 140, 156, and 172;

(e) a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 110, 126, 142, 158 and 174; and

(f) a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 112, 128, 144, 160 and 176.

[0029] In one embodiment, the isolated human antibody or antigen-binding fragment thereof that binds specifically to toxin A of *Clostridium difficile*, comprises the HCDR1, HCDR2 and HCDR3 amino acid sequences of SEQ ID NO: 148, 150 and 152, respectively and LCDR1, LCDR2 and LCDR3 amino acid sequences of SEQ ID NO: 156, 158 and 160, respectively.

[0030] In one embodiment, the isolated human antibody or antigen-binding fragment thereof that binds specifically to toxin A of *Clostridium difficile*, comprises a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/10, 98/106, 114/122, 130/138, 146/154 and 162/170.

[0031] In one embodiment, the isolated human antibody or antigen-binding fragment thereof that binds specifically to toxin A of *Clostridium difficile*, comprises the HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 146/154.

[0032] In one embodiment, the isolated human antibody or antigen-binding fragment thereof that binds specifically to toxin A of *Clostridium difficile* binds to:

an epitope within the carboxy terminal receptor binding domain of toxin A of *Clostridium difficile*, wherein the antibody comprises a HCVR/LCVR amino acid sequence pair

selected from the group consisting of SEQ ID NOs: 2/10, 98/106, 130/138, 146/154 and 162/170; or

an epitope outside of the carboxy terminal receptor binding domain of toxin A of *Clostridium difficile*, wherein the antibody comprises a HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 114/122.

[0033] In one embodiment, the invention provides a fully human monoclonal antibody or antigen-binding fragment thereof that binds specifically to toxin A of *C. difficile*, wherein the antibody or fragment thereof exhibits one or more of the following characteristics: (i) comprises a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 2, 98,114,130,146 and 162, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (ii) comprises a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 10, 106, 122, 138, 154 and 170, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (iii) comprises a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 8, 104,120,136,152 and 168, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 16, 112, 128, 144, 160 and 176, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (iv) comprises a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 4, 100, 116, 132, 148 and 164, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 6, 102, 118, 134, 150 and 166, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 108, 124, 140, 156 and 172, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 14, 110, 126, 142, 158 and 174, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (v) demonstrates a K_D equal to or less than 10^{-9}M; (vi) demonstrates neutralization of Toxin A (at a concentration of 32pM) with an IC50 ranging from about 7pM to about 65pM in a cell viability assay.

[0034] In one embodiment, the fully human monoclonal antibody or antigen binding fragment thereof that binds specifically to toxin A of *C. difficile* comprises a HCDR1 sequence comprising the formula $X^1 – X^2 – X^3 – X^4 – X^5 – X^6 – X^7 – X^8$ (SEQ ID NO: 387), wherein X^1 is Gly, or Arg, X^2 is Phe, X^3 is Asn, or Thr, X^4 is Phe, X^5 is Gly, Ser, Asn, or Thr, X^6 is Thr, Ser, Asn, or Asp, X^7 is His, Tyr, or Phe and X^8 is Asp, Val, Ala, or Tyr; a HCDR2 sequence comprising the formula

$X^1 – X^2 – X^3 – X^4 – X^5 – X^6 – X^7 – X^8$ (SEQ ID NO: 388), wherein X^1 is Leu, or Ile, X^2 is Thr, Gly, Ser, or Trp, X^3 is Ser, Thr, Gly, or Phe, X^4 is Thr, Val, Tyr, Val, Asp, or Gly, X^5 is Gly, X^6 is Gly, Asp, Ser, or Ala, X^7 is Ser, Thr, Asn, or Ala, and X^8 is Ala, Thr, Glu, Lys, or absent; a HCDR3 sequence comprising the formula $X^1 – X^2 – X^3 – X^4 – X^5 – X^6 – X^7 – X^8 – X^9 – X^{10} – X^{11}$ - $X^{12} – X^{13} – X^{14} – X^{15} – X^{16} – X^{17}– X^{18}$ - $X^{19} – X^{20} – X^{21} – X^{22} – X^{23} – X^{24}$ (SEQ ID NO: 389), wherein X^1 is Ala, X^2 is Lys, or Arg, X^3 is Thr, Asp, or Ser, X^4 is Phe, Arg, His, Ala, or Leu, X^5 is Asn, Gly, or Lys, X^6 is Trp, Gly, Asp, or Ile, X^7 is Asn, Ala, or Phe, X^8 is Ser, Asn, Tyr, Gly, or Asp, X^9 is Tyr, Ile, Ala, Thr, Glu, or Leu, X^{10} is Phe, Tyr, Ser, Gly, or absent, X^{11} is Asp. Ser, Gly, or absent, X^{12} is Tyr, Phe, Ser, Pro, or absent, X^{13} is Tyr, Leu, or absent, X^{14} is Tyr, Phe, or absent, X^{15} Gly, Asn, Asp, or absent, X^{16} is Met, Arg, Tyr, or absent, X^{17} is Asp, or absent, X^{18} is Tyr, Val, or absent, X^{19} is Tyr, or absent, X^{20} is Tyr, or absent, X^{21} is Gly, or absent, X^{22} is Met, or absent, X^{23} is Asp, or absent, X^{24} is Val, or absent; a LCDR1 sequence comprising the formula $X^1 – X^2 – X^3 – X^4 – X^5 – X^6 – X^7$ (SEQ ID NO: 390), wherein X^1 is Gln, X^2 is Ser, Asp, or Thr, X^3 is Ile, or Val, X^4 is Ser, X^5 is Thr, Asn, or Ser, X^6 is Tyr, Trp, Phe, or Ser and X^7 is Tyr, or absent; a LCDR2 sequence comprising the formula $X^1 – X^2 – X^3$ (SEQ ID NO: 391), wherein X^1 is Gly, Ala, Lys, or Thr, X^2 is Ala, Thr, or Val and X^3 is Ser; and a LCDR3 sequence comprising the formula $X^1 – X^2 – X^3 – X^4 – X^5 – X^6 – X^7 – X^8 – X^9 – X^{10}$ (SEQ ID NO: 392), wherein X^1 is Gln or absent, X^2 is Gln, Lys, or absent, X^3 is Tyr, Asn, or absent, X^4 is Gly, Asn, Thr, Tyr, His, or absent, X^5 is Asn, Ser, or absent, X^6 is Ser, Ala, Tyr, Asp, Trp, or absent, X^7 is Leu, Pro, Ser, or absent, X^8 is Tyr, Phe, Arg, Pro, or absent, X^9 is Thr, Tyr, or absent, and X^{10} is Thr.

[0035] In one embodiment, the invention provides an isolated human monoclonal antibody or antigen-binding fragment thereof that binds specifically to toxin B of *Clostridium difficile*, wherein the antibody comprises the three heavy chain CDRs (HCDR1, HCDR2 and HCDR3) contained within any one of the HCVR amino acid sequences selected from the group consisting of SEQ ID NOs: 178, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338 and 354; and the three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within any one of the LCVR amino acid sequences selected from the group consisting of SEQ ID NOs: 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346 and 362.

[0036] In one embodiment, the isolated human antibody or antigen-binding fragment thereof that binds specifically to toxin B of *Clostridium difficile* comprises a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 178, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338 and 354.

[0037] In one embodiment, the isolated human antibody or antigen-binding fragment thereof that binds specifically to toxin B of *Clostridium difficile* comprises a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346 and 362.

[0038] In one embodiment, the isolated human antibody or antigen-binding fragment thereof that binds specifically to toxin B of *Clostridium difficile* comprises (a) a HCVR having an amino

acid sequence selected from the group consisting of SEQ ID NOs: 178, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338 and 354; and (b) a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346 and 362.

[0039] In one embodiment, the isolated human antibody or antigen-binding fragment thereof that binds specifically to toxin B of *Clostridium difficile* comprises

(a) a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 180, 196, 212, 228, 244, 260, 276, 292, 308, 324, 340 and 356;

(b) a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 182, 198, 214, 230, 246, 262, 278, 294, 310, 326, 342 and 358;

(c) a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 184, 200, 216, 232, 248, 264, 280, 296, 312, 328, 344 and 360;

(d) a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 188, 204, 220, 236, 252, 268, 284, 300, 316, 332, 348 and 364;

(e) a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 190, 206, 222, 238, 254, 270, 286, 302, 318, 334, 350 and 366; and

(f) a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 192, 208, 224, 240, 256, 272, 288, 304, 320, 336, 352 and 368.

[0040] In one embodiment, the isolated human antibody or antigen-binding fragment thereof that binds specifically to toxin B of *Clostridium difficile*, comprises the HCDR1, HCDR2 and HCDR3 amino acid sequences of SEQ ID NO: 276, 278 and 280, respectively and LCDR1, LCDR2 and LCDR3 amino acid sequences of SEQ ID NO: 284, 286 and 288, respectively.

[0041] In one embodiment, the isolated human antibody or antigen-binding fragment thereof that binds specifically to toxin B of *Clostridium difficile* comprises a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 178/186, 194/202, 210/218, 226/234, 242/250, 258/266, 274/282, 290/298, 306/314, 322/330, 338/346 and 354/362.

[0042] In one embodiment, the isolated human antibody or antigen-binding fragment thereof that binds specifically to toxin B of *Clostridium difficile* comprises the HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 274/282.

[0043] In one embodiment, the isolated human antibody or antigen-binding fragment thereof that binds specifically to toxin B of *Clostridium difficile* binds to:

an epitope within the carboxy terminal receptor binding domain of toxin B of *Clostridium difficile*, wherein the antibody comprises a HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 178/186; or

an epitope outside of the carboxy terminal receptor binding domain of toxin B of *Clostridium difficile*, wherein the antibody comprises a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 194/202, 210/218, 226/234, 242/250, 258/266, 274/282 and 290/298.

[0044] In one embodiment, the invention provides a fully human monoclonal antibody or antigen-binding fragment thereof that binds specifically to toxin B of *C. difficile*, wherein the antibody or fragment thereof exhibits one or more of the following characteristics: (i) comprises a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 178, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338 and 354, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (ii) comprises a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NO:186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346 and 362, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (iii) comprises a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NO:184, 200, 216, 232, 248, 264, 280, 296, 312, 328, 344 and 360, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NO:192, 208, 224, 240, 256, 272, 288, 304, 320, 336, 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; (iv) comprises a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 180,196, 212, 228, 244, 260, 276, 292, 308, 324, 340 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 HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 182, 198, 214, 230, 246, 262, 278, 294, 310, 326, 342 and 358, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 188, 204, 220, 236, 252, 268, 284, 300, 316, 332, 348 and 364, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 190, 206, 222, 238, 254, 270, 286, 302, 318, 334, 350 and 366, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (v) demonstrates a K_D equal to or less than 10^{-9}M; (vi) demonstrates neutralization of Toxin B (at a concentration of 0.03pM) with an IC50 ranging from about 25pM to about 320pM in a cell viability assay.

[0045] In one embodiment, the fully human monoclonal antibody or antigen binding fragment thereof that binds specifically to toxin B of *C. difficile* comprises a HCDR1 sequence comprising the formula $X^1 – X^2 – X^3 – X^4 – X^5 – X^6 – X^7 – X^8 – X^9 – X^{10}$ (SEQ ID NO: 393), wherein X^1 is Gly, X^2 is Phe, Asp, or Tyr, X^3 is Thr, Asn, Ser, or Val, X^4 is Phe, or Val, X^5 is Ser, Arg, Lys, Glu, or Thr, X^6 is Ser, Ile, Asp, or Arg, X^7 is Phe, Tyr, or Asn, X^8 is Gly, Ala, Ser, or Tyr; X^9 is Ala, or absent and X^{10} is Ala or absent; a HCDR2 sequence comprising the formula $X^1 – X^2 – X^3 – X^4 – X^5 – X^6 – X^7 – X^8 – X^9$ (SEQ ID NO: 394), wherein X^1 is Ile, or Thr, X^2 is Ser, Gly, Tyr, or Asn,

X^3 is Thr, Gly, Tyr, Trp, Pro, or Ser, X^4 is Asp, Ser, Asn, Arg, Lys, or Asp, X^5 is Gly, Ser, or Thr, X^6 is Ser, Asp, Gly, Lys, or Asn, X^7 is Lys, Arg, Asn, Ser, Trp, or Gly, X^8 is Lys, Thr, Ile, or Tyr, X^9 is His, or absent; a HCDR3 sequence comprising the formula $X^1 – X^2 – X^3 – X^4 – X^5 – X^6 – X^7 – X^8 – X^9 – X^{10} – X^{11}$ - $X^{12} – X^{13} – X^{14} – X^{15} – X^{16}$ (SEQ ID NO: 395), wherein X^1 is Ala, or Val, X^2 is Arg, Lys, Thr, or Ser, X^3 is Val, Gly, Asp, Arg, or Tyr, X^4 is Gly, Trp, Arg, Lys, or Asn, X^5 is Glu, Tyr, Arg, Ser, or Trp, X^6 is Leu, Tyr, Ser, Pro, or Asn, X^7 Leu, Asp, Tyr, Ser, or Asp, X^8 is Asn, Ser, Phe, Lys, Arg, Asp, or Gly, X^9 is Tyr, Gly, Phe, Asp, Trp, or Val, X^{10} is Ser, Tyr, Asn, Asp, or absent, X^{11} is Tyr, Leu, Val, Gly, or absent, X^{12} is Tyr, Leu, Phe, Val, or absent, X^{13} is Asn, Gly, Asp, Phe, or absent, X^{14} is Tyr, Met, Asp, or absent, X^{15} Asp, Tyr, or absent, and X^{16} is Val, or absent; a LCDR1 sequence comprising the formula $X^1 – X^2 – X^3 – X^4 – X^5 – X^6 – X^7$ (SEQ ID NO: 396), wherein X^1 is Gln, Leu, or Arg, X^2 is Gly, Asp, or Ser, X^3 is Ile, or Val, X^4 is Arg, Ser, Gly, or Tyr, X^5 is Ser, or Asn, X^6 is Trp, His, Asn, Phe, Ser, or Asp, and X^7 is Tyr, or absent; a LCDR2 sequence comprising the formula $X^1 – X^2 – X^3$ (SEQ ID NO: 397), wherein X^1 is Ala, Ser, Asp, or Gly, X^2 is Ala, or Thr, and X^3 is Ser; and a LCDR3 sequence comprising the formula $X^1 – X^2 – X^3 – X^4 – X^5 – X^6 – X^7 – X^8 – X^9$ (SEQ ID NO: 398), wherein X^1 is Gln, His, or Leu, X^2 is Gln, X^3 is Ala, Tyr, Arg, Asp, His, or Val, X^4 is Tyr, Gly, Asn, Ser, Ile, or Lys, X^5 is Ser, Leu, Pro, Ile, Asn, Thr, or Gly, X^6 is Phe, Tyr, Trp, or Ser, X^7 is Pro, X^8 is Leu, Pro, Phe, Val, or Tyr and X^9 is Thr.

[0046] In one embodiment, the invention provides an isolated antibody or antigen-binding fragment thereof that competes for specific binding to *C. difficile* toxin A and/or toxin B with an antibody or antigen-binding fragment comprising the complementarity determining regions (CDRs) of a heavy chain variable region (HCVR), wherein the HCVR has an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338 and 354; and the CDRs of a light chain variable region (LCVR), wherein the LCVR has an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346 and 362.

[0047] In a related embodiment, the invention provides an isolated antibody or antigen-binding fragment thereof that competes for specific binding to *C. difficile* toxin A and/or toxin B with an antibody or antigen-binding fragment comprising the heavy and light chain CDRs contained within heavy and light chain sequence pairs selected from the group consisting of SEQ ID NOs: 18/26, 34/42, 146/154 and 274/282.

[0048] In one embodiment, the invention provides an isolated antibody or antigen-binding fragment thereof that binds the same epitope on *C. difficile* toxin A and/or toxin B as an antibody or antigen-binding fragment comprising the CDRs of a heavy chain variable region (HCVR), wherein the HCVR has an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338 and 354; and the CDRs of a light chain variable region (LCVR), wherein the

LCVR has an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346 and 362.

[0049] In a related embodiment, the invention provides an isolated antibody or antigen-binding fragment thereof that binds the same epitope on *C. difficile* toxin A and/or toxin B as an antibody or antigen-binding fragment comprising the heavy and light chain CDRs contained within heavy and light chain sequence pairs selected from the group consisting of SEQ ID NOs: 18/26, 34/42, 146/154, 274/282.

[0050] In certain embodiments of the invention, the antibodies may interact with, or bind to, amino acid residues 468-863 of the carboxy terminal receptor binding domain of toxin A produced by *Clostridium difficile*, the sequence of which is shown in SEQ ID NO: 375. This region corresponds to amino acid residues ranging from residues 2315-2710 of SEQ ID NO: 378 (full length toxin A). In certain embodiments of the invention, the antibodies may interact with, or bind to, an epitope in the carboxy terminal receptor binding domain of toxin A produced by *Clostridium difficile*, wherein the epitope is selected from the group consisting of residues 468-488 of SEQ ID NO: 375, residues 510-530 of SEQ ID NO: 375, residues 602-610 of SEQ ID NO: 375, residues 644-703 of SEQ ID NO: 375, residues 724-794 of SEQ ID NO: 375, residues 799-814 of SEQ ID NO: 375 and residues 858-863 of SEQ ID NO: 375. These residues correspond to the amino acid sequences found in the full length toxin A sequence having SEQ ID NO: 378, with the particular regions identified as residues 2315-2335 of SEQ ID NO: 378, residues 2357-2377 of SEQ ID NO: 378, residues 2449-2457 of SEQ ID NO: 378, residues 2491-2550 of SEQ ID NO: 378, residues 2571-2641 of SEQ ID NO: 378, residues 2646-2661 of SEQ ID NO: 378 and residues 2705-2710 of SEQ ID NO: 378. In one embodiment, the antibody that binds to or interacts with an epitope in the carboxy terminal receptor binding domain of toxin A produced by *Clostridium difficile*, selected from the group consisting of residues 468-488 of SEQ ID NO: 375, residues 510-530 of SEQ ID NO: 375, residues 602-610 of SEQ ID NO: 375, residues 644-703 of SEQ ID NO: 375, residues 724-794 of SEQ ID NO: 375, residues 799-814 of SEQ ID NO: 375 and residues 858-863 of SEQ ID NO: 375 comprises the HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 146/154. In one embodiment, the antibody that binds to or interacts with an epitope in the carboxy terminal receptor binding domain of toxin A produced by *Clostridium difficile*, selected from the group consisting of residues 468-488 of SEQ ID NO: 375, residues 510-530 of SEQ ID NO: 375, residues 602-610 of SEQ ID NO: 375, residues 644-703 of SEQ ID NO: 375, residues 724-794 of SEQ ID NO: 375, residues 799-814 of SEQ ID NO: 375 and residues 858-863 of SEQ ID NO: 375 is combined with a second antibody that binds specifically to toxin B of *Clostridium difficile* in a pharmaceutical composition. In one embodiment, this second antibody that interacts with or binds to toxin B of *Clostridium difficile* comprises the HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 274/282.

[0051] In a second aspect, the invention provides nucleic acid molecules encoding anti-toxin A and/or anti-toxin B antibodies or fragments thereof. Recombinant expression vectors carrying the nucleic acids of the invention, and host cells into which such vectors have been introduced, are also encompassed by the 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.

[0052] In one embodiment, the 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, 145, 161, 177, 193, 209, 225, 241, 257, 273, 289, 305, 321, 337 and 353 or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof.

[0053] In one embodiment, the HCVR is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 17, 33, 145 and 273.

[0054] In one embodiment, the antibody or fragment thereof further comprises 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, 153, 169, 185, 201, 217, 233, 249, 265, 281, 297, 313, 329, 345 and 361, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof.

[0055] In one embodiment, the LCVR is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 25, 41, 153 and 281.

[0056] In one embodiment, the 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, 151, 167, 183, 199, 215, 231, 247, 263, 279, 295, 311, 327, 343 and 359 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; 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, 159, 175, 191, 207, 223, 239, 255, 271, 287, 303, 319, 335, 351 and 367, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0057] In one embodiment, the invention provides an antibody or fragment thereof further comprising 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, 147, 163, 179, 195, 211, 227, 243, 259, 275, 291, 307, 323, 339 and 355, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; 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, 149, 165, 181, 197, 213, 229, 245, 261, 277, 293, 309, 325, 341 and 357, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; 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, 155, 171, 187, 203, 219, 235, 251, 267, 283, 299, 315, 331, 347 and 363, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; 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, 157, 173, 189, 205, 221, 237, 253, 269, 285, 301, 317, 333, 349 and 365, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0058] In a third aspect, the invention features a human antibody or antigen-binding fragment specific for toxin A and/or toxin B of *C. difficile* comprising a HCVR encoded by nucleotide sequence segments derived from V_H, D_H and J_H germline sequences, and a LCVR encoded by nucleotide sequence segments derived from V_K and J_K germline sequences, with combinations as shown in Table 2.

[0059] The invention encompasses antibodies having a modified glycosylation pattern. In some applications, modification to remove undesirable glycosylation sites may be useful, or e.g., removal of a fucose moiety 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).

[0060] In a fourth aspect, the invention provides a pharmaceutical composition comprising at least one isolated fully human monoclonal antibody or antigen-binding fragment thereof that binds to either toxin A or toxin B of *C. difficile*, or that binds to both toxin A and toxin B of *C. difficile* and a pharmaceutically acceptable carrier or diluent. In one embodiment, the invention provides a pharmaceutical composition comprising an isolated fully human monoclonal antibody or antigen-binding fragment thereof that binds specifically to only toxin A of *C. difficile* and a pharmaceutically acceptable carrier or diluent. In one embodiment, the invention provides a pharmaceutical composition comprising an isolated fully human monoclonal antibody or antigen-binding fragment thereof that binds specifically to only toxin B of *C. difficile* and a pharmaceutically acceptable carrier or diluent. In one embodiment, the invention provides a pharmaceutical composition comprising two fully human monoclonal antibodies or antigen-binding fragments thereof, one that binds specifically to toxin A and one that binds specifically to toxin B of *C. difficile* and a pharmaceutically acceptable carrier or diluent. In one embodiment, the invention provides a pharmaceutical composition comprising one dual binding fully human monoclonal antibody (an antibody that binds to both toxin A and toxin B) and a pharmaceutically acceptable carrier or diluent. In one embodiment, the invention provides a pharmaceutical composition comprising two dual binding fully human monoclonal antibodies (an antibody that binds to both toxin A and toxin B) and a pharmaceutically acceptable carrier or diluent. The dual antibodies used in the pharmaceutical composition may recognize and/or bind to the same epitope on toxin A or toxin B, or may recognize and/or bind to different epitopes on toxin A or toxin B. It is to be understood that any combination of antibodies as described herein

may be used in a pharmaceutical composition to achieve the desired results in the patient population in need of such therapy. For example, two antibodies that recognize and/or bind only toxin A may be used in a composition. Alternatively, two antibodies that recognize and/or bind only toxin B may be used in a composition. In one embodiment, one antibody that recognizes/binds to only toxin A or toxin B may be combined with a dual binding antibody in a composition. In one embodiment, one antibody that recognizes/binds to only toxin A may be combined with one antibody that recognizes/binds to only toxin B and this combination may be used in a composition.

[0061] In one embodiment, the pharmaceutical composition comprises a fully human monoclonal antibody that binds to the carboxy terminal receptor binding domain of both toxin A and toxin B of *C. difficile* having any one or more of the characteristics described herein. The antibody that binds to the carboxy terminal receptor binding domain of both toxin A and toxin B of *C. difficile* binds toxin A and toxin B with a K_D equal to or less than 10^{-7}M.

[0062] In one embodiment, the composition comprises an antibody that binds both toxin A and toxin B of *C. difficile* and has a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 18/26, 34/42, 50/58, 66/74 and 82/90.

[0063] In one embodiment, the composition comprises an antibody that binds both toxin A and toxin B of *C. difficile* and has a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 18/26 and 34/42.

[0064] In one embodiment, the pharmaceutical composition comprises at least one antibody that binds a *Clostridium difficile* toxin, wherein the antibody is selected from:

a) an isolated antibody or antigen-binding fragment thereof that specifically binds toxin A of *Clostridium difficile*, wherein the antibody comprises the three heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) contained within any one of the heavy chain variable region (HCVR) amino acid sequences selected from the group consisting of SEQ ID NOs: 2, 98, 114, 130, 146 and 162; and the three light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3) contained within any one of the light chain variable region (LCVR) amino acid sequences selected from the group consisting of SEQ ID NOs: 10, 106, 122, 138, 154 and 170;

b) an isolated antibody or antigen-binding fragment thereof that specifically binds toxin B of *Clostridium difficile*, wherein the antibody comprises the three heavy chain CDRs (HCDR1, HCDR2 and HCDR3) contained within any one of the HCVR amino acid sequences selected from the group consisting of SEQ ID NOs: 178, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338 and 354; and the three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within any one of the LCVR amino acid sequences selected from the group consisting of SEQ ID NOs: 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346 and 362; and

c) an isolated antibody or antigen-binding fragment that binds to/cross reacts with both toxin A and toxin B of *Clostridium difficile*, wherein the antibody comprises the three heavy

chain CDRs (HCDR1, HCDR2 and HCDR3) contained within any one of the HCVR amino acid sequences selected from the group consisting of SEQ ID NOs: 18, 34, 50, 66 and 82; and the three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within any one of the LCVR amino acid sequences selected from the group consisting of SEQ ID NOs: 26, 42, 58, 74 and 90.

[0065] In one embodiment, the pharmaceutical composition comprises an isolated first fully human monoclonal antibody or antigen-binding fragment thereof that specifically binds toxin A of *Clostridium difficile,* as described herein, and an isolated second fully human monoclonal antibody or antigen-binding fragment thereof that specifically binds toxin B of *Clostridium difficile*, as described herein, and a pharmaceutically acceptable carrier or diluent.

[0066] In one embodiment, the composition comprises at least one antibody, or an antigen-binding fragment thereof that binds specifically to toxin A of *Clostridium difficile* and at least one antibody, or an antigen-binding fragment thereof that binds specifically to toxin B of *Clostridium difficile*, wherein:

a) the antibody or antigen-binding fragment thereof that binds specifically to toxin A comprises the three heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) contained within any one of the heavy chain variable region (HCVR) amino acid sequences selected from the group consisting of SEQ ID NOs: 2, 98, 114, 130, 146 and 162; and the three light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3) contained within any one of the light chain variable region (LCVR) amino acid sequences selected from the group consisting of SEQ ID NOs: 10, 106, 122, 138, 154 and 170; and wherein

b) the antibody or antigen-binding fragment thereof that binds specifically to toxin B comprises the three heavy chain CDRs (HCDR1, HCDR2 and HCDR3) contained within any one of the HCVR amino acid sequences selected from the group consisting of SEQ ID NOs: 178, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338 and 354; and the three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within any one of the LCVR amino acid sequences selected from the group consisting of SEQ ID NOs: 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346 and 362.

[0067] In one embodiment, the pharmaceutical composition comprises:

a) an isolated first fully human monoclonal antibody, or antigen-binding fragment thereof that specifically binds toxin A of *Clostridium difficile*, which comprises a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 98, 114, 130, 146 and 162; and a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 106, 122, 138, 154 and 170; and

b) an isolated second fully human monoclonal antibody, or antigen-binding fragment thereof that specifically binds toxin B of *Clostridium difficile*, which comprises a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 178, 194, 210,

226, 242, 258, 274, 290, 306, 322, 338 and 354; and a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346 and 362.

[0068] In one embodiment, the pharmaceutical composition comprises an isolated first fully human monoclonal antibody or antigen-binding fragment thereof that specifically binds toxin A of *C. difficile*, which comprises a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/10, 98/106, 114/122, 130/138, 146/154 and 162/170; and an isolated second fully human monoclonal antibody or antigen-binding fragment thereof that specifically binds toxin B of *C. difficile*, which comprises a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 178/186, 194/202, 210/218, 226/234, 242/250, 258/266, 274/282, 290/298, 306/314, 322/330, 338/346 and 354/362.

[0069] In another embodiment, the pharmaceutical composition comprises an isolated first fully human monoclonal antibody or antigen-binding fragment thereof that specifically binds toxin A of *C. difficile*, which comprises a HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 146/154; and an isolated second fully human antibody or antigen-binding fragment thereof that specifically binds toxin B of *C. difficile*, which comprises a HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 274/282.

[0070] In another related embodiment, the pharmaceutical composition comprises:

a) an isolated first human antibody, or antigen-binding fragment thereof that specifically binds toxin A of *Clostridium difficile*, comprising a HCDR1 having the amino acid sequence of SEQ ID NO: 148, a HCDR2 having the amino acid sequence of SEQ ID NO: 150, a HCDR3 having the amino acid sequence of SEQ ID NO: 152, a LCDR1 having the amino acid sequence of SEQ ID NO: 156, a LCDR2 having the amino acid sequence of SEQ ID NO: 158, a LCDR3 having the amino acid sequence of SEQ ID NO: 160;

b) an isolated second human antibody, or antigen-binding fragment thereof that specifically binds toxin B of *Clostridium difficile*, comprising a HCDR1 having the amino acid sequence of SEQ ID NO: 276, a HCDR2 having the amino acid sequence of SEQ ID NO: 278, a HCDR3 having the amino acid sequence of SEQ ID NO: 280, a LCDR1 having the amino acid sequence of SEQ ID NO: 284, a LCDR2 having the amino acid sequence of SEQ ID NO: 286, a LCDR3 having the amino acid sequence of SEQ ID NO: 288; and

c) a pharmaceutically acceptable carrier or diluent.

[0071] In one embodiment, the antibodies of the invention, or compositions containing one or more antibodies of the invention may be used to neutralize either toxin A, or toxin B, or both toxin A and B from any strain of *Clostridium difficile*.

[0072] In one embodiment, the antibodies of the invention, or compositions containing one or more antibodies of the invention may be used to neutralize toxins A and/or B from a hypervirulent strain of *Clostridium difficile*.

[0073] In one embodiment, the antibodies of the invention, or compositions containing one or more antibodies of the invention may be used to neutralize toxins A and/or B from a BI/NAP1/027 strain.

[0074] In one embodiment, the antibodies of the invention, or compositions containing one or more antibodies of the invention, may be used to neutralize toxins A and/or B from a BI/NAP1/027 strain, wherein the BI/NAP1/027 strain is selected from the group consisting of VA5, VA17, 6336 and 6443.

[0075] In one embodiment, the antibody composition comprising a first antibody that binds specifically to toxin A, may be administered alone as a separate composition and the antibody composition comprising the second antibody that binds specifically to toxin B may also be administered as a separate composition. Each composition may be prepared for delivery to the patient in separate syringes, or delivery devices, or vials. When formulated separately as two compositions, both compositions may be delivered separately, with one antibody composition being given immediately prior to the other antibody composition. Alternatively, the two antibody compositions may be mixed together shortly before administration and given concurrently.

[0076] In one embodiment, the invention features a composition, which is a combination of an antibody or antigen-binding fragment of an antibody of the invention, and a second therapeutic agent.

[0077] The second therapeutic agent may be a small molecule drug, a protein/polypeptide, an antibody, a nucleic acid molecule, such as an anti-sense molecule, or a siRNA. The second therapeutic agent may be synthetic or naturally derived.

[0078] The second therapeutic agent may be any agent that is advantageously combined with the antibody or fragment thereof of the invention, for example, a probiotic, an antibiotic, a toxoid, a vaccine specific for *C. difficile*, or a second different antibody against *C. difficile* toxin A and/or toxin B.

[0079] In certain embodiments, the second therapeutic agent may be an agent that helps to counteract or reduce any possible side effect(s) associated with the antibody or antigen-binding fragment of an antibody of the invention, if such side effect(s) should occur.

[0080] It will also be appreciated that the antibodies and pharmaceutically acceptable compositions of the present invention can be employed in combination therapies, that is, the antibodies and pharmaceutically acceptable compositions can be administered concurrently with, prior to, or subsequent to, one or more other desired therapeutics or medical procedures. The particular combination of therapies (therapeutics or procedures) to employ in a combination regimen will take into account compatibility of the desired therapeutics and/or procedures and the desired therapeutic effect to be achieved. It will also be appreciated that the therapies employed may achieve a desired effect for the same disorder (for example, an antibody may be administered concurrently with another agent used to treat the same disorder), or they may achieve different effects (*e.g.,* control of any adverse effects). As used herein, additional

therapeutic agents that are normally administered to treat or prevent a particular disease, or condition, are appropriate for the disease, or condition, being treated.

[0081] When multiple therapeutics are co-administered, dosages may be adjusted accordingly, as is recognized in the pertinent art.

[0082] A fifth aspect of the invention provides a method for treating a patient suffering from a *Clostridium difficile*-associated condition or disease, or for treating at least one symptom or complication associated with the condition or disease, or for preventing the development of a *Clostridium difficile*-associated condition or disease in a patient at risk thereof, the method comprising administering to the patient an effective amount of an antibody or an antigen-binding fragment thereof that binds to *C. difficile* toxin A and/or toxin B; or a pharmaceutical composition comprising an effective amount of an antibody or an antigen-binding fragment thereof that binds to *Clostridium difficile* toxin A and/or toxin B, such that the *Clostridium difficile*-associated condition or disease is either prevented, or lessened in severity and/or duration, or at least one symptom or complication associated with the condition or disease is prevented, or ameliorated, or that the frequency and/or duration of, or the severity of recurrences, or relapses with *Clostridium difficile* is reduced.

[0083] In one embodiment, the invention provides for use of one or more antibodies of the invention, or pharmaceutical compositions comprising one or more antibodies of the invention in the manufacture of a medicament for use in treating a patient suffering from a *Clostridium difficile*-associated condition or disease, or for treating at least one symptom or complication associated with the condition or disease, or for preventing the development of a *Clostridium difficile*-associated condition or disease in a patient at risk thereof, wherein the *Clostridium difficile*-associated condition or disease is either prevented, or lessened in severity and/or duration, or at least one symptom or complication associated with the condition or disease is prevented, or ameliorated, or that the frequency and/or duration of, or the severity of recurrences, or relapses with *Clostridium difficile* is reduced. The at least one symptom or complication associated with the *Clostridium difficile*-associated condition or disease may be selected from the group consisting of anorexia, abdominal pain, abdominal bloating, diarrhea with or without bleeding, dehydration, malnutrition, pseudomembranous colitis, complete or segmental colonic resection, fever and systemic infection (sepsis), death, relapse of the *Clostridium difficile* condition or disease, and rejection of a transplanted tissue or organ.

[0084] In one embodiment, the patient to be treated with the antibodies of the invention, or with the pharmaceutical compositions comprising one or more antibodies of the invention are infected with a hypervirulent isolate of *Clostridium difficile*, such as one belonging to the BI/NAP1/027 group, or may be at risk for developing an infection with a hypervirulent strain, as described herein.

[0085] In a related embodiment, the antibodies of the invention, or a pharmaceutical composition containing one or more antibodies of the invention may be used to neutralize the

toxins produced by a hypervirulent strain of *Clostridium difficile*, such as but not limited to any of those belonging to the BI/NAP1/027 group of strains. Included in these hypervirulent strains are clinical isolates noted herein as VA5, VA17, 6336 and 6443, described herein in Example 10.

[0086] In one embodiment, the patient at risk of developing a *Clostridium difficile*-associated condition or disease, who may benefit from treatment with the antibodies of the invention, or with a composition comprising one or more antibodies of the invention, may be selected from an elderly (≥ 65 years old) patient, a patient who is immunocompromised due to underlying illness or due to administration of immunosuppressive therapeutics, a patient who has some underlying medical condition that may pre-dispose them to acquiring a *Clostridium difficile* infection, a patient hospitalized for an extended period of time (one week or more), a patient who has been treated for an extended period of time (≥ 14 days) with broad spectrum antibiotics, a cancer patient, a transplant patient, and a patient on therapy with agents such as but not limited to a proton pump inhibitor, or histamine H2 receptor inhibitor that are used for treatment of gastrointestinal diseases or conditions to reduce or treat gastric acidity, gastroesophageal reflux disease (GERD), stomach and small intestine ulcers, or heartburn.

[0087] In one embodiment, the patient at risk of developing a *Clostridium difficile*-associated condition or disease is a cancer patient. In a related embodiment, the cancer patient is undergoing treatment with an anti-cancer drug, or undergoing radiotherapy to treat a cancer.

[0088] In one embodiment, the patient at risk of developing a *Clostridium difficile*-associated condition or disease is a transplant patient. In a related embodiment, the transplant patient is a patient receiving a hematopoietic stem cell transplant, or a solid tissue or organ transplant. In certain embodiments, the transplant patient is being treated with an immunosuppressive drug, or any transplant rejection drug, or is a patient who is undergoing treatment with a drug regimen to prevent tissue or organ graft rejection following the transplant.

[0089] In one embodiment, the antibody is administered therapeutically (administered after the infection has been established and given throughout the course of the infection) to a patient suffering from a *Clostridium difficile*-associated condition or disease, or suffering from at least one symptom or complication associated with the condition or disease. In one embodiment, the antibody is administered prophylactically (administered prior to development of the infection) to a patient at risk for developing a *Clostridium difficile*-associated condition or disease, or at risk for developing at least one symptom or complication associated with the *Clostridium difficile* condition or disease. For example, such "patients at risk for developing a *Clostridium difficile* infection" include the elderly (65 years of age or older), or patients who may be immunocompromised due to illness or due to administration of immunosuppressive therapeutics, or patients who have some underlying medical condition that may pre-dispose them to acquiring a *Clostridium difficile* infection, or patients hospitalized for long periods of time (generally one week or longer), or patients who have been treated for a long period of time with

broad spectrum antibiotics (generally 14 days or longer), or patients on therapy with proton pump inhibitors for treatment of gastrointestinal diseases or conditions. Other patients at risk for developing a *Clostridium difficile* infection are those patients that are in need of a tissue or organ transplant, who would be undergoing treatment with immunosuppressive drugs to prevent tissue or organ rejection. This patient population includes individuals in need of either an autologous or allogeneic hematopoietic stem cell transplant. The long hospitalization required for these patients, in addition to receipt of high doses of antibiotic therapy to prevent other types of infections may pre-dispose these patients to acquiring a primary *C. difficile* infection. Alternatively, if a patient in need of such a transplant already suffers from a *C. difficile* infection, or has exhibited symptoms of a *C. difficile* infection, that patient may be prone to a recurrence, or exacerbation of such infection when placed on high dose antibiotic therapy, then followed by immunosuppressive therapy to prevent graft rejection. Furthermore, these transplant patients may be at risk not only for acquiring a *C. difficile* infection, but also may be at risk for rejection of the transplant due to GI related graft versus host disease (GI-GVHD), which appears to be enhanced in transplant patients suffering from infection with *C. difficile* (See Alonso, C.D. *et.al.,* (2012), Clin. Infect. Dis. 54, 1053–1063. The relationship between *C. difficile* infection and GVHD involving the GI tract is unclear at this time, but it is appears that this patient population would benefit from therapy with the anti-toxin A and/or anti-toxin B antibodies of the invention. While it is envisioned that this patient population may be treated therapeutically (after the start of the infection), it is also contemplated that these patients would benefit from prophylactic (prior to infection) administration of any of the antibodies of the invention. The patients who are candidates for treatment with the antibodies of the invention may be administered the compositions comprising one or more antibodies by any route of delivery suitable for administration, including but not limited to intravenous injection, or subcutaneous injection.

[0090] In one embodiment, the pharmaceutical composition comprising the antibodies of the invention is administered to the patient in combination with one or more therapeutic agents useful for treating a *C. difficile* infection.

[0091] In one embodiment, the one or more therapeutic agents may be selected from the group consisting of a toxoid, a probiotic, a *C. difficile* vaccine (*e.g.*, inactivated toxins A and B, such as, but not limited to ACAM-CDIFF™), an antibiotic (e.g. metronidazole, vancomycin or fidaxomicin), another different antibody to *C. difficile* toxin A and/or B, and any other palliative therapy useful for reducing the severity of the *C. difficile* disease or for reducing the frequency of recurrence of the *C. difficile* disease or for ameliorating at least one symptom associated with a *C. difficile*-associated condition or disease.

[0092] In another embodiment, the one symptom or complication associated with the *C. difficile*-associated condition or disease is selected from the group consisting of diarrhea,

pseudomembranous colitis, relapse/recurrence of the *Clostridium difficile* condition or disease, and rejection of a transplanted tissue or organ.

[0093] Other embodiments will become apparent from a review of the ensuing detailed description.

BRIEF DESCRIPTION OF THE FIGURES

[0094] Figure 1 shows the domain structures of Toxin A and Toxin B from *Clostridium difficile* (See Davies AH, *et al.,* Biochem. J. (2011), 436:517-526).

[0095] Figure 2 is a graph showing results of hamster relapse assays as the percentage of hamsters surviving clindamycin and vancomycin treatment following *C. difficile* challenge and the effect of treatment with anti-toxin A and anti-toxin B mAbs. All antibodies were given subcutaneously once a day on days 3-6. Positive control antibodies are comparator antibodies, anti-Toxin A (control I) and anti-Toxin B (control II). Vancomycin was given as an oral dose at 10 mg/kg on days 1-3 to all animals. (● with dotted line: PBS control; △ with dotted line: Negative isotype control at 10 mg/kg; □ with solid line: Control I/Control II at 5 mg/kg each (5/5): ◆ with solid line: H1H3330P/H1H3347P at 5 mg/kg each (5/5)).

[0096] Figure 3 is a graph showing results of hamster relapse assays as the percentage of hamsters surviving clindamycin and vancomycin treatment following *C. difficile* challenge and the effect of anti-toxin A and anti-toxin B mAbs. All antibodies were given subcutaneously once on day 3. Positive control antibodies are comparator antibodies, anti-Toxin A (control I) and anti-Toxin B (control II). Vancomycin was given as an oral dose at 10 mg/kg on days 1-3 to all animals. (△ with dotted line: Negative isotype control at 10 mg/kg; □ with solid line: Control I/Control II at 5 mg/kg each (5/5); ◆ with solid line: H1H3330P/H1H3347P at 5 mg/kg each (5/5); O with solid line: Control I/Control II at 2 mg/kg each (2/2); ⬡ with solid line: H1H3330P/H1H3347P at 2 mg/kg each (2/2)).

[0097] Figure 4 is a graph showing survival results in an acute model of *C. difficile* infection in hamsters. Results are shown as the percentage of hamsters surviving *C. difficile* challenge (day 0) following clindamycin treatment (day -1). All antibodies were given subcutaneously on each of 4 days from day -3 to day 0. Antibodies were administered at 50 mg/kg each (50/50), 16.6 mg/kg each (16.6/16.6), 5.5 mg/kg each (5.5/5.5) and 1.85 mg/kg each (1.85/1.85). (▽ with solid line: Uninfected; ● with dotted line: PBS control; △ with dotted line: Negative isotype control at 100 mg/kg; ◆ with solid line: H1H3330P/H1H3347P at 50 mg/kg each (50/50); O with solid line: H1H3330P/H1H3347P at 16.6 mg/kg each (16.6/16.6); □ with solid line: H1H3330P/H1H3347P at 5.5 mg/kg each (5.5/5.5); ┼ with a solid line: H1H3330P/H1H3347P at 1.85 mg/kg each (1.85/1.85).

[0098] Figure 5 is a graph showing survival results in an acute model of *C. difficile* infection in hamsters. Results are shown as the percentage of hamsters surviving *C. difficile* challenge (day 0) following clindamycin treatment (day -1). All antibodies were given subcutaneously on each

of 4 days from day -3 to day 0. Antibodies were administered at 20 mg/kg each (20/20), or at 5 mg/kg each (5/5). (▽ with solid line: Uninfected; ● with dotted line: PBS control; △ with dotted line: Negative isotype control at 40 mg/kg; □ with solid line: Control I/Control II at 20 mg/kg each (20/20); ⊠ with solid line: Control I/Control II at 5 mg/kg each (5/5); ◆ with solid line: H1H3330P/H1H3347P at 20 mg/kg each (20/20); ◊ with solid line: H1H3330P/H1H3347P at 5 mg/kg each (5/5)).

DETAILED DESCRIPTION

[0099] Before the present methods are 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.

[0100] 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. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, preferred methods and materials are now described.

Definitions

[0101] The term "toxin A" (also referred to as "tcdA") refers to the toxin A protein produced by *Clostridium difficile* (also referred to herein as "*C. difficile*"). The amino acid sequence of "toxin A' is provided in GenBank as accession number CAA63564 and is also referred to herein as SEQ ID NO: 378. Toxin A is encoded by the nucleic acid provided herein as SEQ ID NO: 377, and is also found in GenBank as accession number AM180355.

[0102] The term "toxin B" (also referred to as "tcdB") refers to the toxin B protein produced by *Clostridium difficile*. The amino acid sequence of "toxin B' is provided in GenBank as accession number CAJ67492 and is also referred to herein as SEQ ID NO: 380. Toxin B is encoded by the nucleic acid provided herein as SEQ ID NO: 379, and is also found in GenBank as accession number AM180355.

[0103] The "carboxy terminal receptor binding domain of toxin A and toxin B of *Clostridium difficile*" refers to the portion of toxin A and toxin B from *C. difficile* that is responsible for binding to the target cell, thus allowing for subsequent receptor mediated endocytosis. As described herein, the amino acid sequence of the carboxy terminal receptor binding domain of toxin A is shown in SEQ ID NO: 375. The amino acid sequence of the carboxy terminal receptor binding domain of toxin B is shown in SEQ ID NO: 376. The various domains of toxin A and toxin B from *C. difficile* are illustrated in Figure 1 and further described in Davies *et al.* (Davies, AH, *et al.*, Biochem. J. (2011), 436:517-526).

[0104] The "BI/NAP1/027" designation for *Clostridium difficile* refers to a highly virulent group

of isolates of *Clostridium difficile* that has been associated with an increase in morbidity and mortality throughout Europe and North America (Loo, VG, *et al.*, (2005), N Engl J Med, 353:2442-9; McDonald, LC *et al.* (2006), Emerg Infect Dis, 12:409-15; McDonald, LC, *et al.*,(2005), N Engl J Med, 353:2433-41; Redelings, MD, *et al.*, (2007), Emerg Infect Dis 13:1417-9). The "BI/NAP1/027" designation further refers to North American pulsed-field type I (NAP1), ribotype 027, and group BI by restriction endonuclease analysis. It was originally identified in the 1980s, but was not originally identified as being resistant to the newer fluoroquinolone agents and was not epidemic prior to 2000 (Warny, M. et al., (2005), Lancet 366:1079-84; Kelly, CP, et al., N Engl J Med 359:1932-40). The "BI/NAP1/027" strain of *Clostridium difficile* is also characterized by increased toxin A and toxin B production, by the presence of an additional toxin (binary toxin), and increased resistance to fluoroquinolones (McDonald, LC, *et al.*,(2005), N Engl J Med, 353:2433-41; Warny, M. et al., (2005), Lancet 366:1079-84).

[0105] The term "antibody", as used herein, is intended to refer to immunoglobulin molecules comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds (*i.e.*, "full antibody molecules"), as well as multimers thereof (*e.g.* IgM) or antigen-binding fragments thereof. Each heavy chain is comprised of a heavy chain variable region ("HCVR" or "V_H") and a heavy chain constant region (comprised of domains C_H1, C_H2 and C_H3). Each light chain is comprised of a light chain variable region ("LCVR or "V_L") and a light chain constant region (C_L). The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), 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 certain embodiments of the invention, the FRs of the antibody (or antigen binding fragment 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.

[0106] Substitution of one or more CDR residues or omission of one or more CDRs is also possible. Antibodies have been described in the scientific literature in which one or two CDRs can be dispensed with for binding. Padlan *et al.* (1995 FASEB J. 9:133-139) analyzed the contact regions between antibodies and their antigens, based on published crystal structures, and concluded that only about one fifth to one third of CDR residues actually contact the antigen. Padlan also found many antibodies in which one or two CDRs had no amino acids in contact with an antigen (see also, Vajdos *et al.* 2002 J Mol Biol 320:415-428).

[0107] CDR residues not contacting antigen can be identified based on previous studies (for example residues H60-H65 in CDRH2 are often not required), from regions of Kabat CDRs lying outside Chothia CDRs, by molecular modeling and/or empirically. If a CDR or residue(s)

thereof is omitted, it is usually substituted with an amino acid occupying the corresponding position in another human antibody sequence or a consensus of such sequences. Positions for substitution within CDRs and amino acids to substitute can also be selected empirically. Empirical substitutions can be conservative or non-conservative substitutions.

[0108] The fully human anti-toxin A and/or anti-toxin B monoclonal 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. 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.

[0109] The present invention also includes fully human anti-toxin A and/or anti-toxin B monoclonal 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-toxin A and anti-toxin B 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.

[0110] 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 mAbs 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 mAbs in which CDR sequences derived from the germline of another mammalian species (e.g., mouse), have been grafted onto human FR sequences.

[0111] The term "specifically binds," or “binds specifically to”, 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. Specific binding can be characterized by an equilibrium dissociation constant of at least about $1x10^{-6}$ M or less (*e.g.*, a smaller K_D denotes a tighter binding). Methods for determining whether two molecules specifically bind are well known in the art and include, for example, equilibrium dialysis, surface plasmon resonance, and the like. As described herein, antibodies have been identified by surface plasmon resonance, *e.g.*, BIACORE™, which bind specifically to either toxin A, or specifically to toxin B from *C. difficile*, while others have been identified that bind specifically to the carboxy terminal receptor binding domain of both toxin A and B. Moreover, multi-specific antibodies that bind to toxin A or toxin B and one or more additional antigens or a bi-specific that binds to two different regions of toxin A or toxin B are nonetheless considered antibodies that “specifically bind”, as used herein.

[0112] The term “high affinity” antibody refers to those mAbs having a binding affinity to toxin A or toxin B, expressed as K_D, of at least 10^{-8} M; preferably 10^{-9} M; more preferably 10^{-10}M, even more preferably 10^{-11} M, even more preferably 10^{-12} M, as measured by surface plasmon resonance, e.g., BIACORE™ or solution-affinity ELISA.

[0113] By the term “slow off rate”, “Koff” or “kd” is meant an antibody that dissociates from toxin A or toxin B, or both, with a rate constant of 1×10^{-3} s^{-1} or less, preferably 1×10^{-4} s^{-1} or less, as determined by surface plasmon resonance, *e.g.*, BIACORE™.

[0114] 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. The terms "antigen-binding portion" of an antibody, or "antibody

fragment", as used herein, refers to one or more fragments of an antibody that retain the ability to bind to toxin A or toxin B or both.

[0115] The specific embodiments, antibody or antibody fragments of the invention may be conjugated to a therapeutic moiety ("immunoconjugate"), such as an antibiotic, a second anti-toxin A or B antibody, or a *C. difficile* vaccine, or a toxoid, or any other therapeutic moiety useful for treating a disease or condition caused by *C. difficile*.

[0116] An "isolated antibody", as used herein, is intended to refer to an antibody that is substantially free of other antibodies (Abs) having different antigenic specificities (*e.g.,* an isolated antibody that specifically binds toxin A or toxin B, or a fragment thereof, is substantially free of Abs that specifically bind antigens other than toxin A or toxin B.

[0117] A "blocking antibody" or a "neutralizing antibody", as used herein (or an "antibody that neutralizes toxin A and/or toxin B activity"), is intended to refer to an antibody whose binding to toxin A and/or toxin B results in inhibition of at least one biological activity of toxin A and/or toxin B. For example, an antibody of the invention may aid in preventing the primary disease caused by *C. difficile.* Alternatively, an antibody of the invention may demonstrate the ability to prevent a recurrence or relapse of the disease caused by *C. difficile*, or at least one symptom caused by *C. difficile* infection, including diarrhea or psudomembranous colitis. This inhibition of the biological activity of toxin A and/or toxin B can be assessed by measuring one or more indicators of toxin A and/or toxin B biological activity by one or more of several standard *in vitro* assays (such as a neutralization assay, as described herein) or *in vivo* assays known in the art (for example, animal models to look at protection from challenge with *C. difficile* following administration of one or more of the antibodies described herein).

[0118] The term "surface plasmon resonance", as used herein, refers to an optical phenomenon that allows for the analysis of real-time biomolecular interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIACORE™ system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, N.J.).

[0119] The term "K_D ", as used herein, is intended to refer to the equilibrium dissociation constant of a particular antibody-antigen interaction.

[0120] 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. The term "epitope" also refers to a site on an antigen to which B and/or T cells respond. It also refers to a region of an antigen that is bound by an antibody. Epitopes may be defined as structural or functional. Functional epitopes are generally a subset of the structural epitopes and have those residues that directly contribute to the affinity of the interaction. Epitopes may also be conformational, that is, composed of non-linear amino acids. In certain embodiments, epitopes may include determinants that are chemically active surface groupings of molecules such as amino acids,

sugar side chains, phosphoryl groups, or sulfonyl groups, and, in certain embodiments, may have specific three-dimensional structural characteristics, and/or specific charge characteristics.

[0121] 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 90%, and more preferably at least about 95%, 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.

[0122] 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 90% sequence identity, even more preferably at least 95%, 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 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: 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 45. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[0123] Sequence similarity for polypeptides 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 with 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 (1997) Nucleic Acids Res. 25:3389 402.

[0124] In specific embodiments, the antibody or antibody fragment for use in the method of the invention may be mono-specific, bi-specific, or multi-specific. Multi-specific antibodies may be specific for different epitopes of one target polypeptide or may contain antigen-binding domains specific for epitopes of more than one target polypeptide. 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 bi-specific 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 an 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 mAbs; N44S, K52N, and V82I (IMGT; N384S, K392N, and V422I by EU) in the case of IgG2 mAbs; 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 mAbs. Variations on the bi-specific antibody format described above are contemplated within the scope of the present invention.

[0125] By the phrase “therapeutically effective amount” is meant an amount that produces the desired effect for which it is administered. The exact amount will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (see, for example, Lloyd (1999) The Art, Science and Technology of Pharmaceutical Compounding).

General Description

[0126] *Clostridium difficile* is a gram-positive, spore-forming, toxin producing bacterium, which

is a leading cause of nosocomial antibiotic-associated diarrhea and colitis in humans (Bartlett, J.G. *et al.* (1978), N. Engl. J. Med. 298:531-534; Kyne, L., *et al.* (2001), Clin. N. Am. 30:753-777). The perturbation of the colonic environment resulting from administration of broad-spectrum antibiotics leads to colonization of the gut by the bacterium (Johnson, S.C. *et al.* (1990), Lancet 336:97-100). A large percentage of this patient population that becomes colonized with *C. difficile* develops diarrhea, which in certain instances leads to pseudomembranous colitis, which is believed to be due to the production of two exotoxins by *C. difficile*, toxin A and toxin B. Treatment consists of the discontinuation of the offending antibiotic, or alterations in the dosing of the offending antibiotic, or no change in the offending antibiotic, followed by the administration of metronidazole, vancomycin, or fidaxomicin. While this treatment regimen is usually successful, many patients relapse when therapy is discontinued (Fekety, R., (1997), Am. J. Gastroenterology, 92:739-750). Furthermore, in many instances, the *C. difficile* bacterium becomes resistant to the therapy used, thus leading to treatment failures and in some instances increased mortality rates (Dworczynski, A. *et al.* (1991), Cytobios. 65:149-153; Fekety, R. *et al.* (1993), JAMA, 269:71-75). Accordingly, there is a need for more effective therapies to combat this disease and/or to prevent the recurrence of this disease in patients colonized with *C. difficile*. In addition, there is a need to treat patients who are at risk for developing a *C. difficile* infection by prophylactic administration of an effective agent. Included in this at risk patient population are the elderly, in particular, patients 65 years of age and older, although patients younger than 65 may be at greater risk depending on the presence of any underlying disease that may predispose them to infection with *C. difficile*. Patients that have been infected with *C. difficile* previously may be at greater risk of recurrences. Other patients at risk include patients who are pre-disposed to a *C. difficile* infection because of an underlying medical condition, or patients who are hospitalized for long periods of time (at least one week or longer) and/or, who are on long term treatment (≥ 14 days) with broad spectrum antibiotics, as well as patients who are on proton pump inhibitors to treat gastroesophageal reflux disease (GERD), stomach and small intestine ulcers and inflammation of the esophagus. These agents include dexlansoprazole, esomeprazole, lansoprazole, omeprazole, pantoprazole sodium, or rabeprazole sodium. Other agents that are under study for placing a patient at risk for developing a *C. difficile* infection include histamine-H2 receptor blockers, such as cimetidine, famotidine, nizatidine and ranitidine. Other studies noted an age-specific incidence of *C. difficile*-associated diarrhea, more specifically, an increase in patients after the age of 50 years, and an increase in mortality rate in patients after the age of 60 (Loo, VG, *et al.*, (2005), N Engl J Med 353:2442—9). This study was in fact, consistent with an earlier study that showed an age-related increase in the incidence of positive assays for *C. difficile* toxin (Karlström, O. *et al.* (1998), Clin Infect Dis 26:141-5).

[0127] To address the need for more effective therapies against *C. difficile*, many studies have been conducted to determine if anti-toxin A and/or B antibodies, when used alone, or as adjunct

therapy, could be used as a means of treating this disease, or at least as a means of preventing the recurrence of the diarrhea or colitis associated with *C. difficile* infection. (Corthier, et al. (1991), Infect. Immun. 59(3):1192-1195; Kink, J.A. and Willilams, J.A., (1998), Infect. Immun. 66(5):2018-2025; Lowy, I. et al. (2010), N. Engl. J. Med. 362(3):197-205; Babcock, G.J., et al.; (2006), Infection and Immunity, 74(11):6339-6347). More particularly, animal models of infection with *C. difficile* have been used to study the effect of antibodies to toxin A and/or toxin B from *C. difficile* on primary infection, as well as on relapse rates *in vivo* (Corthier, G. *et al.* (1991), Infect. Immun. 59(3):1192-1195; Kink, J.A. et al. (1998), Infect. Immun. 66(5):2018-2025; Babcock, G.J. *et al.* (2006), 74(11):6339-6347). The results in animal models of *C. difficile* showed significant protection, thus prompting further clinical trials using anti-toxin A and anti-toxin B antibodies in human patients with the disease (Lowy, I., (2010), N. Engl. J. Med. 362(3):197-205).

[0128] The antibodies described herein demonstrate specific binding to toxin A and/or to toxin B of *C. difficile* and may be useful for treating patients suffering from infection with *C. difficile*. The use of such antibodies may be an effective means of treating patients suffering from a primary infection with *C. difficile*, or they may be used to prevent a relapse of the disease and the accompanying symptoms associated with the disease, or may be used to lessen the severity of the diarrhea or colitis associated with a primary infection or with the recurrence of the infection. They may be used alone or as adjunct therapy with other therapeutic moieties or modalities known in the art for treating *C. difficile* infections, such as, but not limited to, antibiotic therapy, for example, with metronidazole, vancomycin, or fidaxomicin. They may be used in conjunction with a *C. difficile* vaccine, or with use of a toxoid, or with a second or third different antibody specific for toxin A and/or B.

[0129] In certain embodiments of the invention, combinations of the antibodies of the invention may be used to treat an infection caused by a hypervirulent strain of *C. difficile*. The most notable hypervirulent epidemic isolate group to date is one referred to as “BI/NAP1/027”. This has been associated with outbreaks of *C. difficile* infections throughout Europe and North America. Isolates that fall into this designation are characterized by increased toxin A and toxin B production, by the presence of an additional toxin (binary toxin) and by an increased resistance to fluoroquinolones (McDonald, LC, *et al.,* (2005), N Engl J Med 353:2433-41; Warny, ME, *et al.,* (2005), Lancet 366:1079-84). This group of isolates may also be referred to as the North American pulsed-field type 1 (NAP1), ribotype 027, group BI strains. This group of strains contains an 18 base pair *tcdC* gene deletion and the binary toxin, which it produces is encoded by *cdtA* and *cdtB* genes. It has been reported that this group produces toxin A and toxin B in quantities 16 and 23 times, respectively, greater than control strains (Warny, ME, *et al.,* (2005), Lancet 366:1079-84). Since the antibodies of the present invention have been shown to neutralize the toxin produced by four different clinically isolated *C. difficile* BI/NAP1/027 strains (VA5, VA17, 6336 and 6443), it is envisioned that compositions comprising

the antibodies of the present invention may be administered therapeutically to patients suffering from an infection with the above-noted hypervirulent strains of *C. difficile*, or may be administered prophylactically to patients who are at risk for developing an infection with the hypervirulent strains noted herein, as well as with any other clinically relevant hypervirulent strains. The means by which to identify these strains are known to those skilled in the art, and these methods may include pulsed-field gel electrophoresis (PFGE) of *C. difficile* isolates (See for example, Fawley, WN, *et al*., (2002), J. Clin Microbiol 40:3546-7), PCR analyses for binary toxin genes and partial deletions of the *tcdC* gene (See, for example, Gonçalves, C. *et al*. (2004), J Clin Microbiol 42:1933-9; and Cohen, SH et al., (2000), J Infect Dis 181:659-63), and restriction-endonuclease analyses (See, for example, Clabots, CR, *et al*., (1993), J Clin Microbiol 31:1870-5).

[0130] In certain embodiments, the antibodies of the invention are obtained from mice immunized with a primary immunogen, such as a native, inactivated, toxin A (See GenBank accession number CAA63564 (SEQ ID NO: 378)), or toxin B (See GenBank accession number CAJ67492 (SEQ ID NO: 380)) from *C. difficile*, or with a recombinant, but inactivated form of the toxins, or toxin fragments, followed by immunization with a secondary immunogen, or with an immunogenically active fragment of the native toxin. Animals may be immunized with either inactivated toxin A alone or inactivated toxin B alone, or with both inactivated toxin A and inactivated toxin B concurrently. The toxins can be inactivated prior to use as an immunogen using standard procedures for preparing toxoids, including by treatment with formaldehyde, glutaraldehyde, peroxide, or oxygen treatment (Relyveld, et al. *Methods in Enzymology*, 93:24, 1983, Woodrow and Levine, eds. *New Generation Vaccines*, Marcel Dekker, Inc., New York, 1990). Another means of inactivation is by use of UDP-dialdehyde (Genth et al., (2000), Infect. Immun. 68(3):1094-1101), which may act to preserve the native structure of the toxin compared to other inactivation methods, thereby enhancing the likelihood of eliciting antibodies that are more reactive with the native toxin.

[0131] Alternatively, mutant toxins from *C. difficile*, which exhibit reduced toxicity, may be produced using standard recombinant techniques and used as immunogens (See, for example, US 5,085,862; 5,221,618; 5,244,657; 5,332,583; 5,358,868; and 5,433,945). Such mutants may contain deletions or point mutations in the active site of the toxin.

[0132] The immunogen may be a biologically active and/or immunogenic fragment of native toxin A or toxin B, or DNA encoding the active fragment thereof. The fragment may be derived from the N-terminal or C-terminal domain of either toxin A or toxin B. The fragment may be derived from any of the known domains of toxin A or toxin B (See Figure 1), including the glucosylating enzymatic domain (A), the autocatalytic processing domain (C), the translocating domain (D) or the binding domain (B). In certain embodiments of the invention, the immunogen is the carboxy terminal receptor binding domain of toxin A that ranges from about amino acid residues 1832-2710 of SEQ ID NO: 378. In certain embodiments of the invention, the

immunogen is the carboxy terminal receptor binding domain of toxin A that is shown in SEQ ID NO: 375. In certain embodiments of the invention, the immunogen is the carboxy terminal receptor binding domain of toxin B that ranges from about amino acid residues 1834-2366 of SEQ ID NO: 380. In certain embodiments of the invention, the immunogen is the carboxy terminal receptor binding domain of toxin B that is shown in SEQ ID NO: 376.

[0133] The full-length amino acid sequence of toxin A from *C. difficile* is shown as SEQ ID NO: 378.

[0134] The full-length amino acid sequence of toxin B from *C. difficile* is shown as SEQ ID NO: 380.

[0135] In certain embodiments, antibodies that bind specifically to *C. difficile* toxin A or toxin B may be prepared using fragments of the above-noted regions, or peptides that extend beyond the designated regions by about 5 to about 20 amino acid residues from either, or both, the N or C terminal ends of the regions described herein. In certain embodiments, any combination of the above-noted regions or fragments thereof may be used in the preparation of toxin A or toxin B specific antibodies. In certain embodiments, any one or more of the above-noted regions of toxin A or toxin B, or fragments thereof may be used for preparing monospecific, bispecific, or multispecific antibodies.

Antigen-Binding Fragments of Antibodies

[0136] Unless specifically indicated otherwise, the term "antibody," as used herein, shall be understood to encompass antibody molecules comprising two immunoglobulin heavy chains and two immunoglobulin light chains (*i.e.*, "full antibody molecules") as well as antigen-binding fragments thereof. 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. The terms "antigen-binding portion" of an antibody, or "antibody fragment", as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to either toxin A and/or toxin B of *C. difficile*. An antibody fragment may include a Fab fragment, a $F(ab')_2$ fragment, a Fv fragment, a dAb fragment, a fragment containing a CDR, or an isolated CDR. 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.

[0137] 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.

[0138] 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.

[0139] 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)).

[0140] As with full antibody molecules, antigen-binding fragments may be mono-specific or multi-specific (*e.g.*, bi-specific). A multi-specific 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 multi-specific antibody format, including the exemplary bi-specific 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.

Preparation of Human Antibodies

[0141] Methods for generating human antibodies in transgenic mice 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 toxin A and/or toxin B of *C. difficile*.

[0142] Using VELOCIMMUNE® technology (see, for example, US 6,596,541, Regeneron Pharmaceuticals, VELOCIMMUNE®) or any other known method for generating monoclonal antibodies, high affinity chimeric antibodies to toxin A and/or toxin B of *C. difficile* are initially isolated having a human variable region and a mouse constant region. The VELOCIMMUNE® technology involves generation of a transgenic mouse having a genome comprising human heavy and light chain variable regions operably linked to endogenous mouse constant region loci such that the mouse produces an antibody comprising a human variable region and a mouse constant region in response to antigenic stimulation. The DNA encoding the variable regions of the heavy and light chains of the antibody are isolated and operably linked to DNA encoding the human heavy and light chain constant regions. The DNA is then expressed in a cell capable of expressing the fully human antibody.

[0143] Generally, a VELOCIMMUNE® mouse is challenged with the antigen of interest, and lymphatic cells (such as B-cells) are recovered from the mice that express antibodies. The lymphatic cells may be fused with a myeloma cell line to prepare immortal hybridoma cell lines, and such hybridoma cell lines are screened and selected to identify hybridoma cell lines that produce antibodies specific to the antigen of interest. DNA encoding the variable regions of the heavy chain and light chain may be isolated and linked to desirable isotypic constant regions of the heavy chain and light chain. Such an antibody protein may be produced in a cell, such as a CHO cell. Alternatively, DNA encoding the antigen-specific chimeric antibodies or the variable domains of the light and heavy chains may be isolated directly from antigen-specific lymphocytes.

[0144] Initially, high affinity chimeric antibodies are 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.

[0145] In general, the antibodies of the instant invention possess very high affinities, typically

possessing K_D of from about 10^{-12} through about 10^{-9} M, when measured by binding to antigen either immobilized on solid phase or in solution phase. The mouse constant regions are replaced with desired human constant regions to generate the fully human antibodies of the invention. 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

[0146] The anti-toxin A and anti-toxin B 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 toxin A or toxin B. 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 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 antibody or antibody fragment that is essentially bioequivalent to an antibody or antibody fragment of the invention.

[0147] 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.

[0148] In one embodiment, two antigen-binding proteins are bioequivalent if there are no clinically meaningful differences in their safety, purity, and potency.

[0149] 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.

[0150] 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.

[0151] Bioequivalence may be demonstrated by *in vivo* and/or *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.

[0152] Bioequivalent variants of the 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 antibody variants comprising amino acid changes, which modify the glycosylation characteristics of the antibodies, *e.g.*, mutations that eliminate or remove glycosylation.

Biological Characteristics of the Antibodies

[0153] In general, the antibodies of the present invention may function by binding to either toxin A or to toxin B of *C. difficile*, or to both toxin A and toxin B of *C. difficile* (cross-reactive antibodies), or to a fragment of either A or B.

[0154] In certain embodiments, the antibodies of the present invention may bind to an epitope located in at least the C-terminal receptor binding domain of toxin A and/or toxin B of *C. difficile*. In one embodiment, the antibodies may bind to the C-terminal region of toxin A, ranging from amino acid residue 1832-2710 of the carboxy terminal receptor binding domain of toxin A, which spans amino acid residues 1832-2710 of SEQ ID NO: 378. In certain embodiments of the invention, the antibodies may bind the carboxy terminal receptor binding domain of toxin A that is shown in SEQ ID NO: 375. In certain embodiments of the invention, the antibodies may interact with, or bind to, amino acid residues 468-863 of the carboxy terminal receptor binding domain of toxin A produced by *Clostridium difficile*, the sequence of which is shown in SEQ ID NO: 375. In certain embodiments of the invention, the antibodies may interact with, or bind to, an epitope in the carboxy terminal receptor binding domain of toxin A produced by *Clostridium difficile*, wherein the epitope is selected from the group consisting of residues 468-488 of SEQ ID NO: 375, residues 510-530 of SEQ ID NO: 375, residues 602-610 of SEQ ID NO: 375, residues 644-703 of SEQ ID NO: 375, residues 724-794 of SEQ ID NO: 375, residues 799-814 of SEQ ID NO: 375 and residues 858-863 of SEQ ID NO: 375. In one embodiment, the antibody that binds to or interacts with an epitope in the carboxy terminal receptor binding domain of toxin A produced by *Clostridium difficile*, selected from the group consisting of residues 468-488 of SEQ ID NO: 375, residues 510-530 of SEQ ID NO: 375, residues 602-610

of SEQ ID NO: 375, residues 644-703 of SEQ ID NO: 375, residues 724-794 of SEQ ID NO: 375, residues 799-814 of SEQ ID NO: 375 and residues 858-863 of SEQ ID NO: 375 comprises the HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 146/154. In one embodiment, the antibody that binds to or interacts with an epitope in the carboxy terminal receptor binding domain of toxin A produced by *Clostridium difficile*, selected from the group consisting of residues 468-488 of SEQ ID NO: 375, residues 510-530 of SEQ ID NO: 375, residues 602-610 of SEQ ID NO: 375, residues 644-703 of SEQ ID NO: 375, residues 724-794 of SEQ ID NO: 375, residues 799-814 of SEQ ID NO: 375 and residues 858-863 of SEQ ID NO: 375 is combined with a second antibody that binds specifically to toxin B of *Clostridium difficile* in a pharmaceutical composition. In one embodiment, this second antibody that interacts with or binds to toxin B of *Clostridium difficile* comprises the HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 274/282.

[0155] In certain embodiments of the invention, the antibodies may bind to the carboxy terminal receptor binding domain of toxin B that ranges from about amino acid residues 1834-2366 of SEQ ID NO: 380. In certain embodiments of the invention, the antibodies may bind to the carboxy terminal receptor binding domain of toxin B that is shown in SEQ ID NO: 376.

[0156] In certain embodiments, the antibodies of the present invention may function by blocking or inhibiting the toxicity associated with toxin A of *C. difficile* by binding to any other region or fragment of the full length native toxin A protein, the amino acid sequence of which is shown in SEQ ID NO: 378, which is encoded by the nucleic acid sequence shown in SEQ ID NO: 377.

[0157] In certain embodiments, the antibodies of the present invention may function by blocking or inhibiting the toxicity associated with toxin B of *C. difficile* by binding to any other region or fragment of the full length native toxin B protein, the amino acid sequence of which is shown in SEQ ID NO: 380, which is encoded by the nucleic acid sequence shown in SEQ ID NO: 379.

[0158] In certain embodiments, the antibodies of the present invention may be bi-specific antibodies. The bi-specific antibodies of the invention may bind one epitope in toxin A and may also bind one epitope in toxin B. In certain embodiments, the bi-specific antibodies of the invention may bind two different epitopes in toxin A. In certain embodiments, the bi-specific antibodies of the invention may bind two different epitopes in toxin B. In certain embodiments, the bi-specific antibodies of the invention may bind to two different sites within the same domain on either one of toxin A or toxin B, or may bind to the same domain on both toxin A and toxin B.

[0159] In one embodiment, the invention provides a fully human monoclonal antibody or antigen-binding fragment thereof that binds to the carboxy terminal receptor binding domain of both toxin A and toxin B of *C. difficile*, wherein the antibody or fragment thereof exhibits one or more of the following characteristics: (i) comprises a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 18, 34, 50, 66 and 82, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (ii) comprises a LCVR having an amino acid sequence selected from the

group consisting of SEQ ID NO: 26, 42, 58, 74 and 90, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (iii) comprises a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 24, 40, 56, 72 and 88, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 32, 48, 64, 80 and 96, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (iv) comprises a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 20, 36, 52, 68 and 84, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 22, 38, 54, 70 and 86, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 28, 44, 60, 76 and 92, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 30, 46, 62, 78 and 94, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (v) binds to toxin A and toxin B with a K_D equal to or less than 10^{-9}M.

[0160] In one embodiment, the invention provides a fully human monoclonal antibody or antigen-binding fragment thereof that binds specifically to toxin A (but not to toxin B) of *C. difficile*, wherein the antibody or fragment thereof exhibits one or more of the following characteristics: (i) comprises a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 2, 98,114,130,146 and 162, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (ii) comprises a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 10, 106, 122, 138, 154 and 170, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (iii) comprises a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 8, 104,120,136,152 and 168, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 16, 112, 128, 144, 160 and 176, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (iv) comprises a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 4, 100, 116, 132, 148 and 164, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a HCDR2 domain having an amino acid sequence

selected from the group consisting of SEQ ID NO: 6, 102, 118, 134, 150 and 166, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 108, 124, 140, 156 and 172, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 14, 110, 126, 142, 158 and 174, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (v) demonstrates a K_D equal to or less than 10^{-9}M; (vi) demonstrates neutralization of Toxin A (at a concentration of 32pM) with an IC50 ranging from about 7pM to about 65pM in a cell viability assay.

[0161] In one embodiment, the invention provides a fully human monoclonal antibody or antigen-binding fragment thereof that binds specifically to toxin B (but not to toxin A) of *C. difficile*, wherein the antibody or fragment thereof exhibits one or more of the following characteristics: (i) comprises a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 178, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338 and 354, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (ii) comprises a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NO:186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346 and 362, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (iii) comprises a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NO:184, 200, 216, 232, 248, 264, 280, 296, 312, 328, 344 and 360, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NO:192, 208, 224, 240, 256, 272, 288, 304, 320, 336, 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; (iv) comprises a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 180,196, 212, 228, 244, 260, 276, 292, 308, 324, 340 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 HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 182, 198, 214, 230, 246, 262, 278, 294, 310, 326, 342 and 358, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 188, 204, 220, 236, 252, 268, 284, 300, 316, 332, 348 and 364, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 190, 206, 222, 238, 254, 270, 286, 302, 318,

334, 350 and 366, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (v) demonstrates a K_D equal to or less than 10^{-9}M; (vi) demonstrates neutralization of Toxin B (at a concentration of 0.03pM) with an IC50 ranging from about 25pM to about 320pM in a cell viability assay.

[0162] Certain anti-toxin A or anti-toxin B antibodies of the present invention are able to bind to and neutralize the toxicity of either toxin A, or toxin B, or both, of *C. difficile*, as determined by *in vitro* or *in vivo* assays. The ability of the antibodies of the invention to bind to and neutralize the activity of the toxins may be measured using any standard method known to those skilled in the art, including binding assays, or neutralization of toxicity (protection from cell death) assays, as described herein.

[0163] Non-limiting, exemplary *in vitro* assays for measuring binding activity are illustrated in Examples 4, 5 and 6, herein. In Examples 4 and 5, the binding affinities and kinetic constants of human anti-toxin A or anti-toxin B antibodies were determined by surface plasmon resonance and the measurements were conducted on a T200 Biacore instrument. In Example 6, the binding studies were conducted using size exclusion chromatography. In Example 7, a neutralization bioassay was developed in Vero cells to detect cell viability after treatment with toxin A or B and antibodies to either toxin A or to toxin B.

[0164] The present invention also includes anti-toxin A or B antibodies and antigen binding fragments thereof which bind to at least one biologically active fragment of any of the following proteins, or peptides: SEQ ID NO: 378 (full length toxin A), residue numbers 1832-2710 of SEQ ID NO: 378 (C-terminal domain of toxin A); SEQ ID NO: 380 (full length toxin B), residues 1834-2366 of SEQ ID NO: 380; SEQ ID NO: 375 (carboxy terminal receptor binding domain of toxin A); or SEQ ID NO: 376. The present invention also provides for antibodies that interact with or bind to an epitope within the carboxy terminal receptor binding domain of toxin A produced by *Clostridium difficile*, or an antigen binding fragment thereof, wherein the epitope is contained within residues ranging from about residue 468 to about 863 of SEQ ID NO: 375. In one embodiment, the epitope for an antibody that binds toxin A is selected from the group consisting of residues 468-488 of SEQ ID NO: 375, residues 510-530 of SEQ ID NO: 375, residues 602-610 of SEQ ID NO: 375, residues 644-703 of SEQ ID NO: 375, residues 724-794 of SEQ ID NO: 375, residues 799-814 of SEQ ID NO: 375 and residues 858-863 of SEQ ID NO: 375. Any of the toxin A or toxin B peptides described herein, or fragments thereof, may be used to generate anti-toxin A or anti-toxin B antibodies.

[0165] The peptides may be modified to include addition or substitution of certain residues for tagging or for purposes of conjugation to carrier molecules, such as, KLH. For example, a cysteine may be added at either the N terminal or C terminal end of a peptide, or a linker sequence may be added to prepare the peptide for conjugation to, for example, KLH for immunization. The antibodies specific for toxin A or toxin B may contain no additional labels or moieties, or they may contain an N-terminal or C-terminal label or moiety. In one embodiment,

the label or moiety is biotin. In a binding assay, the location of a label (if any) may determine the orientation of the peptide relative to the surface upon which the peptide is bound. For example, if a surface is coated with avidin, a peptide containing an N-terminal biotin will be oriented such that the C-terminal portion of the peptide will be distal to the surface.

Epitope Mapping and Related Technologies

[0166] 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, for example, a routine cross-blocking assay such as that described Antibodies, Harlow and Lane (Cold Spring Harbor Press, Cold Spring Harb., NY) can be performed. Other methods include alanine scanning mutational analysis, peptide blot analysis (Reineke (2004) Methods Mol Biol 248:443-63), peptide cleavage analysis crystallographic studies and NMR 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. 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 and exchangeable protons within amino acids that are protected by the antibody complex undergo deuterium-to-hydrogen back-exchange at a slower rate than exchangeable protons within amino acids that are not part of the interface. As a result, amino acids that form part of the protein/antibody interface may retain deuterium and therefore exhibit relatively higher mass compared to amino acids not included in the interface. After dissociation of the antibody, the target protein is subjected to protease cleavage and mass spectrometry analysis, thereby revealing the deuterium-labeled residues that 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.

[0167] The term "epitope" refers to a site on an antigen to which B and/or T cells respond. B-cell epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents, whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation.

[0168] Modification-Assisted Profiling (MAP), also known as Antigen Structure-based Antibody Profiling (ASAP) is a method that categorizes large numbers of monoclonal antibodies (mAbs) directed against the same antigen according to the similarities of the binding profile of each antibody to chemically or enzymatically modified antigen surfaces (US 2004/0101920). Each

category may reflect a unique epitope either distinctly different from or partially overlapping with epitope represented by another category. This technology allows rapid filtering of genetically identical antibodies, such that characterization can be focused on genetically distinct antibodies. When applied to hybridoma screening, MAP may facilitate identification of rare hybridoma clones that produce mAbs having the desired characteristics. MAP may be used to sort the antibodies of the invention into groups of antibodies binding different epitopes.

[0169] In certain embodiments, the anti-toxin A or anti-toxin B antibody or antigen-binding fragments thereof binds an epitope within any one of the regions exemplified in Figure 1, or in SEQ ID NOS: 378, or 380, or at least one of the carboxy terminal receptor binding domains of toxin A or toxin B, or to a fragment thereof, wherein the carboxy terminal receptor binding domain of toxin A is shown in SEQ ID NO: 375, and wherein the carboxy terminal receptor binding domain of toxin B is shown as SEQ ID NO: 376.

[0170] The present invention includes anti-toxin A or anti-toxin B antibodies that bind to the same epitope as any of the specific exemplary antibodies described herein in Table 1. Likewise, the present invention also includes anti-toxin A or anti-toxin B antibodies that compete for binding to toxin A or B or a toxin A or B fragment with any of the specific exemplary antibodies described herein in Table 1.

[0171] One can easily determine whether an antibody binds to the same epitope as, or competes for binding with, a reference anti-toxin A or anti-toxin B 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-toxin A or anti-toxin B antibody of the invention, the reference antibody is allowed to bind to a toxin A or B protein or peptide under saturating conditions. Next, the ability of a test antibody to bind to the toxin A or B molecule is assessed. If the test antibody is able to bind to toxin A or B following saturation binding with the reference anti-toxin A or anti-toxin B antibody, it can be concluded that the test antibody binds to a different epitope than the reference anti-toxin A or anti-toxin B antibody. On the other hand, if the test antibody is not able to bind to the toxin A or B molecule following saturation binding with the reference anti-toxin A or anti-toxin B antibody, then the test antibody may bind to the same epitope as the epitope bound by the reference anti-toxin A or anti-toxin B antibody of the invention.

[0172] To determine if an antibody competes for binding with a reference anti-toxin A or anti-toxin B antibody, the above-described binding methodology is performed in two orientations: In a first orientation, the reference antibody is allowed to bind to a toxin A or B molecule under saturating conditions followed by assessment of binding of the test antibody to the toxin A or B molecule. In a second orientation, the test antibody is allowed to bind to a toxin A or B molecule under saturating conditions followed by assessment of binding of the reference antibody to the toxin A or B molecule. If, in both orientations, only the first (saturating) antibody is capable of binding to the toxin A or B molecule, then it is concluded that the test antibody and the reference antibody compete for binding to toxin A or B. 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 identical epitope as the reference antibody, but may sterically block binding of the reference antibody by binding an overlapping or adjacent epitope.

[0173] Two antibodies bind to the same or overlapping epitope if each competitively inhibits (blocks) binding of the other to the antigen. That is, 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 have 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 have overlapping epitopes if some amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

[0174] 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, surface plasmon resonance, flow cytometry or any other quantitative or qualitative antibody-binding assay available in the art.

Immunoconjugates

[0175] The invention encompasses a human anti-toxin A or anti-toxin B monoclonal antibody conjugated to a therapeutic moiety ("immunoconjugate"), such as an agent that is capable of reducing the severity of primary infection with *C. difficile*, or to ameliorate at least one symptom associated with *C. difficile* infection, including diarrhea or colitis, or the severity thereof. Such an agent may be a second different antibody to either or both toxin A or toxin B of *C. difficile*, or a toxoid, or a *C. difficile* vaccine. The type of therapeutic moiety that may be conjugated to the anti-toxin A or anti-toxin B antibody and will take into account the condition to be treated and the desired therapeutic effect to be achieved. Alternatively, if the desired therapeutic effect is to treat the sequelae or symptoms associated with *C. difficile* infection, or any other condition resulting from such infection, such as, but not limited to, pseudomembranous colitis, it may be advantageous to conjugate an agent appropriate to treat the sequelae or symptoms of the condition, or to alleviate any side effects of the antibodies of the invention. Examples of suitable agents for forming immunoconjugates are known in the art, see for example, WO 05/103081.

Multi-specific Antibodies

[0176] The antibodies of the present invention may be mono-specific, bi-specific, or multi-specific. Multi-specific 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 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 multi-specific antibody with a second binding specificity. For example, the present invention includes bi-specific antibodies wherein one arm of an immunoglobulin is specific for toxin A of *C. difficile*, or a fragment thereof, and the other arm of the immunoglobulin is specific for toxin B of *C. difficile*, or a second therapeutic target, or is conjugated to a therapeutic moiety. In certain embodiments of the invention, one arm of an immunoglobulin is specific for an epitope on the C-terminal domain of toxin A or a fragment thereof, and the other arm of the immunoglobulin is specific for an epitope on the C-terminal domain of toxin B, or a fragment thereof.

[0177] 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 bi-specific 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 Administration and Formulations

[0178] The invention provides therapeutic compositions comprising the anti-toxin A or anti-toxin B antibodies or antigen-binding fragments thereof of the present invention. The administration of therapeutic compositions in accordance with the invention will be administered with suitable carriers, excipients, and other agents that are incorporated into formulations to 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.

[0179] The dose of each of the antibodies of the invention may vary depending upon the age and the size of a subject to be administered, target disease, conditions, route of administration, and the like. When the antibodies of the present invention are used for treating a *C. difficile* infection in a patient, or for treating one or more symptoms associated with a *C. difficile* infection, such as the diarrhea or colitis associated with a *C. difficile* infection in a patient, or for preventing a relapse of the disease, or for lessening the severity of the disease, it is advantageous to administer each of the antibodies of the present invention intravenously or subcutaneously normally at a single dose of about 0.01 to about 30 mg/kg body weight, more preferably about 0.1 to about 20 mg/kg body weight, or about 0.1 to about 15 mg/kg body weight, or about 0.02 to about 7 mg/kg body weight, or about 0.03 to about 5 mg/kg body weight, or about 0.05 to about 3 mg/kg body weight, or about 1 mg/kg body weight, or about 3.0 mg/kg body weight, or about 10 mg/kg body weight, or about 20 mg/kg body weight. Multiple doses may be administered as necessary. Depending on the severity of the condition, the frequency and the duration of the treatment can be adjusted. In certain embodiments, the antibodies or antigen-binding fragments thereof of the invention can be administered as an initial dose of at least about 0.1 mg to about 800 mg, about 1 to about 600 mg, about 5 to about 300 mg, or about 10 to about 150 mg, to about 100 mg, or to about 50 mg. In certain embodiments, the initial dose may be followed by administration of a second or a plurality of subsequent doses of the antibodies or antigen-binding fragments thereof in an amount that can be approximately the same or less than that of the initial dose, wherein the subsequent doses are separated by at least 1 day to 3 days; at least one week, at least 2 weeks; at least 3 weeks; at least 4 weeks; at least 5 weeks; at least 6 weeks; at least 7 weeks; at least 8 weeks; at least 9 weeks; at least 10 weeks; at least 12 weeks; or at least 14 weeks.

[0180] 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, transdermal, 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.

[0181] The pharmaceutical composition can be also delivered in a vesicle, in particular a liposome (see, for example, Langer (1990) Science 249:1527-1533).

[0182] In certain situations, the pharmaceutical composition can be delivered in a controlled release system. In one embodiment, a pump may be used. In another embodiment, polymeric materials can be used. 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.

[0183] 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.

[0184] 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.

[0185] Numerous reusable pen and autoinjector delivery devices have applications in the subcutaneous delivery of a pharmaceutical composition of the present invention. Examples include, but certainly are not limited to AUTOPEN™ (Owen Mumford, Inc., Woodstock, UK), DISETRONIC™ pen (Disetronic Medical Systems, Burghdorf, 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 certainly are not limited to the SOLOSTAR™ pen (sanofi-aventis), the FLEXPEN™ (Novo Nordisk), and the KWIKPEN™ (Eli Lilly), the SURECLICK ™ Autoinjector (Amgen, Thousands 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.

[0186] 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.

Administration Regimens

[0187] According to certain embodiments of the present invention, multiple doses of an antibody to toxin A and/or B of *Clostridium difficile* may be administered to a subject over a defined time course. The methods according to this aspect of the invention comprise sequentially administering to a subject multiple doses of an antibody to toxin A and/or B. As used herein, "sequentially administering" means that each dose of antibody to toxin A and/or B is administered to the subject at a different point in time, *e.g.*, on different days separated by a predetermined interval (*e.g.*, hours, days, weeks or months). The present invention includes methods which comprise sequentially administering to the patient a single initial dose of an antibody to toxin A and/or B, followed by one or more secondary doses of the antibody to toxin A and/or B, and optionally followed by one or more tertiary doses of the antibody to toxin A and/or B.

[0188] The terms "initial dose," "secondary doses," and "tertiary doses," refer to the temporal sequence of administration of the antibody to toxin A and/or B. Thus, the "initial dose" is the dose which is administered at the beginning of the treatment regimen (also referred to as the "baseline dose"); the "secondary doses" are the doses which are administered after the initial dose; and the "tertiary doses" are the doses which are administered after the secondary doses. The initial, secondary, and tertiary doses may all contain the same amount of antibody to toxin A and/or B, but generally may differ from one another in terms of frequency of administration. In certain embodiments, however, the amount of antibody to toxin A and/or B contained in the initial, secondary and/or tertiary doses vary from one another (*e.g.*, adjusted up or down as appropriate) during the course of treatment. In certain embodiments, two or more (*e.g.*, 2, 3, 4, or 5) doses are administered at the beginning of the treatment regimen as "loading doses"

followed by subsequent doses that are administered on a less frequent basis (*e.g.*, "maintenance doses").

[0189] In one exemplary embodiment of the present invention, each secondary and/or tertiary dose is administered 1 to 26 (*e.g.*, 1, 1½, 2, 2½, 3, 3½, 4, 4½, 5, 5½, 6, 6½, 7, 7½, 8, 8½, 9, 9½, 10, 10½, 11, 11½, 12, 12½, 13, 13½, 14, 14½, 15, 15½, 16, 16½, 17, 17½, 18, 18½, 19, 19½, 20, 20½, 21, 21½, 22, 22½, 23, 23½, 24, 24½, 25, 25½, 26, 26½, or more) weeks after the immediately preceding dose. The phrase "the immediately preceding dose," as used herein, means, in a sequence of multiple administrations, the dose of antibody to toxin A and/or B which is administered to a patient prior to the administration of the very next dose in the sequence with no intervening doses.

[0190] The methods according to this aspect of the invention may comprise administering to a patient any number of secondary and/or tertiary doses of an antibody to toxin A and/or B. For example, in certain embodiments, only a single secondary dose is administered to the patient. In other embodiments, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) secondary doses are administered to the patient. Likewise, in certain embodiments, only a single tertiary dose is administered to the patient. In other embodiments, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) tertiary doses are administered to the patient.

[0191] In embodiments involving multiple secondary doses, each secondary dose may be administered at the same frequency as the other secondary doses. For example, each secondary dose may be administered to the patient 1 to 2 weeks after the immediately preceding dose. Similarly, in embodiments involving multiple tertiary doses, each tertiary dose may be administered at the same frequency as the other tertiary doses. For example, each tertiary dose may be administered to the patient 2 to 4 weeks after the immediately preceding dose. Alternatively, the frequency at which the secondary and/or tertiary doses are administered to a patient can vary over the course of the treatment regimen. The frequency of administration may also be adjusted during the course of treatment by a physician depending on the needs of the individual patient following clinical examination.

Therapeutic Uses of the Antibodies

[0192] Due to their interaction with toxin A and/or toxin B of *C. difficile,* the present antibodies are useful for treating the primary *C. difficile* disease or condition, or at least one symptom associated with the disease or condition, such as diarrhea or colitis, or for preventing a relapse of the disease, or for lessening the severity, duration, and/or frequency of recurrences of the disease. The antibodies of the invention are also contemplated for prophylactic use in patients at risk for developing or acquiring a *C. difficile* infection. These patients include the elderly (for example, in anyone 65 years of age or older), or patients immunocompromised due to illness or treatment with immunosuppressive therapeutics, or patients who may have an underlying medical condition that predisposes them to a *C. difficile* infection (for example, cancer,

inflammatory bowel disease, pre-liver transplant patients with ascites accumulation), or patients that are hospitalized for long periods of time (for example, in some cases this time period may vary from as little as two or three days, but generally can be from one week, to two weeks or longer), making them prone to acquiring nosocomial infections, or patients on long term treatment (≥ 14 days) with broad spectrum antibiotics (in some instances, patients may acquire the infection within 24 hours if the gut is disregulated, but in other instances this may take much longer, for example, one week or longer), or patients on therapy with proton pump inhibitors for treatment of gastrointestinal disorders. It is contemplated that the antibodies of the invention may be used alone, or in conjunction with a second agent, or third agent for treating the *C. difficile* infection, or for alleviating at least one symptom or complication associated with the *C. difficile* infection, such as the diarrhea or colitis associated with, or resulting from such an infection. The second or third agents may be delivered concurrently with the antibodies of the invention, or they may be administered separately, either before or after the antibodies of the invention.

[0193] In yet a further embodiment of the invention the present antibodies are used for the preparation of a pharmaceutical composition for treating patients suffering from a *C. difficile* infection, including those infections caused by a clinically relevant hypervirulent strain of *Clostridium difficile*, or the diarrhea and colitis associated with a *C. difficile* infection. In yet another embodiment of the invention the present antibodies are used for the preparation of a pharmaceutical composition for reducing the severity of a primary infection with *C. difficile*, or for reducing the severity, duration of, and/or number of recurrences with *C. difficile*. In a further embodiment of the invention the present antibodies are used as adjunct therapy with any other agent useful for treating *C. difficile* infections, including probiotics, antibiotics, toxoids, vaccines, or any other palliative therapy known to those skilled in the art.

Combination Therapies

[0194] The methods of the present invention, according to certain embodiments, comprise administering to the subject one or more additional therapeutic agents in combination with an antibody to toxin A and/or toxin B of *Clostridium difficile*. As used herein, the expression "in combination with" means that the additional therapeutic agents are administered before, after, or concurrent with the pharmaceutical composition comprising the anti-toxin A and/or B antibodies. The term “in combination with” also includes sequential or concomitant administration of the anti-toxin A and/or B antibodies and a second therapeutic agent.

[0195] For example, when administered "before" the pharmaceutical composition comprising the anti-toxin A and/or B antibodies, the additional therapeutic agent may be administered about 72 hours, about 60 hours, about 48 hours, about 36 hours, about 24 hours, about 12 hours, about 10 hours, about 8 hours, about 6 hours, about 4 hours, about 2 hours, about 1 hour, about 30 minutes, about 15 minutes or about 10 minutes prior to the administration of the

pharmaceutical composition comprising the anti-toxin A and/or B antibodies. When administered "after" the pharmaceutical composition comprising the anti-toxin A and/or B antibodies, the additional therapeutic agent may be administered about 10 minutes, about 15 minutes, about 30 minutes, about 1 hour, about 2 hours, about 4 hours, about 6 hours, about 8 hours, about 10 hours, about 12 hours, about 24 hours, about 36 hours, about 48 hours, about 60 hours or about 72 hours after the administration of the pharmaceutical composition comprising the anti-toxin A and/or B antibodies. Administration "concurrent" or with the pharmaceutical composition comprising the anti-toxin A and/or B antibodies means that the additional therapeutic agent is administered to the subject in a separate dosage form within less than 5 minutes (before, after, or at the same time) of administration of the pharmaceutical composition comprising the anti-toxin A and/or B antibodies, or administered to the subject as a single combined dosage formulation comprising both the additional therapeutic agent and the anti-toxin A and/or B antibodies.

[0196] Combination therapies may include an anti-toxin A or anti-toxin B antibody of the invention and any additional therapeutic agent that may be advantageously combined with an antibody of the invention, or with a biologically active fragment of an antibody of the invention.

[0197] For example, a second or third therapeutic agent may be employed to aid in reducing the bacterial load in the gut, such as an antibiotic that is bacteriostatic or bacteriocidal with respect to *C. difficile*. Exemplary antibiotics include vancomycin, metronidazole, or fidaxomicin. The antibodies may also be used in conjunction with other therapies, such as toxoids, vaccines specific for *C. difficile,* or probiotic agents, such as *Saccharomyces boulardii*.

Diagnostic Uses of the Antibodies

[0198] The anti-toxin A or anti-toxin B antibodies of the present invention may also be used to detect and/or measure toxin A or B in a sample, *e.g.*, for diagnostic purposes. It is envisioned that confirmation of an infection thought to be caused by *C. difficile* may be made by measuring the presence of either toxin A or toxin B through use of any one or more of the antibodies of the invention. Exemplary diagnostic assays for toxin A or toxin B may comprise, *e.g.*, contacting a sample, obtained from a patient, with an anti-toxin A or anti-toxin B antibody of the invention, wherein the anti-toxin A or anti-toxin B antibody is labeled with a detectable label or reporter molecule or used as a capture ligand to selectively isolate toxin A or toxin B protein from patient samples. Alternatively, an unlabeled anti-toxin A or anti-toxin B 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, β-galactosidase, horseradish peroxidase, or luciferase. Specific exemplary assays that can be used to detect or measure toxin A or toxin B in a sample include enzyme-linked immunosorbent assay (ELISA),

radioimmunoassay (RIA), and fluorescence-activated cell sorting (FACS).

[0199] Samples that can be used in *C. difficile* diagnostic assays according to the present invention include any tissue or fluid sample obtainable from a patient, which contains detectable quantities of either *C. difficile* toxin A or toxin B protein, or fragments thereof, under normal or pathological conditions. Generally, levels of toxin A or toxin B in a particular sample obtained from a healthy patient (*e.g.*, a patient not afflicted with a disease or condition associated with the presence of *C. difficile*) will be measured to initially establish a baseline, or standard, level of toxin A or toxin B from *C. difficile*. This baseline level of toxin A or toxin B can then be compared against the levels of toxin A or toxin B measured in samples obtained from individuals suspected of having a *C. difficile* related disease or condition, or symptoms associated with such disease or condition.

EXAMPLES

[0200] 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 *Clostridium difficile* toxin A and/or toxin B

[0201] An immunogen comprising any one of the following can be used to generate antibodies to *C. difficile* toxin A and/or toxin B. In certain embodiments, the antibodies of the invention are obtained from mice immunized with a primary immunogen, such as a full length, native, inactivated, toxin A (See GenBank accession number CAA63564 (SEQ ID NO: 378)), and/or toxin B (See GenBank accession number CAJ67492 (SEQ ID NO: 380)) from *C. difficile*, or with a recombinant, but inactivated form of the toxins, or toxin fragments, or a toxoid, followed by immunization with a secondary immunogen, or with an immunogenically active fragment of the native toxin. Animals may be immunized with either inactivated toxin A alone or inactivated toxin B alone, or with both inactivated toxin A and inactivated toxin B, concurrently. The toxins can be inactivated prior to use as an immunogen using standard procedures for preparing toxoids, including by treatment with formaldehyde, glutaraldehyde, peroxide, or oxygen treatment (Relyveld, et al. *Methods in Enzymology*, 93:24, 1983, Woodrow and Levine, eds. *New Generation Vaccines*, Marcel Dekker, Inc., New York, 1990). Another means of inactivation is by use of UDP-dialdehyde (Genth et al., (2000), Infect. Immun. 68(3):1094-1101), which may act to preserve the native structure of the toxin compared to other inactivation

methods, thereby enhancing the likelihood of eliciting antibodies that are more reactive with the native toxin. Alternatively, mutant toxins from *C. difficile*, which exhibit reduced toxicity, may be produced using standard recombinant techniques and used as immunogens (See, for example, US 5,085,862; 5,221,618; 5,244,657; 5,332,583; 5,358,868; and 5,433,945). Such mutants may contain deletions or point mutations in the active site of the toxin.

[0202] In certain embodiments, the antibodies of the invention are obtained from mice immunized with a primary immunogen, such as a biologically active and/or immunogenic fragment of native toxin A or toxin B, or DNA encoding the active fragment thereof. In certain embodiments, the immunogen may be a peptide from the N terminal or C terminal end of toxin A and/or toxin B, or a fragment derived from the N or C terminal peptide of toxin A and/or toxin B. In certain embodiments of the invention, the immunogen is the carboxy terminal receptor binding domain of toxin A that ranges from about amino acid residues 1832-2710 of SEQ ID NO: 378. In certain embodiments of the invention, the immunogen is the carboxy terminal receptor binding domain of toxin A that is shown in SEQ ID NO: 375. In certain embodiments of the invention, the immunogen is the carboxy terminal receptor binding domain of toxin B that ranges from about amino acid residues 1834-2366 of SEQ ID NO: 380. In certain embodiments of the invention, the immunogen is the carboxy terminal receptor binding domain of toxin B that is shown in SEQ ID NO: 376.

[0203] Accordingly, in one embodiment, the antibodies of the invention were obtained from mice immunized with either an inactivated full length toxin A (toxoid), or an inactivated full length toxin B (toxoid), or both toxoids. Furthermore, in one embodiment, antibodies were obtained from mice immunized with a polypeptide comprising amino acid sequences from the carboxy-terminal receptor binding domain of *C. difficile* toxin A, or with a polypeptide comprising amino acid sequences from the carboxy-terminal receptor binding domain of *C. difficile* toxin B, or both, concurrently.

[0204] In certain embodiments, antibodies that bind specifically to *C. difficile* toxin A or toxin B may be prepared using fragments of the above-noted regions, or peptides that extend beyond the designated regions by about 5 to about 20 amino acid residues from either, or both, the N or C terminal ends of the regions described herein. In certain embodiments, any combination of the above-noted regions or fragments thereof may be used in the preparation of toxin A or toxin B specific antibodies. In certain embodiments, any one or more of the above-noted regions of toxin A or toxin B, or fragments thereof may be used for preparing monospecific, bispecific, or multispecific antibodies.

[0205] The full length proteins, or carboxy-terminal fragments thereof, that were used as immunogens, as noted above, were 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 *C. difficile* toxin A and/or toxin B-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 *C. difficile* toxin A and/or toxin B-specific antibodies. Using this technique, and the various immunogens described above, several anti-*C. difficile* toxin A and toxin B, as well as cross-reactive, chimeric antibodies (*i.e.*, antibodies possessing human variable domains and mouse constant domains) were obtained; certain exemplary antibodies generated in this manner were designated as H1H3067N, H1H3134N, H1H3117N, H1M3123N, H1M3121N and H1M3124N.

[0206] Anti-*C. difficile* toxin A and toxin B 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-C. difficile toxin A and toxin B antibodies (*i.e.*, antibodies possessing human variable domains and human constant domains) were obtained; exemplary antibodies generated in this manner were designated as follows: H1H3328P, H1H3324P, H1H3325P, H1H3330P, H1H3350P, H1H3347P, H1H3335P, H1H3344P. H1H3339P, H1H3337P, H1H3343P, H1H3411P, H1H3354P, H1H3317P, H1H3355P, H1H3394P and H1H3401P.

[0207] The biological properties of the exemplary 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

[0208] Table 1 sets forth the heavy and light chain variable region amino acid sequence pairs of selected antibodies specific for toxin A and/or toxin B from *C. difficile* and their corresponding antibody identifiers. Antibodies are typically referred to herein according to the following nomenclature: Fc prefix (e.g. “H4H”, “H1M, “H2M”), followed by a numerical identifier (e.g. “3117” as shown in Table 1), followed by a “P” or “N” suffix. Thus, according to this nomenclature, an antibody may be referred to as, e.g. “H1H3117”. The H4H, H1M, and H2M 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 H1M or H2M antibody can be converted to an H4H antibody, and vice versa, but in any event, the variable domains (including the CDRs), which are indicated by the numerical identifiers shown in Table 1, will remain the same. Antibodies having the same numerical antibody designation, but differing by a letter suffix of N, B or P 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, B and P 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.

Antibody Comparators

[0209] Anti-toxin A and anti-toxin B controls were included in the following Examples for comparative purposes. Isotype matched negative controls were also used in the Examples. One anti-toxin A control antibody is designated herein as Control I and is an anti-toxin A antibody with heavy and light chain variable domain sequences of the "3D8" antibody as set forth in US7625559 and US2005/0287150. One anti-toxin B antibody is designated herein as Control II and is an anti-toxin B antibody with heavy and light chain variable domain sequences of the "124-152" antibody as set forth in US7625559 and US2005/0287150. Another anti-toxin A antibody is designated herein as Control III and is an anti-toxin A antibody with heavy and light chain variable domain sequences of the "3358" antibody as set forth in US2009/0087478.

Table 1

	SEQ ID NOs:							
Antibody Designation	HCVR	HCDR1	HCDR2	HCDR3	LCVR	LCDR1	LCDR2	LCDR3
H1H3117N	2	4	6	8	10	12	14	16
H1H3134N	18	20	22	24	26	28	30	32
H1H3067N	34	36	38	40	42	44	46	48
H1H3121N	50	52	54	56	58	60	62	64
H1H3123N	66	68	70	72	74	76	78	80
H1H3124N	82	84	86	88	90	92	94	96
H1H3324P	98	100	102	104	106	108	110	112
H1H3325P	114	116	118	120	122	124	126	128
H1H3328P	130	132	134	136	138	140	142	144
H1H3330P	146	148	150	152	154	156	158	160
H1H3350P	162	164	166	168	170	172	174	176
H1H3317P	178	180	182	184	186	188	190	192
H1H3335P	194	196	198	200	202	204	206	208
H1H3337P	210	212	214	216	218	220	222	224
H1H3339P	226	228	230	232	234	236	238	240
H1H3343P	242	244	246	248	250	252	254	256
H1H3344P	258	260	262	264	266	268	270	272
H1H3347P	274	276	278	280	282	284	286	288
H1H3354P	290	292	294	296	298	300	302	304
H1H3355P	306	308	310	312	314	316	318	320
H1H3394P	322	324	326	328	330	332	334	336
H1H3401P	338	340	342	344	346	348	350	352
H1H3411P	354	356	358	360	362	364	366	368

Example 3. Variable Gene Utilization Analysis

[0210] To analyze the structure of antibodies produced, the nucleic acids encoding antibody variable regions were cloned and sequenced. From the nucleic acid sequence and predicted amino acid sequence of the antibodies, gene usage was identified for each Heavy Chain Variable Region (HCVR) and Light Chain Variable Region (LCVR). Table 2 sets forth the gene usage for selected antibodies in accordance with the invention.

Table 2

Antibody	Antibody Identifier	HCVR			LCVR	
	HCVR/LCVR	V_H	D_H	J_H	V_K	J_K
H1H3067N	34/42	3-30	6-6	4	4-1	4
H1H3134N	18/26	3-33	3-10	4	4-1	3
H1H3117N	2/10	3-23	1-7	4	3-20	2
H1H3123N	66/74	3-48	4-11	6	1-5	1
H1H3121N	50/58	3-48	5-18	6	1-5	1
H1H3124N	82/90	3-48	3-22	6	1-5	1
H1H3328P	130/138	3-13	3-10	6	1-27	3
H1H3324P	98/106	3-13	3-10	6	1-27	3
H1H3325P	114/122	3-23	3-10	6	1-5	1
H1H3330P	146/154	3-33	1-7	4	1-39	5
H1H3350P	162/170	3-11	7-27	4	3-15	2
H1H3347P	274/282	3-23	1-26	4	1-16	3
H1H3335P	194/202	3-23	1-26	4	1-16	3
H1H3344P	258/266	3-23	2-15	4	1-16	3
H1H3339P	226/234	3-23	1-26	4	1-16	3
H1H3337P	210/218	3-23	1-26	5	1-16	3
H1H3343P	242/250	3-23	1-26	4	1-16	3
H1H3411P	354/362	3-23	1-1	6	1D-12	2
H1H3354P	290/298	6-1	2-8	4	3-11	2
H1H3317P	178/186	3-30	3-10	4	1D-12	4
H1H3355P	306/314	3-9	1-26	6	1-6	3
H1H3394P	322/330	1-2	2-2	4	3-20	4
H1H3401P	338/346	3-30	1-1	4	1D-12	2

Example 4. Antibody Binding to Toxin A and/or Toxin B from *C. difficile* as Determined by Surface Plasmon Resonance

[0211] Binding affinities and kinetic constants of human monoclonal anti-*Clostridium difficile* toxin A and/or B antibodies were determined by surface plasmon resonance at 37°C (Tables 3-5). Measurements were conducted on a T200 Biacore instrument.

[0212] Antibodies, expressed as human IgG1 Fc (AbPID prefix H1H) or hybridoma (AbPID prefix HxM), were captured onto an anti-human or anti-mouse-Fc sensor surface, respectively

(Mab capture format). Soluble full-length toxin A or B (TechLab), ranging from 5 to 10 nM, was injected over the antibody-captured surface. Antibody-antigen association was monitored for 150 seconds while dissociation in buffer was monitored for 480 seconds. Kinetic analysis was performed to calculate K_D and half-life of antigen/antibody complex dissociation using Biacore T200 evaluation software 1.0.

[0213] As seen in Tables 3-5, three types of antibodies were isolated: antibodies that bound both toxin A and toxin B ("dual binders", see Table 3), antibodies that bound only toxin A (Table 4), and antibodies that bound only toxin B (Table 5). Several antibodies were identified that bound both toxin A and toxin B, including those designated as H2M3121N, H2M3123N, H2M3124N, H1H3067N and H1H3134N and thus were classified as dual binders. Isolated anti-toxin A antibodies bound toxin in the sub-nanomolar (nM) range similar to the isotype matched comparator Mab (control I; see US patent US7625559 for comparator sequences for clone 3D8 (A toxin Ab) and clone 124-152 (B toxin Ab)), while only a few anti-toxin B binders showed affinities in the range of control II isotype matched comparator Mab (clone 124-152) (~200-300pM). Binding dissociation equilibrium constants and dissociative half-lives were calculated from the kinetic rate constants as: $K_D = k_d / k_a$; $T_{1/2}$ (min) = $(\ln 2/k_d)/60$

Table 3: Biacore affinities of anti-*C. difficile* Dual Binding mAbs at 37°C

Binding at 37°C / Mab Capture Format					
AbPID	**Analyte (Toxin)**	**ka (Ms^{-1})**	**kd (s^{-1})**	**K_D (Molar)**	**T½ (min)**
H2M3121N	Toxin A	9.69E+05	1.66E-04	1.72E-10	69
	Toxin B	6.11E+04	7.58E-05	1.24E-09	152
H2M3123N	Toxin A	1.23E+06	5.93E-04	4.81E-10	19
	Toxin B	3.97E+04	6.54E-05	1.65E-09	176
H2M3124N	Toxin A	1.14E+06	1.98E-04	1.74E-10	58
	Toxin B	3.31E+05	1.00E-06	3.02E-12	11550
H1H3067N	Toxin A	1.44E+05	3.45E-05	2.40E-10	335
	Toxin B	2.54E+03	6.43E-04	2.53E-07	18
H1H3134N	Toxin A	1.02E+05	2.82E-06	2.78E-11	4096
	Toxin B	2.99E+03	9.73E-04	3.25E-07	12

Table 4: Biacore affinities of anti-*C. difficile* Toxin A mAbs at 37°C

Binding at 37°C / Mab Capture Format					
AbPID	**Analyte**	**ka (Ms^{-1})**	**kd (s^{-1})**	**K_D (Molar)**	**T½ (min)**
H1H3117N	Toxin A	4.38E+05	3.84E-05	7.93E-11	332
H1H3324P	Toxin A	2.51E+05	3.50E-06	1.39E-11	3297
H1H3325P	Toxin A	5.27E+05	5.51E-05	1.05E-10	209
H1H3328P	Toxin A	3.82E+05	3.66E-05	9.57E-11	316
H1H3330P	Toxin A	2.50E+05	1.37E-04	5.47E-10	85
H1H3350P	Toxin A	4.02E+05	4.05E-06	1.01E-11	2854
Control I	Toxin A	3.77E+05	3.24E-05	8.59E-11	58

Table 5: Biacore affinities of anti-*C. difficile* Toxin B mAbs at 37°C

AbPID	Analyte	ka (Ms^{-1})	kd (s^{-1})	K_D (Molar)	T½ (min)
H1H3317P	Toxin B	6.50E+05	7.78E-05	1.20E-10	149
H1H3335P	Toxin B	1.77E+05	4.14E-04	2.34E-09	28
H1H3337P	Toxin B	2.41E+05	9.45E-04	3.93E-09	12
H1H3339P	Toxin B	2.76E+05	5.37E-04	1.95E-09	22
H1H3343P	Toxin B	2.84E+05	4.48E-04	1.58E-09	26
H1H3344P	Toxin B	2.04E+05	8.65E-04	4.24E-09	13
H1H3347P	Toxin B	3.39E+05	8.13E-04	2.40E-09	14
H1H3354P	Toxin B	NB	NB	NB	
H1H3355P	Toxin B	NB	NB	NB	
H1H3394P	Toxin B	4.86E+05	1.62E-04	3.33E-10	72
H1H3401P	Toxin B	4.20E+05	2.41E-04	5.74E-10	48
H1H3411P	Toxin B	2.35E+05	1.59E-04	6.77E-10	73
Control II	Toxin B	2.11E+06	4.59E-04	2.18E-10	25

NB = no binding under the conditions tested

Example 5. Determination of the binding domain for anti-*Clostridium difficile* toxin A and B antibodies using Surface Plasmon Resonance

[0214] Studies were done to determine if anti-*Clostridium difficile* toxin A and/or B antibodies bound to the C-term receptor-Binding Domain (CBD) of each toxin. In these studies, two experimental Biacore formats were employed. The first utilized captured anti-*C. difficile* antibody surfaces in which 100nM of CBD-toxin A-Fc (SEQ ID NO:375) or CBD-toxin B-Fc (SEQ ID NO:376) was flowed over and the responses (RU) recorded. The CBD-toxin reagents were formatted in both human and mouse Fc to enable both hybridoma and human Fc formatted antibody analysis. The second format employed antigen (CBD-Fc) captured surfaces in which 500nM of anti-*C. difficile* mAb was flowed over. In this format, hybridoma or human Fc formatted antibodies were flowed over human and mouse Fc captured antigens, respectively. In both formats a response that was significantly above background (>50 RU) was considered binding to the CBD of toxin A or B (see Table 6). For both anti-toxin A and anti-toxin B antibodies, epitopes that were within and outside the CBD were obtained. Both control I (3D8 antibody from US7625559 and US 2005/0287150) and control II (124-152 antibody from US7625559 and US 2005/0287150) were mapped to the CBD of their respective toxins in agreement with previous reports (data not shown; see US 2005/0287150 and US7625559).

Table 6: Determination of the domain of binding for *C. difficile* antibodies

	C-term Toxin A Binding		C-term Toxin B Binding		
mAb	mAb Capture 100nM CBD-A Binding (RU)	CBD-A Capture 500nM mAB Binding (RU)	mAB Capture 100nM CBD-B Binding (RU)	CBD- B Capture 500nM mAb Binding (RU)	Domain Binding#

H2aM3067	-2	237	25	369	C-Term
H1M3117	-3	350	-1	21	C-Term A
H2aM3121	0	23	2	10	Non CBD
H2aM3123	1	23	1	14	Non CBD
H2aM3124	0	29	0	19	Non CBD
H1M3134	-1	195	23	394	C-Term
H1H3324P	269	224	19	-8	C-Term A
H1H3325P	17	3	7	-8	Non CBD
H1H3328P	354	227	35	-6	C-Term A
H1H3330P	441	515	40	-4	C-Term A
H1H3335P	13	5	13	-6	Non CBD
H1H3337P	-17	8	-24	-2	Non CBD
H1H3339P	19	2	14	-2	Non CBD
H1H3343P	11	3	9	-4	Non CBD
H1H3344P	5	5	4	-2	Non CBD
H1H3347P	42	-13	44	7	Non CBD
H1H3354P	-19	-2	-24	-4	Non CBD

Non CBD indicates no binding to C-term receptor Binding Domain of Toxin-A or -B.

Example 6. Determination of the domain of binding for anti-*Clostridium difficile* toxin A and B antibodies using Size Exclusion Chromatography

[0215] As a complimentary method for determining if anti-*Clostridium difficile* toxin A and/or B antibodies bound the C-term receptor Binding Domain (CBD), size exclusion chromatography (SEC) was utilized. Briefly, the CBD of toxin A (SEQ ID NO: 375) or the CBD of toxin B (SEQ ID NO: 376), at ~500nM was mixed with excess antibody at specified molar ratios (1:5 and 1:20; CBD:Mab) in phosphate buffered saline containing 5% glycerol pH 7.4 (PBS/G) and incubated at room temperature for 1 hour.

[0216] Any precipitation visible after 1 hr was recorded as +++ (strong), ++ (moderate), + (minimal), or – (not observed). Following centrifugation (5 min. @ 16,000 x g), the mixture of antibody and CBD was subjected to SEC analysis using a Superose 6 column (GE Healthcare) with PBS/G as the mobile phase. Protein peaks corresponding to complexes larger than the antibody or CBD alone were interpreted as binding to the C-terminal domain.

[0217] The results demonstrated that CBD binding corresponds well with that predicted from the domain of binding inferred from SPR (Biacore) and CBD studies (see example 5). One notable exception was H1H3134N, where binding to CBD-A was not observed via SEC but K_D values indicated dual binding properties for the antibody.

Table 7: Domain of binding for anti-*Clostridium difficile* toxin A and B antibodies

mAb	Precipitation with CBD-A	Observed CBD-A binding via SEC	Precipitation with CBD-B	Observed CBD-B binding via SEC	Domain Binding Via Biacore
H2M3067N	+++	Yes	NT	NT	C-Term A/B

H1M3117N	+++	Yes	NT	NT	C-Term A
H2M3121N	-	No	NT	NT	Non CBD
H2M3123N	-	No	NT	NT	Non CBD
H2M3124N	-	No	NT	NT	Non CBD
H1M3134N	-	No	NT	NT	C-Term A/B
H1H3317P	NT	NT	-	Yes	NT
H1H3324P	+	Yes	NT	NT	C-Term A
H1H3325P	-	No	NT	NT	Non CBD
H1H3328P	-	Yes	NT	NT	C-Term A
H1H3330P	-	Yes	N.D.	N.D.	C-Term A
H1H3335P	NT	NT	-	No	Non CBD
H1H3337P	NT	NT	-	No	Non CBD
H1H3339P	NT	NT	-	No	Non CBD
H1H3343P	NT	NT	-	No	Non CBD
H1H3344P	NT	NT	-	No	Non CBD
H1H3347P	NT	NT	-	No	Non CBD
H1H3350P	-	Yes	NT	NT	NT
H1H3354P	NT	NT	-	No	Non CBD

NT= Not Tested. TBD= To Be Done
Non CBD indicates no binding to C-term receptor Binding Domain of Toxin-A or -B.

Example 7. Determination of the neutralization potency of anti-*Clostridium difficile* toxin A and/or toxin B antibodies

[0218] To determine the neutralization potency (IC_{50}) of anti-*C. difficile* antibodies *in vitro*, a cell viability assay was conducted. Briefly, Vero cells ($1.25x10^3$) cultured in MEM alpha medium, supplemented with 10 % FBS, were seeded into 96-well microplates and incubated for 16-18 hours at 37°C, in 5% CO_2. Anti-*C. difficile* toxin antibodies, at various concentrations (0-66nM), were added to the cells and subsequently incubated with *C. difficile* toxin A (32 or 25pM) or toxin B (0.03pM or 0.01pM) for 48hrs. Controls not containing toxin (100% viability) and controls containing toxin but no antibody (100% relative lethality) were utilized. All dilutions of antibody were conducted in triplicate. Following the 2-day incubation, cell viability was measured by adding tetrazolium salt (WST-1; Roche Biochemicals), waiting for 4hrs to allow for color development and then recording absorbance at 450 nm. Absorbance values were analyzed by a four-parameter logistic equation over an 11-point response curve (GraphPad Prism).

[0219] The results showed that ten antibodies displayed neutralization against toxin A with IC_{50} values ranging from 7pM to 65pM at 25-32pM constant toxin A (Table 8A). Of note, H1H3330P demonstrated neutralization potency equal to that of Control III (isotype matched comparator antibody, clone 3358 as set forth in US2009/0087478) and potency of approximately 20 fold greater than control I (see US2005/0287150 for clone 3D8). Several toxin-B neutralizing antibodies showed significantly greater potency than control II (isotype matched comparator antibody, see clone 124-152 of US2005/0287150) with IC_{50}s ranging from 25-120pM at 0.03pM constant toxin B (Table 8B). Antibodies H1M3067N and H1M3134N, while able to bind both

toxin A and B showed only neutralization activity against toxin A. While the reason for this is not yet known, one possible explanation for this finding may be that while antibodies can bind at many sites in the repetitive regions of the C terminal portion of the toxin, other parts of the same toxin domain may still be capable of interacting with the mammalian membrane, thus allowing entry of the toxin into the cell.

Table 8A: Neutralization potency (IC_{50}) for selected mAbs against Toxin A

mAb	Trial 1 (IC_{50}) 32pM Toxin A	Trial 2 (IC_{50}) 32pM Toxin A	Trial 3 (IC_{50}) 32pM Toxin A	Trial 4 (IC_{50}) 32pM Toxin A	Trial 5 (IC_{50}) 25pM Toxin A
H1M3067N	64pM	44pM	NT	NT	NT
H1M3117N	29pM	11pM	NT	NT	NT
H2aM3121N	65pM	35pM	NT	NT	NT
H2aM3123N	65pM	24pM	NT	NT	NT
H2aM3124N	41pM	21pM	NT	NT	NT
HIM3134N	NT	NT	NT	38pM	NT
H1H3324P	NT	NT	NT	33pM	NT
H1H3325P	NT	NT	NT	33pM	NT
H1H3328P	NT	NT	112pM	NT	NT
H1H3330P	NT	NT	7pM	NT	7pM
Control I	NT	NT	NT	NT	199pM
Control III	18pM	6pM	10pM	11pM	9pM

NT: Not tested

Table 8B: Neutralization potency (IC_{50}) for selected Mabs against Toxin B

mAb	Trial 1 (IC_{50}) 0.1pM Toxin B	Trial 2 (IC_{50}) 0.1pM Toxin B	Trial3 (IC_{50}) 0.03pM Toxin B
H1M3067N	No Neutralization		
HIM3134N	No Neutralization		
HIH3317P	No Neutralization	NT	NT
H1H3335P	730pM	380pM	120pM
H1H3337P	1730pM	980pM	320pM
H1H3339P	480pM	270pM	90pM
H1H3343P	280pM	200pM	50pM
H1H3344P	580pM	400pM	40pM
H1H3347P	130pM	90pM	25pM
H1H3350P	No Neutralization	NT	NT
H1H3340P	NT	No Neutralization	NT
H1H3411P	NT	8pM#	NT
Control II	1800pM	1500pM	290pM

Antibody only partially protect (40-50%) at highest concentration
NT: Not Tested

Example 8. Generation of a Bi-specific Antibody

[0220] Various bi-specific antibodies are generated for use in practicing the methods of the invention. For example, *C. difficile* toxin A or toxin B-specific antibodies are generated in a bi-specific format (a "bi-specific") in which variable regions binding to distinct domains of toxin A and/or B are linked together to confer dual-domain and/or dual toxin specificity within a single binding molecule. Appropriately designed bi-specifics may enhance overall toxin neutralization efficacy through increasing both specificity and binding avidity. Variable regions with specificity for individual domains, as shown in Figure 1, (*e.g.*, segments of the N-terminal domain, which is the glucosylating enzymatic domain (designated as domain 'A'), or the autocatalytic processing domain (designated as domain 'C'), or the translocating domain (designated as domain 'D'), or the carboxy terminal receptor binding domain (designated as domain 'B') or that can bind to different regions within one domain, are paired on a structural scaffold that allows each region to bind simultaneously to the separate epitopes, or to different regions within one domain. In one example for a bi-specific, heavy chain variable regions (V_H) from a binder with specificity for one domain are recombined with light chain variable regions (V_L) from a series of binders with specificity for a second domain to identify non-cognate V_L partners that can be paired with an original V_H without disrupting the original specificity for that V_H. In this way, a single V_L segment (*e.g.,* V_L1) can be combined with two different V_H domains (*e.g.,* V_H1 and V_H2) to generate a bi-specific comprised of two binding "arms" (V_H1- V_L1 and V_H2- V_L1). Use of a single V_L segment reduces the complexity of the system and thereby simplifies and increases efficiency in cloning, expression, and purification processes used to generate the bi-specific (See, for example, USSN13/022759 and US2010/0331527).

[0221] Alternatively, antibodies that bind both toxin A and/or toxin B and a second target, such as, but not limited to, for example, a second different anti-toxin A or anti-toxin B antibody, or a toxoid, or a vaccine, may be prepared in a bi-specific format using techniques described herein, or other techniques known to those skilled in the art. Antibody variable regions binding to distinct toxin A regions may be linked together with variable regions that bind to relevant sites on, for example, toxin B, to confer dual-antigen specificity within a single binding molecule. Appropriately designed bi-specifics of this nature serve a dual function. For example, in the case of a bi-specific antibody that binds both toxin A and toxin B, one may be able to better neutralize both toxin A and toxin B concurrenty, without the need for administration of a composition containing two separate antibodies. Variable regions with specificity for toxin A, are combined with a variable region with specificity for toxin B and are paired on a structural scaffold that allows each variable region to bind to the separate antigens.

[0222] The bi-specific binders are tested for binding and functional blocking of the target antigens, for example, toxin A and toxin B, in any of the assays described above for antibodies. For example, standard methods to measure soluble protein binding are used to assess the bispecific interaction, such as Biacore, ELISA, size exclusion chromatography, multi-angle laser

light scattering, direct scanning calorimetry, and other methods. Binding of bi-specific antibodies to both toxin A and toxin B is determined through use of an ELISA binding assay in which synthetic peptides representing the different toxins are coated onto the wells of microtiter plates, and binding of a bi-specific is determined through use of a secondary detection antibody. Binding experiments can also be conducted using surface plasmon resonance experiments, in which real-time binding interaction of peptide to antibody is measured by flowing a peptide or bi-specific across a sensor surface on which bi-specific or peptide, respectively, is captured. Functional *in vitro* blocking of both toxin A and toxin B by a bi-specific is determined using any bioassay such as the neutralization assay described herein, or by *in vivo* protection studies in appropriate animal models, such as those described herein.

Example 9. Evaluation of *in vivo* Efficacy of Anti-Toxin A and/or Anti-Toxin B Antibodies against *C. difficile* Infection (CDI) in a Hamster Relapse Model (A) and in an Acute Hamster Model (B)

[0223] The efficacy of antibodies specific for toxin A and/or toxin B from *C. difficile* against infection with *C. difficile* was evaluated in hamsters in two different models, described below. Hamsters, in the presence of clindamycin, are sensitive to *C. difficile* infection and usually die within 2-4 days after infection.

[0224] (A) Relapse model: Male Syrian Golden Hamsters were given an oral suspension containing a mixture of *C. difficile* spores and vegetative cells (10^6 in total) on day -1. Twenty-four hours after infection (day 0), animals received a single subcutaneous injection of clindamycin (10 mg/kg). On days 1-3, hamsters were administered oral vancomycin (10 mg/kg) once per day to ameliorate the infection. The antibiotic vancomycin is used clinically to treat a *C. difficile* infection. After the last vancomycin dose, antibodies were administered subcutaneously q.d. for 4 days (days 3-6), or 1 day (day 3) according to their treatment and dosing group (see Tables 9A and 9B below).

[0225] Two different trials (See Figures 2 and 3) using the relapse model as a surrogate for clinical efficacy were conducted. Both trials compared two antibody combinations:

1. Antibodies designated H1H3330P and H1H3347P
2. Comparator anti-Toxin A (Control I; See US patent 7625559 for clone 3D8 sequence) and comparator anti-Toxin B antibodies (Control II; See US patent 7625559 for clone 124-152 sequence)

[0226] In Trial 1, four doses of antibody were administered at 5 mg/kg of each antibody (a total 10 mg/kg dose). In Trial 2, one dose of antibody was given at either 5 mg/kg or 2 mg/kg of each antibody (a total of either 10mg/kg or 4 mg/kg).

Table 9A Trial 1 Design: Relapse model: Combination Treatments with H1H3330P + H1H3347P or comparator anti-Toxin A + comparator anti-Toxin B

Group	Treatment*	Dose (mg/kg x # doses)	*n*
1	Negative Control (irrelevant) antibody	10 x 4	14
2	Comparator anti-Toxin A and comparator anti-Toxin B	[5/5] x 4	23
3	(H1H3330P + H1H3347P combination)	[5/5] x 4	23
4	No antibody		15

* All animals received vancomycin as noted above.

Table 9B Trial 2 Design: Relapse model: Combination Treatments with H1H3330P + H1H3347P or comparator anti-Toxin A + comparator anti-Toxin B

Group	Treatment*	Dose (mg/kg x # doses)	*n*
1	Negative Control (irrelevant) antibody	10 x 1	14
2	Comparator anti-Toxin A and comparator anti-Toxin B	[5/5] x 1	16
3	(H1H3330P + H1H3347P combination)	[5/5] x 1	16
4	Comparator anti-Toxin A and comparator anti-Toxin B	[2/2] x 1	16
5	(H1H3330P + H1H3347P combination)	[2/2] x 1	16

* All animals received vancomycin as noted above.

[0227] Animals were observed twice a day for the duration of the experiment. General observations included signs for mortality and morbidity, the presence of diarrhea ("wet tail") and overall appearance (activity, general response to handling, touch, ruffled fur). Animals judged to be in a moribund state were euthanized. Criteria used to assign a moribund state were extended periods (5 days) of weight loss, progression to an emaciated state, prolonged lethargy (more than 3 days), signs of paralysis, skin erosions or trauma, hunched posture, and a distended abdomen. Observations continued, with deaths or euthanasia recorded for a period up to 18 days post-infection for the relapse model.

[0228] (B) **Acute model:** Male Syrian Golden Hamsters were treated with clindamycin intraperitoneally at a dose of 10 mg/kg on day -1. On day 0 *C. difficile* spores were diluted with sterile PBS to give 100 spores /300 µl and administered by oral gavage. Antibodies were administered every day for 4 days, beginning on day -3 and continuing to day 0, using a subcutaneous route. The dose of the antibodies is indicated in the figure legend. See also Table 9C below for the study outline.

Table 9C Trial 3: Acute model: Combination Treatments with H1H3330P + H1H3347P at various doses

Group	Treatment	Dose (mg/kg x # doses)	# Animals
1	Uninfected control	-	5
2	Infected control	PBS x 4	10
3	Negative Control (irrelevant) antibody	[100] x 4	14
4	(H1H3330P + H1H3347P combination)	[50/50] x 4	14
5		[16/16] x 4	14
6		[5.5/5.5] x 4	14
7		1.85/1.85 x 4	14

Table 9D Trial 4: Acute model: Combination Treatments with H1H3330P + H1H3347P at various doses

Group	Treatment	Dose (mg/kg x # doses)	# Animals
1	Uninfected control	-	5

2	Infected control	PBS x 4	10
3	Negative Control (irrelevant) antibody	[100] x 4	14
4	(H1H3330P + H1H3347P combination)	[20/20] x 4	14
5		[5/5] x 4	14
6	Comparator anti-Toxin A and comparator anti-Toxin B	[20/20] x 4	14
7		[5/5] x 4	14

[0229] Animals were observed twice a day for the duration of the experiment. General observations included signs for mortality and morbidity, the presence of diarrhea ("wet tail") and overall appearance (activity, general response to handling, touch, ruffled fur). Animals judged to be in a moribund state were euthanized. Criteria used to assign a moribund state were extended periods (5 days) of weight loss, progression to an emaciated state, prolonged lethargy (more than 3 days), signs of paralysis, skin erosions or trauma, hunched posture, and a distended abdomen. Observations continued, with deaths or euthanasia recorded for a period up to10 days for the acute model.

Results

[0230] Statistical analysis of data from hamster models was done using the Log-Rank (Mantel Cox) test. For pairwise comparisons the Bonferroni correction was applied to the critical *P* value.

[0231] In the first trial, which was a multi-dose study using a hamster relapse model, (see Figure 2), combination treatment with H1H3330P plus H1H3347P, or combination treatment with the comparator antibodies, showed an increase in overall survival vs isotype control, or vancomycin alone, (74-78%; combination of anti-toxin A and anti-toxin B antibodies vs 27-43 %; control arms). Specifically, by day 19, 27% of the hamsters receiving PBS alone survived; 43% receiving the isotype control survived; 74% receiving the anti-toxin A plus anti-toxin B comparator antibody combination survived; and 78% receiving the H1H3330P (anti-A antibody) plus H1H3347P (anti-B antibody) combination survived.

[0232] In the second trial, which was a single-dose study using a hamster relapse model (see Figure 3), combination treatment with either H1H3330P plus H1H3347P, or the comparator

antibodies, increased survival as compared to the isotype control (negative control antibody), although there was no discrimination between the 2 mg/kg and 5 mg/kg doses.

[0233] In the first acute model study in hamsters (See Figure 4), treatment with the H1H3330P plus H1H3347P combination showed significant protection of the hamsters from death in a titratable manner compared to the negative controls ($p<0.0001$ for all groups vs isotype controls). The doses titrated from 50 mg/kg to 1.85 mg/kg (of each antibody given as a combination), with the high dose providing protection for all of the animals until day 7 compared to day 1 for the lowest dose.

[0234] In further studies using the acute hamster model (see figure 5), combination treatment with either H1H3330P plus H1H3347P, or a combination of the comparator antibodies, significantly increased survival as compared to the isotype control (Figure 5; Isotype control at 40 mg/kg vs Control I/Control II at 20 mg/kg each, $p<0.0001$; isotype control at 40 mg/kg vs Control I/Control II at 5 mg/kg each, $p=0.0003$; isotype control at 40 mg/kg vs H1H3330P/H1H3347P at 20 mg/kg each, $p<0.0001$; isotype control at 40 mg/kg vs H1H3330P/H1H3347P at 5 mg/kg each, $p<0.0001$). However, treatment with a combination of H1H3330P plus H1H3347P protected the hamsters from death in a manner superior to comparator antibody controls when tested at the low dose of 5 mg/kg of each antibody ($p<0.0001$), whereas there was no significant difference between the combination of H1H3330P plus H1H3347P vs the combination of the comparator antibodies at the higher dose of 20 mg/kg of each antibody ($p=0.73$). This result clearly demonstrates superiority at a low dose in the acute hamster model and suggests that doses of the H1H3330P plus H1H3347P antibodies could be effective in the clinic at lower concentrations compared to the comparator antibodies.

Example 10. The Effect of Anti-Toxin A and Anti-Toxin B Antibodies on Blocking the Cytotoxicity Induced by Culture Supernatant from Several Group BI Hypervirulent *C. difficile* Strains: Comparison with Comparator mAbs

[0235] Patients infected with clinically-hypervirulent BI/NAP1/027 strains have lower cure rates than patients infected with non-BI strains when treated with either fidaxomicin or vancomycin (Petrella, LA, *et al.* (2012), Clinical Infectious Diseases, 55(3): 351-357). Furthermore, BI/NAP1/027 strains are associated with a higher CDI recurrence rate and higher expected mortality rate when compared to prototypic strains (Loo, VG, *et al.* (2005), N Engl J Med, 353:23; Petrella, LA, *et al.* (2012), Clinical Infectious Diseases, 55(3): 351-357. These hypervirulent strains are characterized by an increase in toxin A and B production, the presence of binary toxin and increased resistance to fluoroquinolones. The increase in toxin A and B production is most likely caused by a loss-of-function mutation in *tcdC*, a putative negative regulator of *tcdA* and *tcdB* expression, resulting in sustained toxin production throughout the lifecycle.

[0236] The ability of a 1:1 molar ratio mix of H1H3330P and H1H3347P to neutralize toxin from four clinically-isolated *C. difficile* BI/NAP1/027 strains was tested in a cell-based neutralization assay. The VA5 and VA17 clinically isolated hypervirulent strains were obtained from Case Western Reserve University, Cleveland, OH. The 6336 and 6443 clinically isolated hypervirulent strains were obtained from the Dept. of Veterans Affairs, Edward Hines, Jr. Hospital, Hines, IL. Neutralization assays utilized Vero cells, a monkey kidney epithelial cell line, due to their susceptibility to both *C. difficile* toxins. Cells were incubated with varying amounts of antibody cocktail and a fixed amount of culture supernatant isolated from several *C. difficile* strains for 48 hours. Cytotoxicity was determined by addition of WST-1 reagent, a redox indicator that yields a colorimetric change when reduced; metabolic activity during cell growth reduces WST-1, resulting in increased absorbance at 450 nm.

[0237] Culture supernatant from several clinically isolated BI strains exhibited a wide range of cytotoxic activity on Vero cells, with EC_{50} values for inducing cell cytotoxicity ranging from a 3700-fold dilution for the VA5 strain to 88200-fold dilution for the 6443 strain. A 1:1 molar ratio mix of H1H3330P and H1H3347P blocked cytotoxicity induced by culture supernatants from all group BI strains tested with a more than 34-fold better neutralization potency compared to a 1:1 molar ratio mix of comparator anti-Toxin A (control I; See US patent 7625559 for clone 3D8 sequence) and comparator anti-Toxin B (control II; See US patent 7625559 for clone 124-152 sequence). These data demonstrate that the H1H3330P/H1H3347P antibody pair was able to neutralize culture cytotoxicity with IC_{50} values in the picomolar range (31-45pM) for tested hypervirulent BI strains, compared to the comparator mAb cocktail (IC_{50} range: 1200-1700pM; see Table 10).

Table 10

Strain	EC_{50} (Fold Dilution)	Neutralization Assay			
		[Supernatant] (Fold Dilution)	H1H3330P/ H1H3347P IC_{50} (pM)	Comparator mAb 1/2 IC_{50} (pM)	Fold pair/control
VA5	6900	4700	36	1400	39
VA17	3700	3000	31	1400	45
6336	15200	12100	45	1700	38
6443	88200	57500	35	1200	34

Example 11. Epitope mapping of the anti-toxin A antibody H1H3330P

[0238] The epitope of the C-term receptor-Binding Domain (CBD) of toxin A (SEQ ID:375) bound by H1H3330P was determined using mass spectrometry based proteomics. Briefly, the CBD of toxin A was subjected to trypsin digestion over a 12 hour period and the samples run on 10-14% gradient SDS-PAGE, followed by transfer to nitrocellulose membranes and Western

blot analysis using either the H1H3330P antibody, or the control I antibody (See US patent 7625559 for 3D8 antibody sequence) as primary antibodies. Further analysis of the peptide sequences that bound to the H1H3330P antibody was done using 2D electrophoresis followed by MALDI-TOF MS analysis. The procedures are described in greater detail below.

Limited trypsin digestion of Toxin A

[0239] Recombinant C-term receptor-Binding Domain (CBD) of toxin A (0.4 µg/µl in PBS) was added with sequence grade modified trypsin (Promega, Cat # V511C) at a 1:80 mass ratio and incubated at 37°C for 0-12 hr. The enzyme was inactivated by adding 2 volumes of 1X Laemmli sample buffer and heated at 95°C for 5 min. The samples were stored at -20°C until analysis.

Western blot analysis

[0240] The extent of the proteolysis was first examined by 10-14% gradient SDS-PAGE. The amount of each sample loaded was equivalent to 1 µg initial CBD of Toxin A, and the separated proteins in the gel were visualized with SimplyBlue coomassie stain (Invitrogen, Cat# LC6065).

[0241] The samples digested for 0 hour and 12 hour were then selected for 10-14% gradient SDS-PAGE separation with an equivalent to 50 ng initial CBD of toxin A loaded for each sample. Separated proteins were transferred to a nitrocellose membrane, and probed with primary antibody H1H3330P or Control Antibody I at a concentration of 1 µg/ml in TBST (Tris-buffer saline solution containing 0.05% Tween-20) overnight at 4°C followed by anti-human IgG HRP conjugated secondary antibody (Pierce, Cat # 31412; at 1:15,000 dilution in TBST). Both H1H3330P & Control 1 antibodies had a human IgG1 constant domain. The membrane was incubated with chemiluminescence substrate (Perkin Elmer, Cat # NEL103E001EA) and the image was captured onto X-ray film.

2D Gel Electrophoresis

[0242] To determine which amino acids were represented in the protein band unique to Western blots performed using the H1H3330P antibody, a two-dimensional (2D) gel was performed using the 12 hour trypsin digest of the CBD of toxin A.

In-gel trypsin digestion and peptide mapping by MALDI-TOF MS

[0243] The 2D-gel analysis revealed 4 protein spots, closely clustered in the pH (pI values ~ 9) dimension, which composed the 50kDa band that was visualized by Western blot using H1H3330P. The 4 protein spots with corresponding molecular weights of ~50 kDa from the 2D-gel using the 12 hour trypsin digestion were excised, destained by 50% acetonitrile, reduced by 65 mM DTT, and alkylated by 135 mM iodoacetamide. After dehydration by acetonitrile, 20 ul of

2.5 ng/µl sequence grade trypsin (Promega, Cat # V5111) was added to cover the gel bands and the in-gel digestion was carried out by overnight incubation at 37°C.

[0244] The resulting peptides were desalted by Ziptip C18 (Millipore, Cat# ZTC18S096) and analyzed by Bruker UltrafleXtreme MALDI-TOF-TOF MS. The spectrum was processed by FlexAnalysis software and internally calibrated with autolytic trypsin fragment peaks. The calibrated peak lists were searched against an in-house database containing the sequence of the CBD Toxin A at a mass accuracy of 10 ppm.

Results

[0245] The results showed that blotting with H1H3330P revealed a major protein band at around 50kDa at 12 hours post trypsin digestion, while no protein band having a molecular weight of around 50kDa was observed when blotting was carried out with the control 1 antibody at 12 hours post trypsin digestion.

[0246] To determine which amino acids were represented in the 50kDa protein band, unique to Western blots performed using H1H3330P, a two-dimensional (2D) gel was performed using the 12 hour trypsin digest, as described above. As noted, 2D-gel analysis revealed 4 protein spots, closely clustered in the pH (pI values ~ 9) dimension, which composed the 50kDa band visualized by Western blot using H1H3330P. Mass spectrometry analysis of these 4 protein spots identified 17 matching peptides covering amino acid residues 468-863 of the CBD of toxin A (SEQ ID: 375). This fragment of toxin A (amino acids spanning residue 468 to 863) has a predicted molecular weight of 45 kDa and a predicted isoelectric point of 9.01 corresponding well to the values obtained from 2-D gel analysis.

[0247] This example illustrates that the anti-toxin A antibody H1H3330P has an epitope unique from that of Control 1 (3D8 in US 7625559) and binds the CBD of toxin A within amino acids 468 to 863. Particular amino acid sequences were identified within this region which interacted with the H1H3330P antibody and these amino acid sequences were residues 468-488 of SEQ ID NO: 375, residues 510-530 of SEQ ID NO: 375, residues 602-610 of SEQ ID NO: 375, residues 644-703 of SEQ ID NO: 724-794 of SEQ ID NO: 375, residues 799-814 of SEQ ID NO: 375 and residues 858-863 of SEQ ID NO: 375.

What is claimed is:

1. An isolated antibody or antigen binding fragment thereof that specifically binds to *Clostridium difficile* toxin A,

comprising the three heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) contained within a heavy chain variable region (HCVR) amino acid sequence of SEQ ID NO: 146; and the three light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3) contained within a light chain variable region (LCVR) amino acid sequence of SEQ ID NO: 154.

2. The isolated antibody or antigen-binding fragment thereof of claim 1 wherein the antibody, or antigen-binding fragment thereof comprises: (a) a HCVR having an amino acid sequence of SEQ ID NO: 146; and (b) a LCVR having an amino acid sequence of SEQ ID NO: 154.

3. The isolated antibody of claim 1, or an antigen-binding fragment thereof wherein the antibody comprises:

(a) a HCDR1 domain having an amino acid sequence of SEQ ID NO: 148**;**
(b) a HCDR2 domain having an amino acid sequence of SEQ ID NO: 150;
(c) a HCDR3 domain having an amino acid sequence of SEQ ID NO: 152;
(d) a LCDR1 domain having an amino acid sequence of SEQ ID NO: 156;
(e) a LCDR2 domain having an amino acid sequence of SEQ ID NO: 158; and
(f) a LCDR3 domain having an amino acid sequence of SEQ ID NO: 160.

4. The isolated antibody or antigen binding fragment thereof according to any one of the previous claims that interacts with, or binds to, an epitope within amino acid residues 468-863 of the carboxy terminal receptor binding domain of toxin A produced by *Clostridium difficile*, or an antigen binding fragment thereof, wherein the carboxy terminal receptor binding domain of toxin A comprises the amino acid sequence of SEQ ID NO: 375.

5. The isolated antibody or antigen binding fragment thereof according to any one of the previous claims that interacts with or binds to an epitope within the carboxy terminal receptor binding domain of toxin A produced by *Clostridium difficile*, or an antigen binding fragment thereof, wherein the epitope is selected from the group consisting of residues 468-488 of SEQ ID NO: 375, residues 510-530 of SEQ ID NO: 375, residues 602-610 of SEQ ID NO: 375, residues

644-703 of SEQ ID NO: 375, residues 724-794 of SEQ ID NO: 375, residues 799-814 of SEQ ID NO: 375 and residues 858-863 of SEQ ID NO: 375.

6. A pharmaceutical composition comprising one or more antibodies of any one of the previous claims and a pharmaceutically acceptable carrier or diluent.

7. The pharmaceutical composition of claim 6, wherein the composition further comprises at least one antibody, or an antigen-binding fragment thereof that binds specifically to toxin B of *Clostridium difficile*, wherein:

the antibody or antigen-binding fragment thereof that binds specifically to toxin B comprises the three heavy chain CDRs (HCDR1, HCDR2 and HCDR3) contained within any one of the HCVR amino acid sequences selected from SEQ ID NOs: 178, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338 and 354; and the three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within any one of the LCVR amino acid sequences selected from SEQ ID NOs: 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346 and 362.

8. The pharmaceutical composition according to claims 6 to 7, wherein the antibody or an antigen-binding fragment thereof that specifically binds toxin A of *Clostridium difficile* comprises a HCVR/LCVR amino acid sequence pair of SEQ ID NO: 146/154, and wherein the antibody or an antigen-binding fragment thereof that specifically binds toxin B of *Clostridium difficile* comprises a HCVR/LCVR amino acid sequence pair of SEQ ID NO: 274/282.

9. The pharmaceutical composition of any of claims 6 to 8, comprising:

a) an isolated first antibody, or antigen-binding fragment thereof that specifically binds toxin A of *Clostridium difficile*, comprising a HCDR1 having the amino acid sequence of SEQ ID NO: 148, a HCDR2 having the amino acid sequence of SEQ ID NO: 150, a HCDR3 having the amino acid sequence of SEQ ID NO: 152, a LCDR1 having the amino acid sequence of SEQ ID NO: 156, a LCDR2 having the amino acid sequence of SEQ ID NO: 158, a LCDR3 having the amino acid sequence of SEQ ID NO: 160;

b) an isolated second antibody, or antigen-binding fragment thereof that specifically binds toxin B of *Clostridium difficile*, comprising a HCDR1 having the amino acid sequence of SEQ ID NO: 276, a HCDR2 having the amino acid sequence of SEQ ID NO: 278, a HCDR3 having the amino acid sequence of SEQ ID NO: 280, a LCDR1 having the amino acid sequence of SEQ ID NO: 284, a LCDR2 having the amino acid sequence of SEQ ID NO: 286, a LCDR3 having the

amino acid sequence of SEQ ID NO: 288; and

c) a pharmaceutically acceptable carrier or diluent.

10. The pharmaceutical composition according to any one of claims 6 to 9, wherein the antibodies contained within the composition are effective at neutralizing toxins A and B from a hypervirulent strain of *Clostridium difficile*.

11. The pharmaceutical composition according to claim 10, wherein the hypervirulent strain of *Clostridium difficile* is a BI/NAP1/027 strain.

12. The pharmaceutical composition of claim 11, wherein the BI/NAP1/027 strain is selected from VA5, VA17, 6336 and 6443.

13. The pharmaceutical composition according to any one of claims 7 to 12, wherein the antibody or antigen-binding fragment thereof that specifically binds *Clostridium difficile* toxin B comprises: a HCVR/LCVR amino acid sequence pair selected from SEQ ID NOs: 178/186, 194/202, 210/218, 226/234, 242/250, 258/266, 274/282, 290/298, 306/314, 322/330, 338/346 and 354/362.

14. The pharmaceutical composition of according to any one of claims 7 to 12, wherein the antibody or antigen-binding fragment thereof that specifically binds *Clostridium difficile* toxin B comprises:

(a) a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 180, 196, 212, 228, 244, 260, 276, 292, 308, 324, 340 and 356;

(b) a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 182, 198, 214, 230, 246, 262, 278, 294, 310, 326, 342 and 358;

(c) a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 184, 200, 216, 232, 248, 264, 280, 296, 312, 328, 344 and 360;

(d) a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 188, 204, 220, 236, 252, 268, 284, 300, 316, 332, 348 and 364;

(e) a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 190, 206, 222, 238, 254, 270, 286, 302, 318, 334, 350 and 366; and

(f) a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 192, 208, 224, 240, 256, 272, 288, 304, 320, 336, 352 and 368.

15. The pharmaceutical composition according to any one of claims 7 to 12, wherein the antibody or an antigen-binding fragment thereof that specifically binds toxin B of *Clostridium difficile* comprises a HCVR/LCVR amino acid sequence pair of SEQ ID NO: 274/282.

16. Use of the isolated antibody or antigen binding fragment thereof according to any one of claims 1 to 5 or the pharmaceutical composition according to any one of claims 6 to 15 in the manufacture of a medicament for use in treating a patient suffering from a *Clostridium difficile*-associated condition or disease, or for treating at least one symptom or complication associated with the condition or disease, or for preventing the development of a *Clostridium difficile*-associated condition or disease in a patient at risk thereof, wherein the *Clostridium difficile*-associated condition or disease is either prevented, or lessened in severity and/or duration, or at least one symptom or complication associated with the condition or disease is prevented, or ameliorated, or that the frequency and/or duration of, or the severity of recurrences, or relapses with *Clostridium difficile* is reduced.

17. The use according to claim 16, wherein the patient at risk of developing a *Clostridium difficile*-associated condition or disease is selected from an elderly patient, a patient who is immunocompromised due to illness or due to administration of immunosuppressive therapeutics, a patient who has some underlying medical condition that may pre-dispose them to acquiring a *Clostridium difficile* infection**,** a patient hospitalized for an extended period of time, a patient who has been treated for an extended period of time with broad spectrum antibiotics, a patient on therapy with a proton pump inhibitor for treatment of gastrointestinal diseases or conditions, a cancer patient, and a transplant patient.

18. The use according to claim 17, wherein the cancer patient is undergoing treatment with an anti-cancer drug, or undergoing radiotherapy to treat a cancer.

19. The use according to claim 17, wherein the transplant patient is a patient receiving a hematopoietic stem cell transplant, or a solid tissue or organ transplant.

20. The use according to claim 17 or 19, wherein the transplant patient is being treated with an immunosuppressive drug, or any transplant rejection drug, or who is undergoing treatment with a drug regimen to prevent tissue or organ graft rejection following the transplant.

21. The use according to claim 16 to 20, wherein the pharmaceutical composition is administered to the patient in combination with a second therapeutic agent.

22. The use according to claim 21, wherein the second therapeutic agent is selected from a toxoid, a *Clostridium difficile* vaccine, an antibiotic, another different antibody to *Clostridium difficile* toxin A and/or B, and any other palliative therapy useful for ameliorating at least one symptom associated with a *Clostridium difficile*-associated condition or disease.

23. The use according to claim 22, wherein the at least one symptom or complication associated with the *Clostridium difficile*-associated condition or disease is selected from the group consisting of anorexia, abdominal pain, abdominal bloating, diarrhea with or without bleeding, dehydration, malnutrition, pseudomembranous colitis, complete or segmental colonic resection, fever and systemic infection (sepsis), death, relapse of the *Clostridium difficile* condition or disease, and rejection of a transplanted tissue or organ.

24. An isolated nucleic acid encoding an antibody or binding fragment thereof according to any one of claims 1 to 6 comprising at least one non-natively occurring regulatory element.

25. An expression vector comprising the nucleic acid of claim 24.

26. An isolated host cell comprising the expression vector of claim 25.